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Cardiovascular and temperature actions of cathinones

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Cardiovascular and Temperature Actions of Cathinones

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Supervisor: Professor J R Docherty

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Declaration:

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree, Doctor of Philosophy (Ph.D.), is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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Abbreviations

anova	analysis of variance
ATP	adenosine triphosphate
BMY7378	8-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-8-azaspiro[4.5]decane-7,9-dione
BRL44408	2-[(4,5-Dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole maleate
Caff*	caffeine
Cath*	cathinone
CO*	cocaine
DAT	dopamine transporter
DBP	diastolic blood pressure
Eph*	ephedrine
F*	female
HR	heart rate
5-HT	5-hydroxytryptamine
KO	knockout
M*	male
MAO	monoamine oxidase
MDA	Methylenedioxyamphetamine
MDEA	Methylenedioxyethylamphetamine
MDMA	Methylendioxymethamphetamine
MHA	methylhexanamine
NA	noradrenaline
NET	noradrenaline transporter
Noreph*	norephedrine
6-OHDA	6-hydroxydopamine
pKi	concentration producing 50% inhibition (-logM)
prop*	propranolol
SBP	systolic blood pressure
s.e.	standard error
SERT	serotonin transporter

sympX*	sympathectomised
TYR*	tyramine
veh*	vehicle
VMAT-2	vesicular monoamine transporter
WT	wild type

* Abbreviations used only in Figures and Tables.

Publications arising from this work

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Alsufyani HA, Docherty JR. Direct and indirect cardiovascular actions of cathinone and MDMA in the anaesthetized rat. Eur J Pharmacol. 2015 Apr 8; 758:142-146.

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Summary

The main aims of these studies were to investigate cardiovascular and temperature actions of the main constituent of khat, cathinone, and to study possible gender differences. Also, since caffeine is often taken together with cathinone, the interaction between caffeine and cathinone was examined. Cathinone had cardiovascular effects in the anaesthetised rat similar to those of methylenedioxymethamphetamine (MDMA), and like MDMA, cathinone acts predominantly as an indirect sympathomimetic acting at the noradrenaline transporter. The indirect actions of cathinone and MDMA were confirmed by the effects of sympathectomy on cardiovascular responses, and by the lack of contractile responses in rat aorta and vas deferens.

No gender differences were found in the cardiovascular actions of cathinone or MDMA, but differences were found for tyramine and ephedrine that may have different modes of action, involving synaptic vesicular transport. Complex effects of ephedrine on blood pressure involve direct α_{1D}-adrenoceptor, but also indirect α_{1A}-adrenoceptor-mediated components.

Cathinone caused marked increases in locomotor activity and effects were greater in female than male rats. The most surprising result was the interaction of cathinone with caffeine. Caffeine significantly increased the peak effect of cathinone on locomotor activity but thereafter decreased the effects of cathinone. Such a complex interaction between caffeine and cathinone may both increase the toxic effects of a single dose of cathinone and cause more frequent dosing with khat/cathinone. In terms of temperature, cathinone alone or caffeine alone had only minor effects on temperature in both male and female rats. The major finding was the interaction between caffeine and cathinone only in male rats. Following caffeine, cathinone produced a significant acute hyperthermia only in male rats, demonstrating a gender difference in the ability of cathinone to cause a hyperthermia.

The results presented in this thesis demonstrate the need to carry out studies in both male and female to demonstrate actions and interactions that may be relevant to the human situation.

Chapter 1

General introduction

Chapter 1

General introduction.

1.1. Prevalence and consequences of the use of stimulants

1.1.1. Natural stimulants: Overview of khat

Drug abuse continues to be a problem and a burden on individuals and communities. Whether natural or synthetic agents, drugs of abuse are used to enhance mood, increase alertness and increase energy. Many of the natural stimulants have similar harmful effects to the synthetic drugs. One of the naturally occurring stimulants, requiring no synthetic processing, is the plant khat (Catha edulis Forsk).

Khat chewing has been known for years as a cultural and a social issue especially in Yemen and East Africa (Weir, 1985; Randall, 1993). It also grows in Kenya, Ethiopia, Djibouti, Somalia and Uganda. The geographical area of khat use that extends from Yemen to east Africa is known as the Khat-belt countries (Figure 1.1). Kennedy et al. (1983) speculated that the plant catha edulis was known by the ancient Egyptians and it was used for medical reasons in Afghanistan back in the 11th century. The origin of the name Catha edulis and coffee comes from 'Kafa' which is a place name in Ethiopia (El Mahi, 1962).

The Catha edulis plant is harvested as a whole (stem and leaves) and kept fresh wrapped in a cloth or banana leaves (Figure 1.2). Khat is chewed into a ball and stored in the corner of the mouth for hours (Figure 1.3). Chewing khat leaves liberates alkaloids which get absorbed through the buccal mucosa into the systemic circulation (Toennes et al., 2003). The typical duration of consuming khat is 3-4 hours with a consumption of almost 300 g (Kalix, 1994; Nencini et al., 1989). During the khat session, chewers prefer to sit in a closed environment as the increase in temperature may intensify the effect of euphoria. Also, khat chewers may smoke a cigarette or have a generous supply of beverages that

Figure 1.1. Khat-belt countries showing the geographical proximity of khat cultivating countries. http://khat-paradox.blogspot.ie

Figure 1.2. Khat (Catha edulis Forsk) leaves.

http://www.esf.org/index.php?id=5160

Figure 1.3. Chewing of khat leaves.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3991038/#!po=70.8333

may contain caffeine. Among other effects, khat chewers reported feelings of euphoria, alertness and increased confidence (Widler et al., 1994; Elmi, 1983). In Yemen, It was reported that 40% of the family income is spent on khat (Drug Enforcement Administration (DEA), 2003).

1.1.2. Other stimulants

A wide range of health consequences arise from the use of drugs or stimulants like cocaine, cathinone, methylendioxymethamphetamine (Ecstasy, MDMA), mephedrone (meow meow) and methylhexanamine (MHA). Caution must be taken when extrapolating data from poly-drug use due to difficulties in terms of verifying information, and in use of case reports.

In Europe, cocaine is the second most common drug of abuse after cannabis with a high prevalence of 4.2% in the UK among young individuals (aged 15-34) (EMCDDA, 2013b). In Ireland, 94 cases of admissions to hospitals in 2010 were due to a non-fatal overdose (Giraudon et al., 2014). A study in the US showed that 5% of cocaine users can develop dependence in their first year of use, but not more that 20% in the long run (Wagner and Anthony, 2002). The most common adverse effects of cocaine are cardiovascular complications, cerebrovascular accidents and neurological impairment. MDMA (ecstasy) first appeared in the 1960's and became popular in the 1990's in dance parties and clubs. Emergency Department admissions due to MDMA has increased in the past years (Drug and Abuse Warning Network, 2010).

Mephedrone (4-methylmethcathinone) is a synthetic cathinone (a β-keto-amphetamine structure) (Wood et al., 2010), chemically related to amphetamine, and is illegally marketed as ''bath salts'' or ''plant food'' and became very popular and preferred by users over cocaine and MDMA (Winstock et al., 2011; Vardakou et al., 2011). It has been recently banned in some countries in Europe including the UK (Morris, 2010). A survey after the ban in the UK of 150 previous mephedrone users reported that 63% are still using the drug, and sourcing the

drug from drug dealers has increased from 41% to 57% (Dargan & Wood, 2010; Measham et al., 2011).

The British Crime Survey conducted a study of the prevalence of mephedrone use, which was found to be the same as the prevalence of MDMA use with 1.4% in 2010/2011 among 16-59 years old (Home Office Statistical Bulletin, 2011). Mephedrone in the UK was reported to have the highest daily use rate of 4.4% under the age of 21 in a survey of 1006 Scottish school and university students (Dargan et al., 2010). Poly-drug use with mephedrone was studied in 89 patients who presented to the emergency department in Scotland revealed that 35% had used mephedrone with other drugs, 30% used it with alcohol and 33% used it alone (Regan et al., 2011).

Some stimulants have been marketed as energy-boosting supplements such as MHA, which has been subsequently banned in many countries (Singer et al., 2013). MHA is administered through inhalation or taken orally (Venhuis et al., 2012). In the UK, the drug was removed in August 2012 amid raised concerns about its safety (press release, 2012). In 2013, the FDA warned the public about the use of MHA and considered it a potential health risk (Singer et al., 2013). Although many deaths have been reported in athletes or soldiers who consumed MHA, a direct effect could not be identified clearly (Chiaramonte, 2012; Claire Squires inquest, 2013). Little is known about the pharmacology of this drug, but there are published reports about its cardiovascular complications (Aviado, 1959).

1.1.3. Legal status of khat

It has been estimated that more than 20 million people use khat around the world (Saha and Dolley, 2006). While khat is deeply rooted in the culture and history of some countries, khat is still legal in Yemen, Djibouti, Ethiopia, Kenya and Somalia. It is still hard to get figures of khat trade around the world but it is known that almost 90% of khat is exported from Ethiopia (Lemessa, 2001). However, increasing transportation and emigration helped spread khat into western

countries including the United States and the UK (Mayberry et al., 1984; Deitschy, 1992). Khat smuggling in the UK could reach 7 tons going through Heathrow Airport every week (Al-Hebshi & Skaug, 2005). In June 2014, the UK banned khat (The Misuse of Drugs Act, 2014). Khat is also illegal in the USA. Gender aspects of khat use will be discussed later.

1.1.4. Chemistry of khat

Leaves from the naturally occurring plant, khat have similar actions to amphetamine, and contains cathine (norpseudoephedrine) and cathinone (β-keto amphetamine) (Figure 1.4) (Kalix,1990). Cathine was first discovered by the scientist Wolfes (1930) but he did not report any stimulant effect. However, Bruecke (1941) was the first who pointed out that Catha edulis contains a more powerful stimulant and that khat chewers prefer to use the plant fresh. Schorno and Steinegger (1979) isolated cathinone which can become less active in older dried plants. Cathinone was named as a 'natural amphetamine' because it is structurally analogous with similar pharmacological effects to amphetamine (Figue 1.4) (Kalix, 1984). Cathinone when chewed orally, is metabolised via a keto-reduction pathway to norephedrine and a very small fraction of unchanged cathinone is excreted (Brenneisen et al., 1986).

Brenneisen et al. (1990) found that a dose of 0.5 mg/kg of cathinone ingested in a gelatin capsule corresponds to 100g of Catha edulis with a peak plasma concentration of 100 ng/ml. The standard dose of khat chewed in one session equivalent to 0.8 mg/kg gave a peak plasma concentration of 127 ng/ml and an estimated half-life of 4 hours (Widler et al., 1994). The peak plasma concentration was reached more slowly due to the time needed to liberate alkaloid from khat after chewing.

Cathinone ($C_9H_{11}NO$)

Cathine ($C_9H_{13}NO$)

Amphetamine ($C_9H_{13}N$)

Figure 1.4. Chemical structure of cathinone and cathine in comparision to amphetamine.

1.2. Health consequences of khat consumption

The use of khat has been linked to many physiological and psychological disorders. The acute effects of khat use were found to be similar to cocaine and amphetamine (Patel, 2000). Although khat toxicity has a similar picture to amphetamine, toxicity is still unlikely to develop as mastication will slow down the release of stimulants. Irritability and tachycardia were the main acute side effects reported (Widler et al., 1994). Addiction (a disorder of the reward system that is re-inforcing in causing repeat exposure) and tolerance (decreased response) to khat use and psychological cravings were also reported in habitual khat users (Nencini and Ahmed, 1989). Habitual use of khat is characterized by moderate khat use with persistent dependence (a state that results in withdrawal symptoms on cessation of use) (Brenneisen et al., 1990). Withdrawal symptoms can result after chronic use of khat that could result in hypotension and depression (Halbach,1972). Psychosis was reported in chronic heavy khat users (Widler et al., 1994). Reproductive health side effects like oligospermia and decrease levels of testosterone were also reported (Islam et al., 1990). The main adverse effects of khat chewing are summarized in Table 1.1.

Khat is not the only source of the second stimulant found in khat, cathine. Excess dosage with pseudoephedrine can lead to the build up of concentrations of its metabolite cathine (Davis et al., 2008).

1.3. Pharmacology of stimulants

Most drugs of abuse such as amphetamines, amphetamine derivatives, cocaine and natural plants like khat target the monoaminergic neurotransmitter systems (noradrenergic, dopaminergic and serotonergic systems) centrally and/or peripherally. Whether these drugs act directly on monoamine receptors or indirectly through the release of neurotransmitters, they affect mood, alertness, locomotor activity, body temperature and cause cardiovascular stimulation. In terms of sports, some stimulants like MHA have a sympathomimetic role to increase mental concentration, reduce fatigue and increase cardiac output and

blood flow to muscles (USADA, 2007). In addition, peripheral stimulation through β_1-adrenoceptors is particularly important in increasing heart rate (HR) and blood pressure. Given the increasing number of designer drugs now becoming available on the street, it is more useful simply to put down markers for typical actions of stimulants in broad classes of drug based on the effects of the main agents in the group. To spend time analyzing the detailed pharmacological actions of each new derivative is not useful, as the agent might be dropped for a newer designer agent very quickly.

Most stimulants act on the monoaminergic system through the release of noradrenaline (NA), dopamine and/or serotonin (5-hydroxytyptamine: 5-HT). The mode of action of cocaine and amphetamine derivatives can overlap through different sets of actions include:

(1) Block of NA, serotonin or dopamine re-uptake: For example, cocaine is a monoamine re-uptake blocker (Trendelenburg,1966). Phenylpropanolamines such as ephedrine and pseudoephedrine act mainly on the noradrenaline (NET), and are less active at the dopamine transporter (DAT), inactive at the serotonin transporter (SERT), but amphetamines are much less selective (Rothman et al., 2001, 2003; see Davis et al., 2008). Cathinone and MDMA, have actions at NET (Cleary & Docherty, 2003).

(2) Stimulation of monoamine receptors: direct actions on α and/or β-adrenoceptors and indirect actions through the release of NA that in turn acts on α and β-adrenoceptors. Agents such as tyramine may displace neurotransmitter from vesicles (Fleckenstein & Burn, 1953), and agents like cocaine block NET. Other agents may have a mixture of both actions.

(3) Monoamine Oxidase (MAO) inhibitors: These probably have no major acute

Table 1.1. The main adverse effects of khat chewing in humans (adapted from:
Cox and Rampes, 2003)

System	Adverse effects
Cardiovascular system	Tachycardia, palpitations, hypertension, arrhythmias, vasoconstriction, myocardial infarction, cerebral infarction.
Gastro-intestinal system	Dry mouth, dental caries, periodontal disease, loss of appetite, chronic gastritis, constipation, hemorrhoids, weight loss, upper gastro-intestinal malignancy, liver diseases.
Genito-urinary system and Obstetric effects	Urinary retention, oligospermia, impotence, libido change, stillbirth, low-birth weight.
Metabolic and endocrine effects	Hyperthermia, perspiration, hyperglycemia
Central nervous system	Euphoria, Irritability, anorexia, psychosis, depression, hallucinations, insomnia, headaches, lethargy.
Ocular effects	Blurred vision, dilated pupils.

action to affect monoaminergic responses (responses to NA, dopamine, serotonin), but chronic actions, such as in their use as antidepressants, could alter striatal dopamine, or indeed other monoamine, release with chronic MAO-B inhibition (Hertting et al.,1961; Lamensdorf et al., 1996).

(4) Presynaptic and postsynaptic monoaminergic receptors at nerve synapses or junctions. Presynaptic autoreceptors (receptors on a nerve for its own neurotransmitter) or heteroreceptors (receptors on a nerve for another neurotransmitter) are often inhibitory receptors. Amphetamine derivatives like MDMA and cathinone reduce nerve release of NA through actions on the inhibitory α_2-adrenoceptors (Lavelle et al., 1999; Rajamani et al., 2001). Postsynaptic monoaminergic receptors at nerve synapses are often stimulatory in nature in which MDMA can act as an agonist, or indirectly by releasing neurotransmitter, to produce stimulation of adrenoceptors, serotonergic receptors and dopaminergic receptors (Lavelle et al.,1999; Bexis and Docherty, 2006). It has been found that blocking α_{2A}-adrenoceptors can reduce hyperthermia to MDMA, suggesting a role for synaptic α_{2A}-adrenoceptors (Bexis and Docherty, 2005, 2006).

1.3.1. Pharmacological actions of stimulants: actions at adrenoceptors

Stimulants that activate the sympathetic nervous system are called sympathomimetics. The neurotransmitter NA is stored mainly in the terminals of sympathetic nerves and released upon stimulation, to mediate the sympathetic effect through actions on adrenoceptors (Perlman and Chalfie, 1977). The stimulants studied in this thesis all have, to varying degrees, actions at α- and/or β-adrenoceptors, to cause pressor and depressor actions, cardiac stimulation and smooth muscle contractions and relaxations. Subtypes of adrenoceptor and the effects mediated by them are next considered.

Adrenoceptors belong to the G-protein linked superfamily of receptors, membrane bound receptors that mediate actions through linkage to a G-protein to cause intracellular effects. These G-proteins may link to activation or inhibition of an enzyme involved in second messenger production or modulation of channel function. There are classically two classes of adrenoceptors, α- and β-adrenoceptors (Ahlquist, 1948), but it is probably more logical to consider three classes: α_1, α_2, and β (Docherty, 2010).

α-Adrenoceptors were the classical smooth muscle receptors involved in contraction in the classification of Ahlquist (1948). These receptors were present on smooth muscle cell membranes to mediate stimulation. Hence, it was surprising when α-adrenoceptors were found to be present in nerve terminals (Starke, 1977). It was found that these α-adrenoceptors that exist in the presynaptic membrane could mediate a negative feedback mechanism on NA release (Langer, 1977; Starke, 1977). Based on this finding, adrenoceptors were classified as pre- and post-junctional adrenoceptors. Berthelsen and Pettinger (1977) classified the excitatory responses as α_1-adrenoceptor mediated and the inhibitory responses as α_2-adrenoceptor mediated. This classification held good until Docherty et al. (1979) suggested that α_2-adrenoceptors can be found postsynaptically and mediate, along with α_1-adrenoceptors, vascular smooth muscle contractions. Hence, α_1 and α_2-adrenoceptors were classified upon their pharmacological actions, and on the actions of selective antagonists. The α_1- and α_2-adrenoceptors were further subdivided with the development of ligand-binding studies into α_{1A}, α_{1B} (Morrow and Creese, 1986), α_{1D} (Perez et al., 1991) and α_{2A}, α_{2B} and α_{2C}-adrenoceptors (Bylund et al., 1992). It was found that α_{2A}-adrenoceptors were the main presynaptic α_2-adrenoceptors (Trendelenburg et al., 1993).

α_1-Adrenoceptors are found post-synaptically and are coupled to $Gq/_{11}$ proteins that mediate their effects through a Ca^{2+} second messenger system (Cotecchia et al., 1990; Docherty, 2010). When agonists bind to the α_1-adrenoceptor, they activate the enzyme phospholipase C which in turn breaks down phosphatidylinositol biphosphate (PIP_2) resulting in inositol triphosphate (IP_3) and

diacylglycerol (DAG). In turn IP_3 increases Ca^{2+} levels causing cell depolarisation and muscle contraction (Berridge and Irvine, 1989; Chen and Rembold, 1995). The predominant subtypes of α_1-adrenoceptors that cause smooth muscle contractions are α_{1A} and α_{1D}-adrenoceptors. Aboud et al. (1993) investigated the subtypes of α_1-adrenoceptor mediating contractions in aorta, spleen and vas deferens. Rat vas deferens and aorta will be discussed separately below.

1.3.2. Rat vas deferens: α_1-adrenoceptors

The rat vas deferens has been extensively studied in investigations of α-adrenoceptor mediated responses, both in terms of nerve and agonist responses. For agonist responses there have been a number of studies investigating the subtype of α_1-adrenoceptor present in rat vas deferens. Some authors suggested that contractions of rat vas deferens to exogenous NA/adrenaline were due mainly to α_{1A}-adrenoceptor stimulation (Han et al., 1987; Honner & Docherty, 1999), others suggested α_{1D}- in addition to α_{1A}-adrenoceptors (Cleary et al., 2004), or even α_{1L}-adrenoceptors ((L: low affinity for prazosin: Ohmura et al., 1992) in addition to α_{1A}-adrenoceptors (Buccioni et al., 2009). The potency of the α_1-adrenoceptor antagonist prazosin has been investigated in many studies of rat vas deferens: low potency (Ohmura et al., 1992); low and high potency (Honner & Docherty, 1999); high potency (Burt et al., 1995; Amobi et al., 1999). This is important as low prazosin potency might suggest the presence of an unusual α-adrenoceptor, the α_{1L}-adrenoceptor (called α_{1L} due to the low potency of prazosin: Ohmura et al., 1992). It had been thought that prazosin was non-selective as an α_1-adrenoceptor antagonist, blocking all subtypes.

Work from our laboratory has shown that contractions of rat vas deferens to NA are mediated by α_{1A}- and α_{1D}-adrenoceptors (Honner & Docherty, 1999; Cleary et al., 2004), and that the α_{1D}-adrenoceptor mediated component is involved in transient phasic contractions but the α_{1A}-adrenoceptor mediated component is involved in tonic contractions. The confusion of prazosin potency can be solved by the report that prazosin has higher potency at α_{1D}-adrenoceptors than at α_{1A}-adrenoceptors (Docherty, 2014). Depending on experimental conditions, the α_{1A}-

or α_{1D}-adrenoceptor action might predominate, explaining the range of prazosin potencies found by authors in the literature. Hence, in rat vas deferens, it was possible to investigate both α_{1A}- and α_{1D}-adrenoceptor mediated actions of stimulants by looking at tonic and phasic components of the contraction to agonists.

The effects of sympathectomy on responses of rat vas deferens were previously studied in our laboratory (Cleary et al., 2004). The rat vas deferens is a highly innervated tissue, which makes it suitable for the study of directly and indirectly mediated actions. In rat vas deferens the number of α_{1D}-adrenoceptors present and the phasic α_{1D}-adrenoceptor mediated component to the contraction to NA are increased by sympathectomy (Cleary et al., 2004). The number of α_{1A} - adrenoceptors present and the tonic contraction to NA involving mainly α_{1A}-adrenoceptors were largely unaffected by sympathectomy (Cleary et al., 2004). Although sympathectomy produced marked effects on responses to agonists, nerve stimulation–mediated contractions of rat vas deferens were only partly reduced (Cleary et al., 2004), suggesting resistance of this densely innervated tissue to sympathectomy or a postjunctional supersensitivity masking the degree of sympathectomy.

In this thesis, the effects of direct and indirect sympathomimetics have been investigated on contractile responses of vas deferens from control and sympathectomised rats. In this study, cardiac and blood pressure actions of cathinone, MDMA and tyramine were investigated in control and sympathectomised animals. Further studies looked at a number of additional agonists including ephedrine, MHA and cathine. These results will be shown in Chapter 7.

The rat vas deferens was also used to try and get a qualitative way of assessing the degree of sympathectomy produced by the 6-hydroxdopamine (6-OHDA) dosing in terms of reduction of functional contractions, since biochemical changes may tell little about changes in functional responses. In this case the vas deferens response to nerve stimulation was employed. The success of this, or otherwise, will be discussed in the discussion.

1.3.3. Rat aorta: α_1-adrenoceptors

The rat aorta is widely employed as a simple preparation for the study of vascular contractions and relaxations. In particular, it has been studied in terms of α_1-adrenoceptor mediated contractions. BMY 7378 is a selective antagonist at α_{1D}-adrenoceptors (Goetz et al., 1995) and has been useful in identifying responses mediated by α_{1D}-adrenoceptors. BMY 7378 potently and competitively blocks contractions to NA in rat aorta (Piascik et al., 1995; Hussain and Marshall, 1997; Honner & Docherty, 1999). Although the α_{1D}-adrenoceptor is the main receptor involved in contractions, it has been shown that both α_{1A}- and α_{1B} −adrenoceptors are involved in trophic effects including smooth muscle growth in rat aorta (Zhang & Faber, 2001).

Contractile responses to NA in rat aorta are mediated largely by α_{1D}-adrenoceptors. The rat aorta is therefore a model system for investigation of actions of stimulants at α_{1D}-adrenoceptors. However, since the rat aorta is a very poorly innervated tissue, only a direct agonist will produce significant contractions. If this is true, sympathectomy should have no effect on responses of rat aorta. Hence, the rat aorta will allow us to identify α_{1D}-adrenoceptor mediated actions of stimulant. In this thesis, contractions to a range of stimulants in aorta from vehicle treated and sympathectomised rats have been investigated. The results are shown in Chapter 8.

1.3.4. α_2-Adrenoceptors

α_2-Adrenoceptors can be found at both presynaptic and postsynaptic membranes. Postsynaptic α_2-adrenoceptors are involved in vasoconstriction (Docherty et al., 1979; Docherty and McGrath 1980), while presynaptic α_2-adrenoceptors are involved in negative feedback inhibition of NA release (Starke, 1977). It was found that α_{2B}-adrenoceptor knockout mice showed a great decrease in pressor responses to the α_2-agonist dexmedetomidine, implying the importance of α_{2B}-adrenoceptors in vasoconstriction (Altman et al., 1999). α_{2A}−Adrenoceptors are also implicated in the inhibition of dopaminergic and

serotonergic pathways implying their role in locomotor activity and behaviour (Millan et al., 1994). α_2-Adrenoceptor agonists can be used in treatment of drug withdrawal (Gowing et al., 2001).

In this work, there are two points of interests in α_2-adenoceptors. Firstly, the inhibition by sympathomimetics of the nerve stimulation evoked contraction to a single stimulus has been studied in rat vas deferens. Presynaptic α-adrenoceptors in rat vas deferens are, like in most adrenergic nerves, of the α_{2A}-adrenoceptor subtype, as has been demonstrated using the selective α_{2A}-adrenoceptor blocker BRL 44408 (Docherty, 1998). Hence, in the present studies, the α_{2A}-adrenoceptor potency of stimulants has been obtained to add to their profile of actions.

In the rat vas deferens, nerve stimulation-evoked contractions produced by a single electrical stimulus have two components: an early purinergic response due to release of ATP as a cotransmitter, and a later α_{1D}-adrenergic component due to release of NA (see diagrammatic representation in Figure 1.5). The vas deferens can easily be divided into two portions with different responses. Taking the prostatic portion, contractions to a single stimulus are mostly purinergic, and calcium channel blockers of the L-type channels such as nifedipine abolish this response (Blakeley et al., 1981; French and Scott, 1981; Brown et al., 1983): this early purinergic component involves calcium entry. Taking the epididymal portion, contractions to a single stimulus are mostly adrenergic, and nifedipine does not affect this response: this later adrenergic component involves internal calcium stores.   Now, since stimulants can act at α_2-adrenoceptors to inhibit nerve-evoked contractions, but can also act at α_1-adrenoceptors to increase contractions, this complication must be considered when looking at α_2-adrenoceptor potency in this model system. One way round the problem is to look at responses in the epididymal portion in the presence of nifedipine, that prevents postjunctional α_{1A}-adrenoceptor actions of agonists, but leaving the postjunctional α_{1D}-adrenoceptor response to the nerve-stimulation released neurotransmitter.

Figure 1.5. Illustration of the contractile response to a single stimulus in prostatic (P: black trace) and epididymal portions (E: orange trace) of rat vas deferens. The early component of the response is largely purinergic (black box below traces) and predominates in the prostatic portion, but the later component is α_1-adrenergic (orange box below traces) and predominates in the epididymal portion. Responses are normalized to 100% of individual maximum. Time scale shows 1 second. Single pulse electrical stimulus is given at arrow.

Secondly, α_2-adrenoceptors have a role in temperature and locomotor responses to stimulants, and these actions are largely central, but this will be discussed later.

1.3.5. β-Adrenoceptors

There are three subtypes of β-adrenoceptors: β_1-, β_2- and β_3-adrenoceptors (Lands et al., 1967; Emorine et al., 1989). They are involved in cardiac contractile actions and smooth muscle relaxations. Stimulation of β_1-adrenoceptors in the heart will increase the rate (chronotropic) as well as the force of contraction (inotropic) (Motomura et al., 1990). β_2-Adrenoceptors are found in the skeletal muscle vasculature (Elfellah et al., 1989) and in airways smooth muscle to mediate bronchodilation (Barnes et al., 1983). β_3-Adrenoceptors are also involved in energy metabolism (Arch et al., 1984; Fisher et al., 1998), in bladder smooth muscle relaxation (Takeda et al., 1999) and in vascular relaxation (Chruscinski et al., 2001; Al Zubair et al., 2008;). Isoprenaline, a classical direct β-adrenoceptor agonist, was found to be more potent than adrenaline and NA at β-adrenoceptors. Isoprenaline will be used in these studies to assess whether chemical sympathectomy changes the direct actions of agonists. Hence, tachycardia and vasodepressor responses to isoprenaline will be investigated in vehicle treated and sympathectomised rats. The tachycardia is presumably mainly β_1-adrenoceptor mediated and the depressor response presumably mainly β_2-adrenoceptor mediated.

1.4. Synthetic stimulants and amphetamine derivatives

Many studies have made an effort to relate acute and chronic neuropsychological and cardiovascular impairment to drug use and addiction. A wide range of health consequences arises from the use of drugs or stimulants like cathinone, MDMA, MHA and ephedrine (Figure 1.6). With the increasing occurrence of designer

Ephedrine ($C_{10}H_{15}NO$)

MHA ($C_7H_{17}N$)

MDMA ($C_{11}H_{15}NO_2$)

MDA ($C_{10}H_{13}NO_2$)

MDEA ($C_{12}H_{17}NO_2$)

Figure 1.6. Chemical structure of MHA, ephedrine, MDMA, MDA, and MDEA.

stimulant drugs of abuse such as mephedrone, it becomes increasingly difficult for pharmacological research to keep up with new derivatives on the street.

In this thesis, a number of stimulants have been employed. Cathinone and cathine were investigated in cardiovascular and *in vitro* studies as the two major active constituents of khat. For comparison, a number of other stimulants were investigated both in cardiovascular and *in vitro* studies: MDMA, tyramine, ephedrine, and MHA. In cardiovascular studies, tyramine was chosen as an indirect sympathomimetic, and isoprenaline was chosen as a direct β-adrenoceptor agonist. MDA and MDEA were only examined *in vitro* in rat vas deferens and aorta, as ephedrine proved more interesting for further investigation. For interaction with caffeine, both cathinone and MDMA were investigated in cardiovascular studies, but only cathinone was examined in telemetry studies.

It should be noted that differences may be expected in the profile of action of stimulants, when comparing direct and indirect sympathomimetics. An indirect agonist would be expected to activate all subtypes of innervated adrenoceptor, if they are found near nerve terminals, but potency might be affected by the density of innervation. Hence, tyramine has α- and β-adrenoceptor actions as an indirect agonist. In contrast, a direct agonist might selectively act on certain subtypes of receptor, for example, isoprenaline with selectivity for β-adrenoceptors with little or no action on α-adrenoceptors.

The actions of some major stimulants will be discussed below.

1.4.1. MDMA, MDA and MDEA

MDMA, known as ''ecstasy'', as well as MDA, first appeared in the 1960's and became popular in the 1990's in dance parties and in clubs. MDEA appeared as a replacement for MDMA (Colado et al., 1999). Emergency Department admissions due to MDMA have increased in the past years (Drug and Abuse

Warning Network, 2010). Although MDMA, MDEA or MDA tablets all are marketed as ecstasy, they may contain a variable mixture of other stimulants like caffeine, amphetamine and ephedrine (Milroy,1999; Green et al., 1995). Users of these stimulants reported feelings of euphoria, increased affection and empathy (Mas et al., 1990; Milroy et al., 1999). The stimulant effect appears after 20-60 minutes and last for 3-4 hours. The typical dose of MDMA in one table is 60-120mg (Morgan, 2000).

In addition to being subject to misuse *per se*, MDA is also a metabolite of MDMA and MDEA. Like MDMA, MDA and MDEA cause a serotonin transporter dependent depletion of 5-HT from synapses (Schmidt et al., 1987), and cause release of DA from striatal slices (Schmidt, 1987). They also have major actions at NET (Schmidt et al., 1987; Wang et al., 1987). In terms of inhibiting uptake of [^{3}H] NA, MDMA was nearly as potent as cocaine (cocaine 6.16±0.15, MDMA 6.05±0.07, -log IC_{50} values); but MDA (5.68±0.06) and MDEA (5.56±0.08) were significantly less potent (Cleary & Docherty, 2003).

McCann et al. (1996) reported urine retention linked to the use of MDMA, which, like pupil dilation, may have α_1- and/or β_3-adrenoceptor mediated components involved in constriction of the bladder neck and relaxation of the detrusor muscle, respectively (Docherty, 2010; Vij & Drake, 2015). In addition, prejunctional α_{2A}-adrenoceptor stimulation by MDMA was proven through studies of neurotransmission on rat vas deferens (Bexis and Docherty, 2006). Also, β_3-adrenoceptor involvement in temperature regulation through activation of brown adipose tissues has been shown (Sprague et al., 2004).

1.4.2. Cathinone

It has been reported that cathinone induces dopamine release from rabbit caudate and rat striatal slices (Kalix 1980; Wagner et al., 1982). Kalix (1982) showed that dopamine release after administration of cathinone was blocked by inhibitors of the dopamine transporter indicating carrier mediated release similar to the actions of amphetamine. Dopamine is involved in the rewarding and

reinforcing effects as cathinone was found to cause drug induced place preference that was decreased by dopamine receptor blockade in rats (Schechter et al., 1991). It was also found that cathinone had one third the potency of amphetamine at inducing 5-HT release in the rat (Kalix, 1983b). Cathinone induces NA release with a potency higher than that of dopamine release (Kalix, 1983). A study showed that cathinone had a local anaesthetic effect similar to lignocaine (Guantai et al., 1987).

Cathinone has major actions at NET. Cathinone has similar potency to MDMA, and indeed tyramine, at NET (approximately 1 μM) (Horn, 1973; Cleary & Docherty, 2003). In terms of inhibiting uptake of [^{3}H] NA, cathinone was nearly as potent as cocaine (cocaine 6.16; cathinone 6.03: -log IC$_{50}$ values) (Cleary & Docherty, 2003).

1.4.3. Cathine

Cathine is also (-)-pseudonorephedrine. In terms of actions at transporters, cathine is virtually inactive at SERT, had moderate potency at DAT, but was nearly as potent as cathinone at NET (30 versus 12 nM) (Rothman et al., 2003). It should be noted that biochemical studies such as these often give higher potencies than obtained in physiological studies (see above). There is no information as to actions of cathine or cathinone at the extraneuronal monoamine transporter (EMT: uptake$_2$), but this is relatively unimportant in the periphery.

1.4.4. Ephedrine

Ephedrine is a natural sympathomimetic amine that has been used for years as a stimulant, nasal decongestant and also to control appetite (Sweetman, 2007). Ephedrine, which is obtained from the plant Ephedra has a similar structure to amphetamine and can act like adrenaline with a longer effect to increase blood pressure and to stimulate the heart (Drew et al., 1978).

Ephedrine, in addition to peripheral cardiovascular actions, also has marked central stimulant actions. In sport ephedrine has been abused as a performance enhancing drug, particularly as a weight loss supplement, and to arouse aggression in contact sports, especially rugby (Wilson, 2004).

The natural form of ephedrine comes from Ephedra (ma-huang), a chinese herbal medicine that has been used in the past in the treatment of asthma (Sweetman, 2007). Paradoxically, many Chinese herbal remedies contain exogenous additives (i.e. not part of the herbal mixture, but added later), including ephedrine (Coghlan et al., 2015). Ephedrine was reported as one of the basic medications needed in the health care system (WHO model list of essential medicine, April, 2015). The medical uses of ephedrine in health care are widespread including as a decongestant and bronchodilator and in the prevention of hypotension during spinal anaesthesia. However, the misuse of ephedrine could lead to life threatening conditions: increase in blood pressure, increase in HR, arrhythmia and stroke (Haller et al., 2000).

Toxic effects of ephedrine can result from taking of dietary supplements that have ephedrine (including ma-huang in Chinese herbal medicine) or by excess dosage of over-the-counter ephedrine (Sweetman, 2007). Toxic effects include hypertension due to α-adrenoceptor actions, and β-adrenoceptor actions to cause tachycardia and possible arrhythmias (see also Davis et al., 2008).

(-)-Ephedrine is the pharmacopoeial preparation and is reported to be about 5 times more potent at NET than (+)-ephedrine (values of 43.1 and 218 nM, respectively (Ma et al., 2007). However, ephedrine is often produced as the racemate mixture.

Previous studies in anaesthetized rats and isolated tissues have suggested that ephedrine could have a mixture of both direct and indirect actions on blood pressure in anaesthetized rats (Kawasuji et al., 1996; Kobayashi et al., 2003), or that the blood pressure actions were mainly direct (Liles et al., 2006). Gene knockout (KO) technology has also been used to answer whether responses to (-)-ephedrine are predominantly directly mediated or due indirectly to the release of

NA, or a mixture of both (Liles et al., 2007). Liles et al. (2007) employed mice with KO of the dopamine-β-hydroxylase gene (DBH-KO), to prevent formation of NA from dopamine, to answer this question. The rises in blood pressure to the archetypal indirect sympathomimetic tyramine were virtually abolished in DBH-KO mice, demonstrating that tyramine acts virtually exclusively by an indirect mechanism in these studies. The α-adrenoceptor-mediated peak pressor response to ephedrine, like those to the direct agonists NA and phenylephrine, was unaffected by DBH-KO. Hence, the conclusion from the above study was that the actions of ephedrine are directly mediated at least in the mouse and in terms of peak blood pressure. However, there is contradictory evidence that ephedrine does not directly activate human α_1- or α_2-adrenoceptors despite the cardiovascular actions quoted above. Furthermore, Ma et al. (2007) suggested that ephedrine can be a weak antagonist of α_2-adrenoceptors. At least, ephedrine has been shown to be a direct agonist at human β-adrenoceptors (Vansal and Feller, 1999). After many years of use of this agent, there is still confusion as to the exact actions of ephedrine: direct agonism, and at which receptor subtypes, or indirect actions as an indirect sympathomimetic.

In this thesis, ($\pm$)-ephedrine and in addition (-)-ephedrine were studied to investigate whether choice of isomer of ephedrine might change the effects of the agent and the relative direct and indirect components.

The tachycardia and pressor and depressor responses to ephedrine were examined in male and female anaesthetised rats to investigate possible gender differences in vehicle treated animals and sympathectomised animals. The complex blood pressure actions of ephedrine will be examined in detail: initial pressor followed by a delayed depressor action. It will be possible to ascertain possible gender differences, but also whether a component of the response to ephedrine is indirectly mediated as revealed by the effects of sympathectomy.

Since ephedrine produced marked β-adrenoceptor mediated tachycardia and depressor responses, isoprenaline was employed as a direct agonist for

comparison with ephedrine. Possible gender differences in actions of isoprenaline and the effects of sympathectomy will be examined to determine the effects on a direct β-adrenoceptor agonist and how they are altered.

1.4.5. MHA

Some stimulants have been marketed as energy-boosting supplements such as MHA, also known as dimethylamylamine (DMAA) or geranamine, which has been subsequently banned in many countries (Singer et al., 2013). Supplements that contain MHA may be listed as "geranium extract", although there is doubt whether this compound is actually present in the geranium but may be an additive. MHA is administered through inhalation or taken orally (Venhuis et al., 2012). It is a vasoconstrictor that was formerly used as a nasal decongestant in nasal inhalers (Venhuis et al., 2012). In the UK, the drug was removed in August 2012 amid raised concerns about its safety (press release, 2012). In 2013, the FDA warned the public about the use of MHA and considered it a potential health risk (Singer et al., 2013). MHA was listed as one of the prohibited substances by the World Anti-Doping Agency in 2014. Although many deaths have been reported in athletes or soldiers who consumed MHA, a direct effect could not be identified clearly (Chiaramonte, 2012; Derave et al., 2014). Little is known about the pharmacology of this drug, but there are published reports about its cardiovascular complications (Aviado, 1959).

The use of the stimulant MHA in sport has shown some serious cardiovascular complications. These effects could be expected to a single dose of oral intake of 50-75 mg to cause an increase in HR and about 100 mg to cause an increase in blood pressure (Venhuis et al., 2012). MHA is considered to have a long half-life that makes repeated doses build up and contribute to its pharmacological side effects (Venhuis et al., 2012). Laboratory studies showed that energy- boosting

supplements may contain a dose of 25-65 mg of MHA (Venhuis et al., 2012).

It was found that when MHA was given intravenously, the LD_{50} was 75.5 mg/kg in rats and 39 mg/kg in mice (Venhuis et al., 2012). There are limited numbers of published reports about MHA in animal models. However, it was found that the pressor responses to oral dosing in dogs were 4 times less then to ephedrine and 2 times less than to amphetamine (Swanson et al, 1948). The mode of action of MHA in terms of direct or indirect actions will be investigated in this thesis.

1.4.6. Cocaine

Cocaine is still employed medically as a local anaesthetic to a limited extent, but is much more widely seen as a drug of abuse. The most frequently reported cardiovascular complications of cocaine use is non-ischaemic acute myocardial infarction (Mouhaffel et al., 1995). A recent study showed that a low dose of 3 mg/kg of intranasal cocaine given to 10 healthy young adults produced a marked constriction of the coronary microcirculation with a profound reduction of blood volume in the coronary vascular bed as seen on echocardiography (Gurudevan et al., 2013). Chronic use of cocaine can lead to atherosclerosis that develops through the build up of low-density lipoprotein and peroxidase in a damaged area of the vascular endothelium (Brownlow and Pappachan, 2002). Cocaine use can increase the myocardial infarction risk by 24 fold within the first hour of use (Mittleman et al., 1999). Although chest pain can be a sign of myocardial infarction, it can also indicate the development of myocarditis due to hypersensitivity or toxicity (dose-dependent) (Pozner et al., 2005). Endocarditis can also develop from bacterial invasion on top of a vascular and valvular injury which was presumed to be due to the effect of cocaine in increasing blood pressure and HR (Egred and Davis,2005; Pozner et al.,2005). Acute and chronic aortic dissection can develop most likely from the increase in systolic blood pressure (Brownlow and Pappachan, 2002).

Although, in this thesis, cocaine was not employed as a stimulant, but only as a blocker of NET in studies both *in vivo* and *in vitro*, cocaine is important to include in this survey of stimulants because the cardiovascular adverse effects of cocaine have been well documented (see above). Amphetamine-like agents such as MDMA and cathinone that act on NET, may also have similar cardiac side effects to cocaine.

1.4.7. Newer stimulants

A new designer drug, mephedrone, was studied in animal models which showed it to have similar effect to amphetamines in terms of increasing serotonin and dopamine concentrations and inhibiting their uptake (Martinez-Clemente et al., 2012; Meng et al., 2012). The administration of mephedrone to mice and rats showed similar effects to human in terms of increasing body temperature, locomotor activity and decrease in social preference (Angoa-Perez et al., 2011; Motbey et al., 2012). A further study on rats showed a dose dependent effect of mephedrone (3 and 15 mg/kg) on cardiac function, blood pressure and HR (Meng et al., 2012). This sympathomimetic stimulation by intravenous mephedrone on anaesthetized rats was minor with 0.3 mg/kg and more evident with 1 mg/kg (Meng et al., 2012).

1.4.8. Tyramine

It was long ago shown that contractions of the cat nictitating membrane to tyramine were greatly diminished by denervation (Fleckenstein & Burn, 1953) and by blockade of NA uptake by cocaine (Fleckenstein & Stockle, 1955). Burn & Rand (1958) showed that treatment with reserpine mimicked the effects of denervation or cocaine in blocking actions of tyramine. These results can be explained as tyramine enters the adrenergic nerve using NET to displace NA. Tyramine may act both on NET, to enter into the nerve terminal, and the vesicular monoamine transporter (VMAT-2) to enter the vesicle to displace NA, but shows higher potency at the vesicular site (Partilla et al., 2006; Berg &

Jensen, 2013) (See Figure 1.7). The indirect effects of tyramine are increased by Monoamine oxidase (MAO) inhibition (see Sullivan & Shulman, 1984), since MAO inactivates NA in the cytoplasm, and so block of this enzyme increases the amount of NA available for displacement.

Tyramine produces tachycardia and pressor responses in the anaesthetized rat. Pressor responses are greatly reduced by sympathectomy with 6-OHDA (Finch et al., 1973). The tyramine pressor test is used to assess peripheral sympathetic nerve function, looking at the amount of tyramine, given by bolus intravenous injection, required to increase the systolic blood pressure by 30 mmHg (Ghose, 1984; see Broadley, 2010). Intravenous tyramine increases blood pressure in man but this may be largely due to cardiac actions to increase stroke volume (Meck et al., 2003).

1.4.9. Isoprenaline

Isoprenaline was of major importance in the discovery and subclassification of adrenoceptors. Ahlquist (1948) described two types of adrenoceptor, α- and β-adrenoceptor, based on the rank order of potency of the series of agonists, but particularly isoprenaline, adrenaline and NA. In Ahlquist's classification, β-adrenoceptors were present in the heart, where they were excitatory, and in smooth muscle where they were inhibitory causing relaxation. Isoprenaline is a direct β-adrenoceptor agonist with actions predominantly at β_1- and β_2-adrenoceptors (Brodde, 1988; Al Zubair et al., 2008).

The major cardiovascular actions of isoprenaline involve β-adrenoceptors. Isoprenaline produces marked tachycardia and increased force of cardiac contraction, actions which are mainly β_1-adrenoceptor mediated, increases SBP by the cardiac stimulant actions, and decreases DBP by a vasodilator action probably involving mainly β_2-adrenoceptors. In the present study,

Figure 1.7. The possible sites of actions of stimulants to increase blood pressure and heart rate. These adrenergic actions are the result of a complex mix of:

 1). Direct adrenoceptor stimulation

 2). Indirect release/ potentiation of actions of NA by actions on NET

 3). Indirect displacement of NA from vesicles by action at the vesicular transporter.

Tyramine, as a classical indirect sympathomimetic displaces NA from vesicles (site 3), but also has actions at the noradrenaline transporter (NET) (site 2). Cathinone acts mainly at site 2.

isoprenaline was used as a direct β-adrenoceptor agonist in comparison with indirect sympathomimietcs.

1.4.10. Actions of stimulants at trace amine receptors

Trace amines, including tyramine, are defined as biologically active amines found in the body in very small amounts, often consumed in the diet (see Broadley, 2010). Tyramine occurs in cheeses, red wine, etc. Actions of trace amines may involve adrenoceptors, other monoamine receptors or specific trace amine receptors (Lindemann & Hoener, 2005). Tyramine in concentrations of 100 μM and above contracts the pig coronary artery, and these effects are not blocked by a cocktail of drugs including α-adrenoceptor antagonists (Herbert et al., 2008). Likewise, cathinone and MDMA contract these arteries at concentrations of 100 μM and 30 μM respectively (Broadley, 2010). Cathinone also contracts the guinea-pig aorta at concentrations of 100 μM and above (Al-Motarreb & Broadley, 2004). In rat aorta, tyramine was found to produce only very small contractions at about 10 μM and above, and these contractions were abolished by a cocktail of drugs including the α_1-adrenoceptor antagonist prazosin (Braodley et al., 2013). Cathinone and MDMA produced contractions of rat aorta at 100 μM and 30 μM and above, respectively, resistant to a cocktail including prazosin (Broadley et al., 2013). Hence, some actions reported above may involve actions at trace amine receptors.

Actions of stimulants as possible agonists at trace amine receptor will be considered in this thesis when investigating contractions produced in the isolated rat vas deferens, and particularly in the rat aorta.

1.5. Cardiovascular Complications of cathinone and MDMA

1.5.1. Animal studies
In this section, I compare the cardiovascular actions of cathinone and MDMA.

1.5.1.1. Cathinone

Cathinone which is the main and the most active component of khat leaves has been studied both *in vivo* and *in vitro* and it has been shown that the alkaloid components act as weak amphetamines (Kalix,1990; Brenneisen et al.,1990; Kalix et al.,1991). Cathinone was found to cause contractions of guinea-pig aortic rings and coronary arteries that were not inhibited by cocaine (Al-Motarreb, 2001; Al-Motarreb et al., 2002). This latter study could suggest a direct effect of the drug (see also Introduction 1.4.10). Cathinone was similar to amphetamine in anaesthetised dogs, causing tachycardia, hypertension and increasing the force of cardiac contraction (Kalix, 1991). A study showed that rabbits when given khat 3 times daily for 2 months with a dose of 1g/kg resulted in increased cardiac enzymes as well as areas of small multiple infarctions of myocardial tissue on pathological examination (Alkadi et al., 2002). The cardiovascular complications of cathinone including tachycardia, increase in blood pressure and increase in cardiac output have been shown in rats (Yanagita, 1979).

1.5.1.2. MDMA

MDMA has been widely studied. The serotonergic system may have a role in cardiovascular stimulation by MDMA in human studies (Liechi and Vollenweider, 2001; Tancer and Johanson, 2007). Even though much MDMA research focused on the serotonergic and dopaminergic involvement, noradrenergic system involvement cannot be underestimated. MDMA was found to increase both systolic and diastolic blood pressure through actions at α_1 and α_2-adrenoceptors and 5-HT_2 receptors in *in vitro* and *in vivo* experiments (McDaid and Docherty, 2001; Bexis and Docherty, 2006). The α_1-adrenoceptor antagonist prazosin was found to turn the increase in blood pressure following MDMA into a decrease in blood pressure that proves adrenoceptor involvement (McDaid and Docherty, 2001).

Agonist actions of MDMA on α_{2A}-adrenoceptors can produce systemic vasoconstriction (Docherty, 1998; Duka et al., 2000) and can contribute to hyperthermia in mice (Bexis and Docherty, 2005).

There is evidence that MDMA, cocaine and cathinone have an ability to produce cardiac stimulation through indirect sympathomimetic activation of NET rather than displacement of NA when MDMA evoked contractions to NA on the rat right ventricle (Al Sahli et al., 2001).

Similar doses of MDMA can increase HR and blood pressure in conscious rats as reported in humans (O'Cain et al., 2000). The increase in HR in rat was most evident at 3mg/kg (i.v.) followed by a drop in HR when higher does are reached (20mg/kg) which could be explained by a reflex bradycardia from elevation of blood pressure (Bexis and Docherty, 2006). Such an effect on HR and blood pressure can also be observed with cocaine (Kiristy-Roy et al., 1990; Branch and Knuepfer, 1992) and amphetamines (O'Cain et al., 2000). A study on humans showed that the β-adrenoceptor blocker pindolol can block the HR effect of MDMA (Hysek et al., 2010) while propranolol was able the block the tachycardia to MDMA in rats (Schindler et al., 2014). A noradrenaline transporter role has been shown in man in a study where it was found that reboxetine, the NA uptake blocker, can actually attenuate the HR and blood pressure effect to MDMA (Hysek et al., 2011). In addition, MDMA has a long duration of action of around 2 hours in producing cardiovascular effects (Schindler et al., 2014).

1.5.2. Human studies and case reports

1.5.2.1. Khat

Khat chewing has been studied on humans to prove its amphetamine like properties. Cathinine in khat leaves or pure cathinone produced similar effects at equivalent doses, with only a slower peak plasma concentration of cathinone from khat leaves due to its slower absorption (Widler et al., 1994). A study on 4 volunteers who chewed khat with a mean dose of 45 mg of cathinone showed an increase in blood pressure after 3 hours of use (Toennes et al., 2003). Another study showed an increase in systolic and diastolic blood pressure and tachycardia that lasted for 4 hours (Widler et al., 1994). It was shown that 79% of

a group of 120 patents admitted in the Yemen with myocardial infarction were khat users (Alkadi et al., 2002). The cardiovascular effect of khat, including tachycardia and increase in blood pressure, could be blocked by the use of β_1-adrenoceptors blocker atenolol while α_1-adrenoceptor blocker indoramin showed no blocking effect (Nageeb et al., 2005).

1.5.2.2. MDMA

MDMA was found to cause major cardiovascular complications and sudden death (Russell et al., 1992). An increase in HR has been reported (Hayner et al., 1986) and an increase in blood pressure (Grob et al.,1996; Vollenweider et al., 1998). MDMA has similar actions to cocaine in cardiovascular complications through the effect involving NA (Al-Sahli et al., 2001) in addition to 5-HT (Liechi and Vollenweider, 2001; Tancer and Johanson, 2007).

The potency of MDMA in releasing NA is higher than for dopamine or serotonin (Rothman et al., 2001). Cardiac morbidity can also be linked to the high level of catecholamines found in the plasma of MDMA users caused by noradrenergic stimulation (Stuerenburg et al., 2002). MDMA is reported to increase arterial blood pressure (Gouzoulis et al., 1993). Studies have been consistent in showing that MDMA (1mg/kg and above) can increase HR (Mas et al., 1999; Kolbrich et al., 2008). It was found that the cardiac stimulants dobutamine and MDMA could produce similar increases in cardiac output, HR and blood pressure, except that MDMA had no measurable positive inotropic effect (Lester et al., 2000).

1.6. Chemical sympathectomy

The studies in this thesis of direct and indirect actions of stimulants, at least *in vivo*, rely heavily on the use of 6-OHDA also known as oxidopamine. Studies in this thesis employing rat aorta with 6-OHDA will seek to confirm its lack of effect in a tissue without significant innervation.

6-OHDA is used as a neurotoxic drug that selectively destroys the adrenergic and dopaminergic nerve terminals (Thoenen & Tranzer, 1968; Haeusler et al., 1969). 6-OHDA enters adrenergic nerves by NET and so accumulates in these nerve terminals to give selectivity for adrenergic nerves. The primary molecular mechanism of 6-OHDA is to damage proteins by oxidation (Sachs & Jensson, 1975; Soto-Otero et al., 2000), and direct inhibition by reactive oxygen species of the mitochrondrial respiratory chain complex 1 has been shown (Glinka et al., 1996). It was found that 6-OHDA could also cause microglial and NADPH complex activation to trigger cell death (Rodrigues-Pallares et al., 2007). Chemical sympathectomy will be used to distinguish between direct and indirect actions of agonists. Chemical sympathectomy with 6-OHDA is a widely used method of producing partial or complete destruction of peripheral sympathetic nerve terminals (Haeusler et al., 1969). In that study, 6-OHDA was given in 2 doses of 20 mg/kg on day 1, and 1 week later 2 doses of 50 mg/kg (Haeusler et al., 1969). In studies of pressor responses in the anaesthetised rat, 6-OHDA was given as 2 x 50 mg/kg on day 1 and 2 x 100 mg/kg on day 7 and the experiments performed on day 8. (Finch et al., 1973). Similar degrees of sympathectomy were obtained with a single dose of 100 mg/kg on day 1 and on day 4 (Cleary et al., 2004). In the present study, 6-OHDA was given in a dose of 40 mg/kg on day 1, and a subsequent dose of 40 mg/kg. This dosing schedule reduced central sedation and possible morbidity. In the studies of this thesis, responses obtained to stimulants in tissues from sympathectomised animals can be regarded as involving direct actions of the agent. If sympathectomy significantly reduces the response to a stimulant, this is evidence for indirect actions of that stimulant.

Studies of 6-OHDA also rely on negative controls. The tachycardia anddepressor responses to the direct agonist isoprenaline were used to assess whether 6-OHDA alters responses by any mechanism other than sympathectomy.

1.7. Caffeine and its interaction with stimulants

Another consideration is the interaction between stimulants of abuse and common legal minor stimulants, the most common of which is caffeine. Caffeine is different from most stimulants, in that it does not act at adrenoceptors. Caffeine mediates its stimulatory effects by adenosine receptor blockade to cause blood vessel contractions and to increase catecholamine concentrations in the blood (Robertson et al., 1978).

Although caffeine is generally safe in reasonable doses, it can be a potential hazard to amphetamine and cocaine users. Caffeine can be intentionally mixed with sports drinks or soft drinks or it might be present in street drugs: for example, it was found that MDMA might have caffeine mixed with it (Parrott, 2004). Furthermore, it has been shown in rats that mixing caffeine with amphetamines or cocaine could lead to death by exacerbating their acute toxicity (Derlet et al., 1992). Both acute and long-term complications have been associated with MDMA and caffeine in combination. A study showed that long term 5-HT loss occurs in various parts of rat brain with co-administration of MDMA and caffeine (McNamara et al., 2006). It has been known that low levels of 5-HT can result in psychological and behavioral issues (Apter et al., 1990). In the same study acute side effects of hyperthermia developed when mixing MDMA (10mg/kg) with caffeine (10mg/kg) compared to MDMA alone (McNamara et al., 2006). In addition, lethality was reported due to exaggerated hyperthermia with administration of 15mg/kg of MDMA with 10mg/kg of caffeine. Caffeine may cause a combination of adenosine A_{2A} receptor antagonism and phosphodiesterase inhibition to account for the exacerbation of MDMA-induced hyperthermia (Vanattou-Saifoudine et al., 2010). Tachycardia was also reported to 10mg/kg of both caffeine and MDMA when given *in vivo*, but no effect on the electrocardiogram of the isolated rat heart, suggesting a central effect rather than a peripheral effect of the drug (McNamara et al., 2007).

1.8. Gender differences in the direct and indirect cardiovascular actions of cathinone and MDMA in the rat

The stimulants cathinone (from khat leaves: see Kalix, 1990) and MDMA produce adrenoceptor mediated tachycardia and vasopressor actions that may be the result of direct receptor stimulation, indirect actions on NET, and/or displacement of NA from nerve synaptic vesicles (Trendelenburg, 1990; Rudnick & Clarke, 1993). In man, khat, cathinone and MDMA increase HR and blood pressure (Brenneisen et al., 1990; Widler et al., 1994; Toennes et al., 2003; Hysek et al., 2011), but it is not clear whether actions are predominantly direct or indirect, or whether there are gender differences.

Initial studies in this thesis employing the anaesthetised rat examined cathinone, MDMA and tyramine in terms of cardiac and blood pressure effects in male rats. However in further studies, gender differences in these responses were examined.

Until recently, the vast majority of studies of stimulants have been carried out employing only male animals, despite the obvious use of stimulants by both males and females in the human population. For instance, khat is chewed by both males and females in approximately equal numbers in Yemen (Nakajima et al., 2014). The object of the present study was to examine gender differences in the direct and indirect cardiovascular actions of the stimulants cathinone and MDMA, in comparison with the archetypal indirect sympathomimetic tyramine, employing sympathectomy to identify indirect actions.

There is definitely an increasing need to consider gender differences in studying drugs and their effects both in animals and human models. Many researchers choose male animals in their experimental studies with the assumption that female animals might show different responses due to hormonal fluctuations in the estrous cycle. It is still important to know the overall gender differences when studying diseases and drug effects. Starting from January 2016, the National Institute of Health requires all research projects to include both genders unless

the study involves gender specific conditions like reproductive health (N.I.H, 2015). Women were found to have a higher dependence rate on drug use, more difficulties when it comes to stopping the use of drugs and less likely to seek treatment compared to men (Ignjatova and Raleva, 2009; Lynch et al, 2002).

The khat chewing habit is a problem that is growing world wide among both sexes. It is important to consider the gender variations in khat users as it has changed dramatically in the past few years. It has been reported in Australia that less women formerly chewed khat as it was attached to a social stigma (Stevenson et al, 1996). However, changing role of women and the increase in socialization helped to redefine the impact of khat use among women. In the UK, Both genders are using khat almost equally as in a way to adapt to the social conditions (ACMD, 2005).

In Yemen, It was estimated that 82% of men have used khat at least once in their lifetime compared to 43% of women (NIDA, 2011). An observational cross-sectional study was done in the Jazan region of Saudi Arabia adjacent to Yeman to show the overall prevalence of khat chewing in that region. It was found that 42.2% of men have chewed khat at least once in their lifetime compared to 11.3 % of women. (Mahfouz et al, 2015).

Cathinones can also cause cardiovascular complications during pregnancy and can also be detected in the urine of infants during lactation among khat chewing mothers (Graziani et al, 2008; Kuczkowski, 2005). Mothers who chewed khat regularly during pregnancy were found to have an increase incidence of low-birth weight infants as well as a finding of a teratogenic effect due to khat use (ACMD,2005; Mwenda et al, 2003). There is still a significant gap in the physiological and psychological effects of cathinones among both genders in human and animal models.

A study has examined the psychological and physiological differences between males and females in MDMA use to show that females are more likely to have psychological symptoms such as changes in mood and sleep disturbance compared to males, while males are more likely to have cardiovascular

symptoms like changes in HR and blood pressure (Ogeil et al, 2013; Liechti et al, 2001). There is evidence of gender differences in relation to the central serotonergic system in which Carlsson and Carlsson (1988) found that the basal 5-HT level is higher in females than in males which could explain the susceptibility of females to psychological symptoms following ecstasy (MDMA) use. Savagean and Beatty (1981) reported that female rats showed more central locomotor activity and more stereotypical behavior than males after injections of 5mg/kg of amphetamine.

1.8.1. Hormonal changes

Females were also found to be more responsive to amphetamine during the high estrogen phase in their menstrual cycle (White et al., 2002). A study was done on ovariectomised female rats to show that female rats with estrogen implants had a much bigger response to 4mg/kg of MDMA than rats with no estrogen implants (Zhou et al., 2003).

It was also shown in cocaine studies that female mice put more effort to self-administer cocaine when estradiol levels are high during estrous cycle (Martini et al., 2014). In human studies, women have greater response to cocaine when estrogen levels are high, and their response was reduced when they were given progesterone (Evans and Foltin, 2006; Evans et al., 2002). This indicates that the hormonal status in women can largely influence the behavior and pattern of drug use. Moreover, there is a major gap in data about the cardiovascular effects of drug abuse in males and females. More experiments are needed to ultimately draw a strong conclusion to help improve treatment options.

1.9. Gender differences in behavioural and temperature effects of cathinones in the rat

Kalix (1980) was the first to report a hypermobility effects on rats after the administration of cathinone. A study found that rats given cathinone showed

stereotypical behaviour (repetitive seemingly purposeless behaviour) that was quantitatively less by half compared to rats that were given amphetamine (Zelger et al, 1980). Another study found that pretreatment with haloperidol, a dopamine receptor antagonist significantly reduced locomotor activity as well as biting and licking produced by cathinone in mice (Connor et al., 2002).

Stimulants such as MDMA (Palenicek et al. 2005; Walker et al. 2007; Vanattou-Saïfoudine et al. 2010; Rodsiri et al. 2011) and d-amphetamine (Garrett and Holtzmann 1994) stimulate locomotor activity. MDMA produced significantly greater locomotor actions in female rats, (Palenicek et al. 2005; Walker et al. 2007) an effect also reported for d-amphetamine (Savageau and Beatty, 1981). Hence, there is a clear gender difference in the potency of some stimulants in producing locomotor activity in the rat, and this thesis will examine gender differences in the effects of cathinone.

The effects of many stimulants, including MDMA, are largely caused by actions as an indirect monoaminergic agonist, acting predominantly at the monoamine transporters to release the monoamines NA, dopamine and 5-HT, with some additional direct receptor mediated actions (see Docherty 2008; Docherty and Green 2010). Indeed, many studies have tended to overlook, or underestimate, adrenergic actions of stimulants. The mechanism of action of stimulants in producing locomotor stimulation may involve release of dopamine (see Docherty and Green, 2010). The dopamine D_2-receptor antagonist sulpiride blocked increased locomotor activity both to d-amphetamine and to caffeine (Garrett and Holtzman 1994), and caffeine blocks presynaptic adenosine A_2 receptors on central dopaminergic nerves to increase dopamine release (Kim and Palmiter 2003). MDMA releases 5-HT to act at 5-HT_{1B}-receptors to increase activity (Rempel et al. 1993), and at 5-HT_2-receptors to release dopamine which also increases activity (Yamamoto et al., 1995). In addition, α_{2A}-adrenoceptors (Lahdesmaki et al. 2003) and 5-HT_{2C}-receptors (Bankson and Cunningham 2002) mediate inhibition of locomotion (Lähdesmäki et al. 2003), and α_{2A}-adrenoceptor or 5-HT_{2C}-receptor antagonism increases the locomotor response to MDMA (Bankson and Cunningham 2002; Bexis and Docherty 2006).

1.9.1. Physiological Regulation of Temperature

Like blood pressure, the body closely monitors and controls Body temperature. This is because body temperature must be maintained within a relatively small range of temperatures to maintain normal body functions. In particular, fairly small rises in body temperature can cause serious consequences in terms of causing protein denaturisation (Roti Roti, 2008) and pathophysiological alterations in nerve function (Sharma and Hoopes, 2003). Body temperature can be seen as simply a balance between input and output, a balance between heat production and loss. Hence, an increase in heat production or a decrease in heat loss, or a combination of the two, will result in a rise in core body temperature. Core body temperature is maintained relatively constant by the body as a balance between heat production and heat loss.

In cold environments, a combination of increased heat production and diminished heat loss can serve to maintain core body temperature. Heat production can be increased in a number of ways: by increased muscle activity such as in locomotion or exercise; by shivering to produce heat; by an increase in cellular metabolism involving adrenergic, sex and thyroid hormones (the first two are of particular interest in this study of gender and the temperature actions of stimulants that act as sympathomimetics). Heat loss can be decreased also in a number of ways: reduction in skin blood flow by vasoconstriction of skin arterioles; changes in posture to reduce effective surface area in terms of surface area to volume. Humans can don heavy clothing, animals can grow thick winter coats, both can seek shelter and both may be subject to evolutionary adaptations to climate.

Peripheral heat production is partly regulated by NA in brown and white fat in the skeletal muscles. It was shown that sympathectomy in rats only partially decreased the hyperthermic response to MDMA that might suggest another thermoregulation process is involved (Sprague et al., 2003). It was found that

thyroidectomy converted the hyperthermic response produced by MDMA to a hypothermia and that the replacement of thyroid hormone partially restored the temperature effect (Bianco et al., 1988). Hence, the hypothalamic-pituitary-thyroid axis and the sympathetic nervous system are involved in temperature control and can be influenced by MDMA and other stimulants.

In warm environments, a combination of decreased heat production and increased heat loss can serve to maintain core body temperature. Hence, heat production is decreased again in response at least partly to hormonal changes, in this case largely decreased production of hormones (for instance, in north Finland, summer levels of T3 increase due to decreased disposal: Leppaluoto et al., 1998). Heat loss is increased by increase in skin blood flow by vasodilatation of skin arterioles and sweating. Removal of clothing, and, in animals, loss of winter coat can contribute. These effects on temperature control are summarized in Figure 1.8.

Cooling down involves heat transfer away from the body to the environment and has four major components. Radiation is the transfer of infrared energy and this occurs from warm to cool objects. Conduction is the transfer of heat again from warm to cool objects, or to the air or water. Convection is the movement of currents of warm air rising from the body from hot to cold and this is what is increased by use of fans or by the wind. This helps conduction of hot air from the skin. Evaporation is the loss of water, effectively water warmed to body temperature, that is lost by the body to the air. This can be obligatory evaporation such as in the airways due to breathing out moist air saturated with water vapour at body temperature (this can be increased by panting) and obligatory loss through the skin. Evaporation can also be active by sweating. These effects are summarized in Figure 1.8.

Figure 1.8. Aspects of the control of body temperature.

However, if the ambient temperature rises above body temperature, heat loss is largely reliant on evaporation by sweating, or by convection due to fans or the wind. The problem with ambient temperature above body temperature with additional high humidity is that sweat loss by evaporation becomes a problem. The "rave" environment may be seen as a condition of high ambient temperature and high humidity.

The involvement of the autonomic nervous system in the control of temperature is largely in terms of control of cutaneous blood flow, effects on heat production and in terms of control of sweat glands. Amphetamine-like agents might be expected to act particularly on cutaneous blood flow and on heat production, with both central and peripheral actions. The diagram of Figure 1.9 shows possible sites of cathinone and other stimulants in altering temperature control: central actions, thermogeneration or cutaneous vaosconstriction.

Since the present studies examine changes in temperature in rats, we also have to consider some special features in which the rat differs from humans in temperature control. The rat tail is a major organ determining heat loss: an increase in tail blood flow and cutaneous dilatation results in increased heat loss. Rat also differs in the extent of panting to increase heat loss and in the importance of brown fat to cause a much more marked increase in heat production in rat than man. Furthermore, in rat piloerection reduces heat loss by trapping air for insulation. Differences in body mass and in body fat distribution between males and females must also be considered. In these studies of age-matched rats, males were always heavier (around 250g) than female (around 200g) rats. Even surface area to volume has to be considered.

1.9.2. Effects of MDMA and cathinone to produce hyperthermia

Hyperthermia is an acute life-threatening emergency with MDMA especially

Figure 1.9.   Possible sites of action of cathinone in influencing body temperature.

when used in rave situations, a high ambient temperature environment with loud music and excessive dancing. In animal studies, it has also been shown that MDMA disrupts thermoregulation, but surprisingly can cause either hyper- or hypothermia depending on a number of factors including ambient temperature (Malberg & Seiden, 1998). The sympathetic nervous system plays an important role in heat distribution and production (Sprague et al., 2003). The mechanisms by which MDMA acts to interfere with thermoregulation still remain to be established, but both central and peripheral monoaminergic mechanisms may be involved, and serotonergic, noradrenergic and dopaminergic neurotransmitter systems have all been implicated in many studies, in both hyperthermia and hypothermia (White et al., 1996). MDMA as an agonist at receptors for these neurotransmitters may act to disrupt temperature control. MDMA is known to increase 5-HT levels and can act as an agonist at 5-HT$_2$-receptors (Fantegrossi et al., 2003). MDMA increases dopamine levels, so that dopamine may also be a factor, as it has been shown that dopamine is involved in the hyperthermia to methamphetamine (Bronstein and Hong, 1995).

In terms of cathinone and temperature, previous studies reported that cathinone (4 mg/kg) did not affect rectal temperature and cathinone (10 mg/kg) produced hyperthermia in rats (Shorthall et al., 2013). However, these studies differed from the present study in terms of methodology: measurement of temperature by rectal thermometer versus telemetry probe, and these differences have to be considered. More studies have examined MDMA. MDMA increased rectal temperature in dark Agouti rats (Mechan et al., 2002) but decreased rectal temperature in Lister-hooded rats (Shorthall et al., 2013), and in telemetry studies, MDMA produced hypothermia in Wistar (Bexis and Docherty, 2006), and Lister-hooded rats (Rodsiri et al., 2011), although repeated dosing produced subsequent hyperthermia (Rodsiri et al., 2011).

Other studies have examined the interaction between caffeine and stimulants in temperature studies. In Sprague-Dawley rats, caffeine did not affect temperature, MDMA produced a small increase in temperature in rectal temperature studies, but a decrease in temperature in telemetry studies, but the combination of caffeine and MDMA produced a hyperthermia in both studies

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(Vanattou-Saïfoudine et al., 2010a, 2010b). Clearly, effects of MDMA, and presumably other stimulants, on temperature in the rat may depend partly on genetics, on experimental protocol, ambient temperature, and differences between rectal and telemetry probe measurement. Stress may be an important factor in determining temperature effects of stimulants, and rectal temperature measurement may therefore be more stressful and not directly comparable with the more refined telemetry studies.

In this thesis, the actions of cathinone are investigated in telemetry studies of temperature and locomotor activity in conscious male and female rats and the interactions with caffeine were examined. As will be reported in results, both gender differences in response to cathinone and gender differences in the interaction with caffeine were found.

1.10. Aims of this Thesis

The overarching aims of this thesis were to study the cardiovascular and temperature actions of cathinone. Specific aims were as follows.

1. Study the direct and indirect actions of cathinone on the cardiovascular system in comparison with MDMA and tyramine *in vivo*, employing sympathectomy to establish direct or indirect actions, and antagonist drugs to confirm the receptors involved.

2. Study possible gender differences in the actions of cathinone on the cardiovascular system in comparison with MDMA and tyramine.

3. Investigation of the cardiovascular actions of a range of stimulants of human interest, including cathine, MHA and ephedrine.

4. Investigate actions of cathinone and other stimulants *in vitro* in rat vas deferens and aorta.

5. Investigate the interaction of caffeine with cathinone on the cardiovascular system.

6. Investigate the interaction of caffeine with cathinone on temperature and central locomotor actions.

Chapter 2.

Methods

Chapter 2. Methods

2.1. General

2.1.1. Animals

Male Wistar rats (250-300g) or age-matched young adult male (230-300g) and female (190-230g) Wistar rats were obtained from Harlan (UK). All studies have been approved by the Health Products Regulatory Agency (HPRA) in Ireland and by the RCSI Research Ethics Committee. The animals were housed in a controlled environment with a 12-hours light, 12-hours dark cycle and were fed a standard rat diet.

Female rats were not investigated for oestrous cycle. The reasons for this were partly on cost grounds as number of animals being housed at any one time was kept to a minimum, so that selecting rats on a given stage of the oestrous cycle would have delayed experiments. Secondly, there was no particular reason to chose any particular stage of the oestrous cycle, and chosing two or more groups of female rats would have greatly prolonged the work and cost of this thesis. Anyway, there was no evidence from the results I obtained that female animals were more variable than male animals in standard deviation or in possible outliers.

Although female rats were age matched with male rats, it should be noted that they were significantly lighter in weight that male rats, but this could not be avoided as male rats grow so much faster than female rats.

2.1.2. Pretreatments

6-OHDA was weighed out freshly and dissolved in ascorbic acid (1 mg/kg) immediately prior to injection. Dissolving in weak acid immediately prior to injection prevented oxidation of the easily oxidized compound. Animals were injected with 6-OHDA (40 mg/kg, i.p.) in ascorbic acid or with vehicle (ascorbic acid 1 mg/kg, i.p.) once per day on two different days, and employed in studies

the day following the last injection. Animals were injected on day 1 but the subsequent second injection was given either on day 2, 3 or 4, to produce a chemical sympathectomy, and animals were investigated on the following day (day 3, 4 or 5). There was no evidence that the day of the second injection affected the degree of sympathectomy, and this dosing schedule was very convenient to fit with the working week.

In initial studies, chemically sympathectomised rats were compared with control untreated rats. These are the studies of the effects of cathinone, MDMA and tyramine and the interaction with cocaine and propranolol in male rats outlined in Chapter 3. In all other studies, chemically sympathectomised rats were compared with ascorbic acid vehicle treated animals. Comparison of results obtained for male rats in Chapter 3 (control versus sympathectomised) with results obtained for male rats in Chapter 4 (vehicle-treated versus sympathectomised) show that responses from control and vehicle-treated are alike with no significant difference.

2.2. Experiments on anaesthetized rat

2.2.1. Study of direct and indirect cardiovascular actions of stimulants in pentobarbitone anaesthetised rats

Rats were anaesthetised with pentobarbitone sodium (60 mg/kg, i.p., and maintenance doses, in volumes of 0.1 ml of 9 mg/kg, as required, i.v.). A midline incision was made in the neck and the carotid artery and jugular vein were exposed by blunt dissection, and cannulated (cannula 3 FG, 1 mm OD) for recording of blood pressure, and for injection of drugs, respectively. The carotid artery cannula contained heparinised saline (heparin sodium 50 I.U./ml, diluted from solution of 5,000 I.U./ml; Wockhardt, Wrexham, U.K.), and the jugular cannula contained normal saline (NaCl 0.9g/100ml). The heparin prevented clotting of the blood and narrowing of the cannula lumen to allow recording the arterial pulse with SBP and DBP. In most studies, only DBP was used in calculations, although in the results of Chapter 3, SBP was also calculated, but

since responses were qualitatively similar and since DBP may more closely inform of direct vasoconstrictor responses causing changes in peripheral resistance, the results will focus on DBP. This is discussed in the Chapter 11 (Discussion). Blood pressure was measured using a Sensonor 840 blood pressure transducer (Sensonor, Horten, Norway) and HR was extracted from blood pressure using a Narco biotachometer coupler (Narco Biosystems, Houston, Texas). Animals were placed on a Harvard Heated Small Animal Table for maintenance of body temperature.   At the end of the experiment, animal was killed by overdose of anaesthetic (i.v.) and exsanguination.

Experiments were as far as possible randomized, limited by the sometimes limited stocks of certain restricted stimulants and lack of availability at certain times of other stimulants and even by the lack at times of the appropriate gender of rat.   Blinding was not deemed feasible due to limit availability of staff and sometimes complex number of drug dilutions.

2.2.1.1. Studies of interactions of cocaine and propranolol with the stimulants cathinone, MDMA and tyramine in anaesthetized male rats

Once the blood pressure and HR recording had stabilized (usually within 15 min), saline vehicle, cocaine (1 mg/kg) or propranolol (1 or 10 mg/kg) were injected intravenously in a dose of 1 ml/kg and flushed in with a volume of 0.5ml/kg saline, in control and chemically sympathectomised male rats.   Dose-response curves were constructed for cathinone, MDMA and tyramine (all 0.001-1 mg/kg) given cumulatively in 1 log unit increments at 2 min intervals (time to peak may differ between cardiac and vascular actions), or until a maximum was reached, intravenously beginning 5 min after injection of saline vehicle or test drug. Peak changes in DBP and HR were measured.

2.2.1.2. Studies of the actions of stimulants in anaesthetized male and female rats

Once the blood pressure and HR recording had stabilized (usually within 15 min), saline vehicle was injected intravenously in a dose of 1 ml/kg and flushed in with a volume of 0.5 ml/kg saline, in vehicle-treated and chemically sympathectomised male and female rats.   Dose-response curves were constructed for all stimulants examined in this study in a dose range of 0.001-1 mg/kg, except for ephedrine (0.001-10 mg/kg) and isoprenaline (0.0001-0.1 mg/kg), given cumulatively in 1 log unit increments at 2 min intervals intravenously beginning 5 min after injection of saline vehicle. Peak changes in DBP and HR were measured.

2.2.1.3. Studies of the interaction of caffeine with cathinone and MDMA in anaesthetized male and female rats

Once the blood pressure and HR recording had stabilized (usually within 15 min), saline vehicle was injected intravenously in a dose of 1 ml/kg and flushed in with a volume of 0.5ml/kg saline, in vehicle-treated male and female rats.   Beginning 5 min after injection of saline, Caffeine (1 mg/kg, 3 mg/kg and 10 mg/kg) or saline vehicle (1 ml/kg) were injected i.v. at 2 min intervals and flushed in with a volume of 0.5 ml/kg saline. Beginning 5 min after injection of caffeine (10 mg/kg), or 5 min after the last dose of vehicle, dose-response curves were constructed for cathinone and MDMA (all 0.001-1 mg/kg) given cumulatively in 1 log unit increments at 2 min intervals, or until a maximum was reached. Peak changes in DBP and HR were measured taking the baseline as just before injection of the first dose of caffeine, or of the equivalent vehicle.   Hence, the response following the lowest dose of cathinone or MDMA (0.001 mg/kg) was taken as the combined response to caffeine or vehicle and that dose of stimulant.

2.3. Isolated tissue preparation experiments

Male (230-300g) and female (190-230g) Wistar rats employed in this study were obtained from Harlan (UK). The studies have been approved by the Department of Health/Health Products Regulatory Agency (HPRA) in Ireland and by the RCSI

Research Ethics Committee. The animals were housed in a controlled environment with a 12-hours light, 12-hours dark cycle and were fed a standard rat diet. Rats were killed by an overdose of 100% CO_2 gas or by overdose of pentobarbitone and exsanguination. The use of CO_2 was mainly in early experiments, and pentobarbitone mainly in later experiments, to match with animals obtained from other sources, as listed below. In initial experiments, untreated animals served as controls for sympathectomy, but in most experiments vehicle-treated animals served as control. There was no difference in response between the two groups. To reduce the number of animals employed in the work overall, a number of animals from the anaesthetised cardiovascular studies or telemetry studies were employed also in vitro. These were as follows: animals not suitable for anaesthetized experiments (died under anaesthesia, significant bleeding on cannulation); animals employed in saline vehicle experiments; animals given short acting agonists (isoprenaline); animals in which agonists had virtually no effect, especially after sympathectomy; animals in telemetry studies killed 4 hours after injection of caffeine or cathinone; a number of culled animals. Responses in these animals did not differ from those in animals killed only for in vitro studies. In all studies, the descending aorta and two vasa deferentia were removed and 4 aortic rings and two vasa employed. Tissues were employed fresh or left overnight in the fridge in Krebs-Henseleit solution until the following morning. This did not affect the response of the vas deferens, but it was found that the aorta needed longer to equilibrate before commencing dose response curves (approximately 1 hour longer). With this small difference in protocol, responses did not differ between tissues used fresh or left overnight.

Experiments were as far as possible randomized, limited by the sometimes limited stocks of certain restricted stimulants, the need to carry out multiple experiments simultaneously, and lack of availability at certain times of other stimulants and even by the lack at times of the appropriate gender of rat. Blinding was not deemed feasible given the number of organ baths employed (6),

the limited availability of staff and the large number of drug stocks and dilutions that had to be prepared each day.

2.3.1. Preparation of the rat vas deferens

Following anaesthesia with pentobarbitone and exsanguination or CO_2 and exsanguination (see above), a midline incision was made in the abdomen and the testis and epididymis exposed. Blunt forceps were placed to separate the vas deferens from the connective tissue. The whole vas deferens was tied with a long thread at the epididymal end and a short thread at the prostatic end, removed from rats and carefully cleared of connective tissue and blood vessels. By convention, the vas was always placed in organ baths with the long thread and the epididymal end attached to the transducer, and the short thread and prostatic end attached to a fixed rod or electrode.

2.3.2. Isometric contractions of rat vas deferens produced by NA and the interaction with cocaine

The whole vas deferens was attached to a fixed rod and attached to a myograph transducer (Grass FT03) under 1 g tension in organ baths at 37°C in Krebs-Henseleit solution of the following composition: (mM): NaCl 119; $NaHCO_3$ 25; D-glucose 11.1; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.0.

Bathing fluid was changed every 15 min, except during dose response curves. Following 30-45 min equilibration, tissues were contracted with NA (10 µM), and washed. Vehicle or cocaine (3 µM) was then added. Bathing fluid was again changed every 15 min for the next hour. Tissues were then contracted with cumulative concentrations of NA in 0.5 log unit increments, beginning with 10 nM until a maximum was reached.

2.3.3. Isometric contractions of the rat vas deferens produced by stimulants

Bathing fluid was changed every 15 min, except during dose response curves. In some experiments, sympathectomy was assessed by electrical stimulation of the nerves (see below) prior to addition of drugs. Following 30 min equilibration, tissues were contracted with NA (10 µM), and washed. Bathing fluid was then changed every 15 min for the next hour. Tissues were then contracted with cumulative concentrations of other test stimulants mostly beginning with 100 nM, in 0.5 log unit increments, until a maximum was reached, or the maximum concentration was administered. The maximum concentration was 1000 μM for most agonists, but 100 μM for cathinone, cathine, MDMA, MDA, MDEA and MHA, due to limited availability.

Contractions are expressed as the cumulative maximum obtained to increasing concentrations of agonist. Contractions to agonists consisted of sustained tonic contractions with phasic spikes superimposed, and these spikes were generally more prevalent following cocaine or sympathectomy.   Contractile responses were measured in terms of total and tonic responses. Total responses were the maximum contraction reached to that concentration, whether very transient or maintained. Tonic response is the more maintained response, measured as the maximum height of a period of maintained contraction. Concentration-response curves were plotted as absolute tension (g). It was found that in sympathectomised rats, spontaneous spikes could be so large that effects of agonists on spikes were difficult to assess, or that the spontaneous spikes were so large that it was misleading to quantify agonist actions. Hence, tonic contractions proved more reliable in comparing vehicle and sympathectomised rats. These tonic contractions were quantified for all agonists.

2.3.4. Electrical stimulation-evoked contractions

The whole vas deferens was attached through a hook electrode and attached by a long thread to a myograph transducer (Grass FT03) under 1 g tension. Tissues were equilibrated between platinum electrodes in organ baths at 37°C in Krebs-

Henseleit solution of the same composition as listed above. Vas deferens was stimulated with a single stimulus or 10 pulses at 1 Hz (0.5 ms pulses, supramaximal voltage) using a Grass S88 stimulator to produce isometric contractions.

The 1 Hz stimulation-evoked nerve responses were employed as a means of assessing degree of sympathectomy, but, as the results will show, they were not very helpful, and nerve-stimulation experiments were discontinued. The single pulse stimulation-evoked nerve responses were also used in experiments to assess prejunctional inhibition of neurotransmission by stimulants (see below).

2.3.4.1. Inhibition by stimulants of electrical stimulation-evoked contractions

Rat vas deferens was attached through a hook electrode through a thread and attached to myograph transducers (Grass FT03) under 1 g tension. Tissues were equilibrated between platinum electrodes in organ baths at 37°C in Krebs-Henseleit solution of the same composition as listed above. Vas deferens was stimulated with a single stimulus every 5 min (0.5 ms pulses, supramaximal voltage) using a Grass S88 stimulator to produce isometric contractions. The calcium entry blocker nifedipine (10 μM) was administered to eliminate the first purinergic component to the contraction, leaving only the second, α_1-adrenoceptor mediated, calcium-store dependent, component. Nifedipine largely prevents exogenous α_{1A}-adrenoceptor mediated postjunctional contractions, allowing investigation of the prejunctional α_2-adrenoceptor mediated inhibition of the α_{1D}-adrenoceptor mediated nerve-evoked contraction that does not involve calcium entry.

When isometric contractile responses to a single stimulus given every 5 min were of a relatively constant level, vehicle or test stimulant was added beginning with 1 nM in 0.5 log unit increments. The test agent was added immediately following a single stimulus, and 5 min later the effect on the next stimulus was assessed.

The next concentration of agent was then added and a response obtained at 5 min, and so on until the maximum concentration. Stimulants produced a concentration dependent inhibition of stimulation evoked contractions, but vehicle had only minor effects.

2.3.5. Preparation of the rat aorta

Following anaesthesia with pentobarbitone and exsanguination or CO_2 overdose and exsanguination (see above), the rib cage was opened, the heart and lungs removed, and the vena cava lifted and removed. The descending aorta was then carefully lifted with forceps holding the surrounding connective tissue, and the connections with the spinal column cut. A section of aorta of about 20-25 mm was obtained. This was transferred to a petry dish in Krebs-Henseleit solution, and aortic rings of 3-5 mm in length were made with a scalpel. The rings were carefully lifted using fine forceps touching only the connective tissue, and a threaded hook was then placed through the lumen for bath suspension of aortic rings. The aortic ring was mounted on a fine fixed rod, and the thread on the hook attached to a myograph transducer under 1 g tension in organ baths at 37°C in Krebs-Henseleit solution of the following composition: (mM): NaCl 119; $NaHCO_3$ 25; D-glucose 11.1; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.0. Additionally, in some experiments, as mentioned in the results, cocaine (3 µM) and/or propranolol (1 µM) were present, to block NA re-uptake and β-adrenoceptors, respectively, when specified. Aortic rings were always prepared as soon as possible after overdose and exsanguination: if animals were left dead for a significant time before dissection, aortic response tended to be poor. Rings were employed fresh, or left in the fridge overnight and used the following morning (see above).

2.3.6. Contraction of rat aorta produced by NA and the interaction with cocaine

Bathing fluid was changed every 15 min, except during dose response curves. Following 30-45 min equilibration (90 min for tissue left overnight in fridge), tissues were contracted with NA (10 µM), and washed. Bathing fluid was then changed every 15 min for the next hour. Vehicle or cocaine (3 µM) was then added Bathing fluid was again changed every 15 min for the next hour. Tissues were then contracted with cumulative concentrations of NA in 0.5 log unit increments, beginning with 1 nM until a maximum was reached.

2.3.7. Contraction of rat aorta produced by stimulants

Bathing fluid was changed every 15 min, except during dose response curves. Following 30-45 min equilibration (90 min for tissue left overnight in fridge), Tissues were contracted with NA (10 µM), and washed. Bathing fluid was then changed every 15 min for the next hour. Tissues were then contracted with cumulative concentrations of stimulants in 0.5 log unit increments, beginning with 1 nM until a maximum was reached. For some stimulants, the maximum concentration added was 1000 µM, but for stimulants in limited supply (cathinone, cathine, MDMA, MDEA, MDA, MHA) the maximum concentration was 100 µM.   In many experiments, following the last concentration of stimulant, NA (10 µM) or KCl (120 mM) was added to test tissue maximum.

2.4. Gender differences in the effects of cathinone and the interaction with caffeine on temperature and locomotor activity in the rat in telemetry studies

2.4.1 Animal preparation

Male (230-300g) and female (190-230g) Wistar rats were obtained from Harlan (UK). Animals were housed in pairs in home cages (plastic, environmentally controlled) and bedding (wood shavings). The animals were housed in a controlled environment with a 12-hour light, 12-hour dark cycle and were fed a standard rat diet. The principles of laboratory animal care were followed, and all studies have been approved by the Health Products Regulatory Agency (HPRA), and by the RCSI Research Ethics Committee.

Animals were implanted with telemetry probes (TA11TA-F1; Data Sciences International, St Paul, MN, U.S.A.) under isoflurane anaesthesia. The skin was cleaned with alcohol, a small skin incision was made and a small incision through the abdominal wall. Since the telemetry probe is about 13 mm wide, the abdominal incision was about 15 mm long, and the skin incision slightly longer at about 25 mm. The implant was placed in the abdominal cavity, and the abdominal wall and skin incision were closed with silk suturing (Mersilk 3-0, Ethicon). Motion and temperature sensors built into the device measure locomotor activity and core body temperature. Animals were given Vetergesic (buprenorphine hydrochloride 0.05 mg/kg, subcutaneously) and returned to the home cage.

On the experimental day, approximately 7 days later (range 6-9), animals were transferred from the home cage to an experimental cage together with bedding, food and water from the home cage and transferred to the telemetry experimental room. A PhysioTel-Receiver (model RPC- 1) (dimensions 330 x 220mm) was placed under each individual animal experimental cage, enabling recording of the locomotor and temperature parameters. Data signals were acquired from 25 min prior to and for 270 min after drug administration, and analysed using the Dataquest A.R.T., Version 4.3. All recordings were obtained at room temperature (22 ^{o}C). The initial 20 min of recording was used to confirm the baseline, but time zero was set after 20 min of recording, and drug injection was at 5 and 35 min after time zero (see Figure 2.1).

Figure 2.1. Timelines for telemetry experiments. Recording started at time -20 min, sampling started at time zero. Drugs were injected subcutaneously. First drug injection was at 5 min (vehicle or caffeine (10 mg/kg)). Second drug injection was at 35 min (vehicle or cathinone (5 mg/kg)). Hence, there were 4 groups for both male and female. Sampling ended at 275 min.

2.4.2. Experimental protocol

Four treatment groups were employed in studies of males and females: vehicle/vehicle; vehicle/cathinone (5 mg/kg); caffeine (10mg/kg)/vehicle; caffeine (10mg/kg)/cathinone (5mg/kg). Groups of n=7 were employed. Animals were randomized for treatment using a random number generator. The experimenter who also carried out initial analysis was blind to the treatment. Each experimental day, a male and a female animal were investigated together as a pair employing the same treatment regime.

Animals were injected slowly subcutaneously (into the thigh area) with vehicle (distilled water, 1 ml/kg) or test drugs. Vehicle or caffeine (10 mg/kg) was injected at 5 min, and vehicle or cathinone (5 mg/kg) was injected at 35 min, all in a volume of 1 ml/kg s.c., and recording continued for another 240 min (see Figure 2.1). A barrier was placed between the cages so that the animals could not see each other. At the end of the recording period, animals were killed by the injection of an overdose of pentobarbitone (100 mg/kg, i.p.) and exsanguination. The telemetry probe was then removed and cleaned in alcohol.

2.4.3. Data analysis and statistics in telemetry studies

Data was initially compiled using Microsoft Excel, and temperature data was converted to change in temperature ($\square$elta temperature: ΔT) from the zero min baseline. Locomotor and $\square$elta temperature data were then transferred to GraphPad Prism for Macintosh computers for statistical analysis and graphical presentation.

Temperature (ΔT) was plotted at 5 min intervals, and locomotor activity was calculated as the total activity over both 5 min and 30 min time periods. Hence, for activity, the 5 min recording is the sum of activity from 0-5 min. Since very occasionally a telemetry signal was lost usually for a few secs during an experiment (perhaps because of the position of the rat), a small number of missing points were replaced by extrapolation from the points either side of the

missed point(s). This was necessary to carry out two-way analysis of variance (anova) where there cannot be missed points.

Results are expressed as mean±s.e.m from 7 experiments. The minimum level for statistical significance was P<0.05. Total responses were compared by one-way anova, followed by Bonferroni (comparing all groups) or Dunnett (comparison with vehicle) test. Time course responses were compared by repeated measures two-way anova, followed by post-test only when anova showed significance of P<0.05. Post-tests were carried out only when differences were significant (P<0.05). Three-way anova was used to compare interactions between pretreatment (vehicle or caffeine), treatment (vehicle or cathinone) and gender. All statistical tests were carried out using GraphPad Prism for MacIntosh.

2.5. Statistics: general

Values are expressed as mean ± standard error of mean (s.e.m.) from n experiments in brackets. The minimum level for statistical significance was P<0.05.

Power analysis was carried out using G*Power 3.1. For isolated tissue work (vas deferens or aorta), we might expect to detect a 3 fold shift in potency (0.5 log unit shift). If SD is 0.22 (from previous rat aorta work), then effect size is 2.27. Since multiple comparisons will be made, usually comparing up to 3 groups with control), a Bonferroni correction is made to the t-statistic, dividing the α error by m (no. of hypotheses, in this case 3), giving corrected α error of 0.017. Using these data, with n=6 there is a 91% power, and with n=7 there is a 95% power, of detecting a 0.5 log unit shift in potency from control, with 4 groups.

In telemetry studies, we might expect to detect a 50% or at least a 100% rise in basal locomotor activity. Using power analysis with G*Power 3.1, if basal activity is 2000 units/30 min, then a rise of 1000 with SD of 500, gives an effect size of 2,

and a power of 90% to detect a 50% rise and a power of 99.9% to detect a 100% rise, in basal activity with 4 groups.

In anaesthetized rats studies, we might expect to be able to detect a 3 fold shift in potency (0.5 log unit shift). Using power analysis with G*Power 3.1, if SD is 0.25, gives an effect size of 2, and a power of 90% to detect a 3 fold shift in potency, with 4 groups of n=7.

Based on this, groups of n=7 were normally chosen for *in vivo* studies. For the main comparisons, e.g. between cathinone and tyramine groups of at least n=7 were generally employed to allow detection of smaller differences in responses. For the supporting studies, e.g. with MDMA, that was predicted to have similar actions to cathinone, groups of at least 5 were employed. As far as possible groups of at least 7 were used in *in vivo* studies, and groups of 6 in the in vitro studies. However, some early studies in thesis were carried out with group less than 6.

In anaesthetised rat studies, effects of test antagonist drugs (cocaine or propranolol) against agonist responses were compared with those of vehicle, responses in sympathectomised rats were compared with those in vehicle-treated or control (untreated) rats and responses in male rats were compared with those in female rats. HR was measured in bpm and DBP in mmHg. DBP was measured in all studies as it more closely reflects vascular actions than SBP (see Alsufyani & Docherty, 2015; see also Discussion). Where dose response curves produced large responses, mean pED_{50} values (log dose, $\square$g/kg, producing 50% of maximum) were calculated by non-linear regression employing GraphPad Prism for MacIntosh. Differences between groups and vehicle were compared using the GraphPad Prism programme by anova and, only when anova showed significance of $P<0.05$, with Bonferroni or Dunnett test for comparison of effects of vehicle with test drug or Bonferroni or Tukey test for comparison of all groups.

In isolated tissue experiments, Agonist pD_2 values ($-logEC_{50}$)(concentration producing 50% of maximum contraction) values were calculated for agonists that produced clear maximum contractions at submaximal concentrations. Since drugs were administered in log unit increments, potency was also calculated in log units. Agonist potency was calculated in each individual experiment using non-linear regression analysis (GraphPad Prism), but to preserve the original responses, the concentration response curves shown in the Figures do not employ curve fitting. For these agonists maximum contractions were also calculated.   Agonist pD_2 values and maximum contractions were compared between vehicle-treated and sympathectomised animals and between males and females, as appropriate. For agonists that did not produce a clear maximum response at submaximal doses, the effects of appropriate concentrations and the maximum contractions were compared between vehicle-treated and sympathectomised animals and between males and females, as appropriate.

In studies of rat vas deferens as a model of sympathectomy, nerve stimulation-evoked responses in sympathectomised rats were compared with the effects of vehicle, by anova and, only when anova showed significance of $P<0.05$, Bonferroni or Dunnett's post-test.   In rat vas deferens electrical stimulation studies, agonist potencies were expressed as the concentration producing 50% reduction of the control contraction to single pulse stimulus, pIC_{50} ($-logIC_{50}$) values, and compared by anova and, only when anova showed significance of $P<0.05$, with Bonferroni (comparing all groups) or Dunnett (comparison with vehicle) Multiple Comparisons Tests.

Graphical and statistical analysis was carried out using GraphPad Prism and Instat for MacIntosh (GraphPad Software, San Diego, CA, USA). To preserve the original responses, the concentration response curves shown in the Figures do not employ curve fitting.

2.6. Drugs

6-hydroxydopamine hydrochloride (Sigma, Ireland);

Caffeine (Alexis Biochemicals, UK);

Cathine hydrochloride (Sigma, Ireland);

Cathinone hydrochloride (gift; NIDA, Bethesda, MD, USA);

Cocaine hydrochloride (Sigma, Ireland);

(±)-Ephedrine hydrochloride (Sigma, Ireland);

(-)-Ephedrine hydrochloride (Sigma, Ireland);

6-hydroxydopamine hydrochloride (Sigma, Ireland);

isoprenaline hydrochloride (Sigma, Ireland);

Methylenedioxyamphetamine (MDA: Sigma, Ireland);

Methylenedioxyethylamphetamine (MDEA: Sigma, Ireland);

Methylenedioxymethamphetamine (MDMA: gift; NIDA);

Methylhexanamine hydrochloride (MHA: Sigma, Ireland);

Noradrenaline hydrochloride (Sigma, Ireland);

Norephedrine hydrochloride (Sigma, Ireland);

Propranolol hydrochloride (Sigma, Ireland);

Tyramine hydrochloride (Sigma, Ireland);

All drugs were dissolved in distilled water and dilutions made up in saline, except for 6-hydroxydopamine, which was dissolved in ascorbic acid (1 mg/ml) immediately prior to injection.

Chapter 3.

Direct and indirect cardiovascular actions of cathinone and MDMA in the anaesthetized male rat

Chapter 3. Direct and indirect cardiovascular actions of cathinone and MDMA in the anaesthetized male rat

The object of this study was to examine the direct and indirect cardiovascular actions of cathinone and MDMA, in comparison with the archetypal indirect sympathomimetic tyramine, employing sympathectomy to establish the profile of action of cathinone and MDMA in vivo.

3.1. Anaesthetized rat: resting blood pressure and HR

In anaesthetized rats, resting DBP was 106±2 mmHg (n=40) in control rats and 103±2 mmHg (n=8) in sympathectomised rats, with resting HR of 323±7 bpm and 314±9 bpm, respectively (no significant differences). Although baseline DBP did not differ between control and sympathectomised rats, blood pressure was subjectively more stable in sympathectomised rats. Baseline DBP (measured 5 min after test drug injection) was not significantly affected by test antagonist drugs as compared to vehicle, but cocaine (1 mg/kg) significantly increased resting HR by 23±3 bpm (n=11) and propranolol (1 and 10 mg/kg) significantly decreased resting HR by 83±12 bpm (n=12) and 79±9 bpm (n=4), respectively.

A typical recording of blood pressure and HR, and the effects of the stimulant cathinone, in an anaesthetized female Wistar rat, is shown in Figure 3.1. The effects of tyramine in an anaesthetized male Wistar rat are shown in Figure 3.2.

3.2. Effects of stimulants on HR

In anaesthetised control rats, cathinone, MDMA and tyramine (all 0.001-1 mg/kg) produced marked tachycardia (Fig. 3.3-3.5), and this tachycardia was abolished by propranolol (1 mg/kg), except in the case of tyramine (Fig. 3.5). However, propranolol (10 mg/kg) produced a further reduction in the tachycardia to tyramine that was significantly reduced as compared to the response in the presence of propranolol (1 mg/kg) (Fig. 3.5).

Fig. 3.1. Heart Rate (HR) (top) and Blood pressure (BP) (bottom) recording from an anaesthetised female vehicle-treated rat: effects of cathinone. HR calibration (bpm) and BP calibration (mmHg) are shown together with time scale. At the arrows, cathinone in cumulative doses of 0.001, 0.01, 0.1 and 1 mg/kg was injected intravenously, producing a dose-dependent tachycardia but only small effects (pressor and depressor) on blood pressure.

Fig. 3.2. Heart Rate (HR) (top) and Blood pressure (BP) (bottom) recording from an anaesthetised male vehicle-treated rat: effects of tyramine. HR calibration (bpm) and BP calibration (mmHg) are shown together with time scale. At the arrows, tyramine doses of 0.001, 0.01, 0.1 & 1 mg/kg were injected intravenously, producing a dose-dependent tachycardia and dose dependent rises in blood pressure.

Figure 3.3. Effects of intravenous injection of cathinone (0.001-1 mg/kg) on heart rate (HR) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine) or propranolol (1 mg/kg) (prop). Error bars indicate s.e. of mean from at least 4 experiments. Asterisks denote significant differences from response in control rats in the absence of test drugs (anova and Dunnett's test; * P<0.05).

Figure 3.4. Effects of intravenous injection of MDMA (0.001-1 mg/kg) on heart rate (HR) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine) or propranolol (1 mg/kg) (prop). Error bars indicate s.e. of mean from at least 4 experiments, except for propranolol. Asterisks denote significant differences from response in control rats in the absence of test drugs (anova and Dunnett's test; * $P<0.05$; ** $P<0.01$).

Figure 3.5. Effects of intravenous injection of tyramine (0.001-1 mg/kg) on heart rate (HR) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine), propranolol (1 mg/kg) (prop) or propranolol (10 mg/kg) (prop 10). Error bars indicate s.e. of mean from at least 4 experiments. Asterisks denote significant differences from response in control rats in the absence of test drugs (anova and Dunnett's test; * P<0.05; ** P<0.01).

Pretreatment with cocaine (1 mg/kg) did not significantly affect the tachycardia to cathinone or MDMA (Fig. 3.3-3.4).   In the case of tyramine, the tachycardia to high doses of tyramine was significantly reduced by cocaine (1 mg/kg) from 104±3 bpm (n=4) to 58±12 bpm (n=4), hence a large tachycardia remained (Fig. 3.5). This reduction in the tachycardia to tyramine may have been at least partly due to the elevated baseline HR following cocaine: marked tachycardia of 100 bpm plus seen to tyramine in the absence of cocaine, leading to near maximal HR, could not be obtained with a raised baseline HR: cocaine (1mg/kg) raised HR by 28±3 bpm (n=4) prior to tyramine. Higher doses of cocaine (not shown) at 5 mg/kg produced a greater tachycardia of 51±6 bpm (n=3). This problem would not occur for MDMA or cathinone that produced smaller tachycardias in the absence of cocaine. Hence, cocaine in this dose does not clearly discriminate between direct and indirect actions, and results obtained are open to interpretation. Cocaine was largely ineffective at blocking tachycardia to the stimulants examined, particularly cathinone and MDMA. This will be discussed further in the Discussion.

Agonist pED_{50} values were calculated for tachycardia with values of 13.8, 162 and 66.1 μg/kg, for cathinone, MDMA and tyramine respectively (see Table 3.1). Tyramine was approximately 3 times more potent at producing tachycardia than increases in DBP (Table 3.1). Cathinone was approximately 12 times more potent than MDMA in producing tachycardia with a significant tachycardia to cathinone (0.01 mg/kg) but only to MDMA (0.1 mg/kg) (Table 3.1). Tyramine was approximately 5 times less potent than cathinone but this was partly due to a significantly larger tachycardia at the highest dose of 1 mg/kg.

In anaesthetised sympathectomised rats, cathinone and MDMA produced only a very small tachycardia, markedly and significantly reduced from responses in control animals (Fig. 3.3-3.5). The tachycardia to cathinone (1 mg/kg) was significantly reduced from 59±8 bpm (n=4) to 16±8 bpm (n=6) by sympathectomy (P<0.05) (Fig. 3.3). The tachycardia to MDMA (1 mg/kg) was significantly reduced from 59±10 bpm (n=7) to 11±5 bpm (n=5) by sympathectomy (P<0.05)

Table 3.1. Relative potencies of tyramine, cathinone, and MDMA at producing tachycardia in anaesthetized male rats, and for tyramine only, potency at producing rises in DBP. Values are pED$_{50}$ (-log μg/kg) ± s.e. of mean, with n, the number of experiments, in brackets. Statistical analysis was carried out using log data. For simplicity, values of ED$_{50}$ (μg/kg), converted from pED$_{50}$, together with the ratio of agonist potency to that of cathinone (x/cath) and ratio of DBP/HR potency (for tyramine) are also shown. NC: not calculated.

agent	HR pED$_{50}$	x/cath	DBP pED$_{50}$	DBP/HR
cathinone	1.14±0.11 (4)	1	NC	
	13.8 μg/kg			
MDMA	2.21±0.08 (7)**	11.7	NC	
	162 μg/kg			
Tyramine	1.82±0.0.08 (4)**	4.8	2.31±0.17	3.1
	66.1 μg/kg		*204 μg/kg*	

Asterisks denote potency of agonist significantly different from potency of cathinone (anova and Bonferroni test: ** $P<0.01$).

(Fig. 3.4). However, the tachycardia to tyramine, although significantly reduced from 104±3 bpm (n=4) to 64±6 bpm (n=5) by sympathectomy, was still marked (Fig. 3.5).

3.3. Effects of stimulants on blood pressure

Blood pressure effects were examined both in terms of DBP (Fig. 3.6-3.8) and SBP (Fig. 3.9-3.11), but since responses were qualitatively similar and since DBP may give a better indication of vascular responses, the results will focus on DBP. Comparison of DBP and SBP for each individual agonist show similar patterns, although changes in SBP tended to be slightly larger.

In anaesthetised control rats, tyramine produced marked pressor responses of 57±4 mmHg (n=4), MDMA (1 mg/kg) produced a small pressor response of 16±2 mmHg (n=7), but cathinone produced no overall pressor response (Fig. 3.6-3.8). Cathinone sometimes produced biphasic responses: pressor followed by depressor, so that the cumulative effect on blood pressure was non-significant (Fig. 3.6). Pretreatment with cocaine (1 mg/kg) did not significantly affect the pressor response to tyramine (Fig. 3.8). Propranolol (10 mg/kg) significantly reduced rises in SBP, but not DBP, produced by tyramine (Figs. 3.8 & 3.11). Hence, propranolol has actions to reduce the effects of tyramine on SBP but not on DBP. In anaesthetised sympathectomised rats, the pressor response to tyramine was virtually abolished, and the small pressor response to MDMA (1 mg/kg) was also abolished (Fig. 3.7 & 3.8).

Fig. 3.6. Effects of intravenous injection of cathinone (0.001-1 mg/kg) on diastolic blood pressure (DBP) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine) or propranolol (1 mg/kg). Error bars indicate s.e. of mean from at least 4 experiments. There were no significant differences.

Fig. 3.7. Effects of intravenous injection of MDMA (0.001-1 mg/kg) on diastolic blood pressure (DBP) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine) or propranolol (1 mg/kg) (prop). Error bars indicate s.e. of mean from at least 3 experiments. Asterisks denote significant differences from response in control rats in the absence of test drugs (anova and Dunnett's test; * P<0.05).

Fig. 3.8. Effects of intravenous injection of tyramine (0.001-1 mg/kg) on diastolic blood pressure (DBP) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine), propranolol (1 mg/kg) (prop) or propranolol (10 mg/kg) (prop 10). Error bars indicate s.e. of mean from at least 4 experiments. Asterisks denote significant differences from response in control rats in the absence of test drugs (anova and Dunnett's test; * P<0.05; ** P<0.01).

Fig. 3.9. Effects of intravenous injection of cathinone (0.001-1 mg/kg) on systolic blood pressure (SBP) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine), or propranolol (1 mg/kg) (prop). Error bars indicate s.e. of mean from at least 4 experiments. There were no significant differences.

Fig. 3.10. Effects of intravenous injection of MDMA (0.001-1 mg/kg) on systolic blood pressure (SBP) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine) or propranolol (1 mg/kg) (prop). Error bars indicate s.e. of mean from at least 3 experiments. Asterisks denote significant differences from response in control rats in the absence of test drugs (anova and Dunnett's test; * P<0.05).

Fig. 3.11. Effects of intravenous injection of tyramine (0.001-1 mg/kg) on systolic blood pressure (SBP) in pentobarbitone anaesthetized control (con) or chemically sympathectomised (sympX) rats, in the presence of prior vehicle (veh), cocaine (1 mg/kg) (cocaine), propranolol (1 mg/kg) (prop) or propranolol (10 mg/kg) (prop 10). Error bars indicate s.e. of mean from at least 4 experiments. Asterisks denote significant differences from response in control rats in the absence of test drugs (anova and Dunnett's test; * P<0.05; ** P<0.01).

3.4. Summary

1. Male Wistar rats were anaesthetized with pentobarbitone for blood pressure and HR recording. Some rats were sympathectomised by treatment with 6-hydroxydopamine.

2. In the anaesthetised rat, cathinone, MDMA and tyramine (all 0.001-1 mg/kg) produced marked tachycardia, tyramine produced marked pressor responses and MDMA produced small but significant pressor responses.

3. The tachycardia to cathinone and MDMA was almost abolished by propranolol (1 mg/kg). Pretreatment with cocaine (1 mg/kg) did not significantly affect the tachycardia to cathinone or MDMA, but reduced the response to tyramine.

4. However, in sympathectomised rats, the tachycardia to cathinone or MDMA was almost abolished, but the tachycardia to tyramine was only partially reduced. Blood pressure effects of tyramine and MDMA were also markedly attenuated by sympathectomy.

5. The results demonstrate firstly, based on the current results showing lack of effect of cocaine, that cocaine may not be the most suitable agent for assessing direct versus indirect actions in cardiovascular studies. Secondly, the use of chemical sympathectomy achieved the desired goal of demonstrating that cardiac β-adrenoceptor mediated actions of cathinone and MDMA are largely indirect.

Chapter 4.

Investigation of gender differences in the cardiovascular actions of direct and indirect sympathomimetic stimulants including cathinone in the anaesthetized rat

Chapter 4. Investigation of gender differences in the cardiovascular actions of direct and indirect sympathomimetic stimulants including cathinone in the anaesthetized rat

In Chapter 3 it was shown that, in control anaesthetised male rats, cathinone, MDMA and tyramine all produce marked tachycardia but only tyramine produced marked pressor responses. However, in sympathectomised rats, the tachycardia to cathinone or MDMA was markedly attenuated, but the tachycardia to tyramine was only partially reduced. This suggested differences in mode of action between tyramine and cathinone.

Until recently, the vast majority of studies of stimulants have been carried out employing only male animals, despite the obvious use of stimulants by both males and females in the human population. The object of this study was to examine gender differences in the direct and indirect cardiovascular actions of the stimulant cathinone in comparison with the archetypal indirect sympathomimetic tyramine, employing sympathectomy to identify indirect actions. MDMA was also studied as an agent with similar actions to cathinone. One difference in methodology from Chapter 3 is that vehicle-treated animals (injected with ascorbic acid vehicle for 6-OHDA)) rather than untreated (i.e. no injection given) control animals were employed in the present chapter.

4.1. Anaesthetized rat: basal DBP and HR

In anaesthetized rats, resting diastolic blood pressure (DBP) was 118±3 mmHg (n=35) and 117±3 mmHg (n=30) in vehicle treated male and female rats, respectively, and 105±3 mmHg (n=21) and 99±4 mmHg (n=21) in sympathectomised male and female rats, respectively. DBP was significantly reduced by sympathectomy (P<0.05 for both male and female), but there were no significant differences between male and female animals. DBP was both significantly reduced and subjectively more stable in sympathectomised rats of either gender.

Resting HR was 345±4 bpm (n=35) and 332±6 bpm (n=30), respectively in vehicle treated male and female rats, and 318±9 bpm (n=21) and 319±11 bpm (n=21), respectively in sympathectomised male and female rats. HR was significantly reduced by sympathectomy only in male rats (P<0.05), but there were no significant differences between male and female animals in resting HR in either vehicle treated or sympathectomised rats.

4.2. Vehicle treated rats: tachycardia

In anaesthetised vehicle treated rats, cumulative injections of saline vehicle produced cumulative increases in HR of 1±1, 1±1, 3±1 & 4±3 bpm (n=7) in male rats, and 2±1, 1±1, 0±2 & 0±2 bpm (n=7) in female rats. In anaesthetised vehicle treated male and female rats cathinone, MDMA and tyramine produced significant tachycardia (all at 0.1-1 mg/kg) (Fig. 4.1-4.3), but there were no significant differences between male and female for cathinone and MDMA (Fig. 4.1 & 4.2 and Table 4.1). Tyramine produced significantly greater tachycardia in doses from 0.1 mg/kg to 1 mg/kg in male than in female rats and this was reflected in significantly greater potency of tyramine in male animals (Fig. 4.3 and Table 4.1).
Potency of agonists at producing tachycardia was calculated for all agonists in vehicle experiments for both male and female rats. However, since clear maximum responses were obtained in few experiments, differences in potency between male and female could reflect differences in maximum responses. With this proviso, potency is discussed below.

Cathinone was significantly more potent than MDMA in producing tachycardia in both male and female rats, approximately 7 (male) or 5 (female) times more potent, with a significant tachycardia to cathinone (0.01 mg/kg) but only to MDMA (0.1 mg/kg) (Fig. 2 and Table 4.1). Cathinone was 1.7 times more potent than tyramine in producing tachycardia in male rats (non-significant), but 3.7 times more potent than tyramine in female rats (significant: P<0.05) (Table 4.1). Differences in relative potency of tyramine were presumably due to the lower potency of tyramine at producing tachycardia in females.
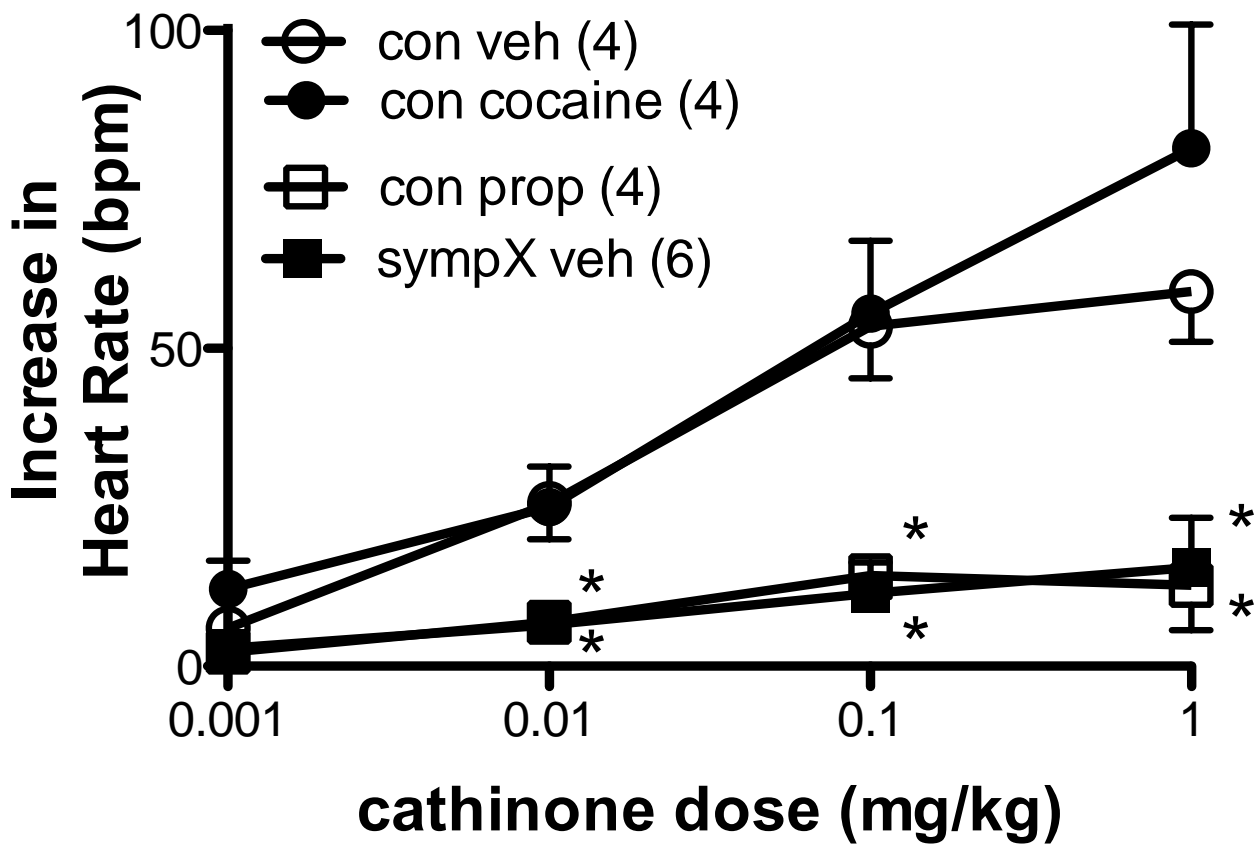
Fig. 4.1. Effects of intravenous injection of cathinone (cath) (0.001-1 mg/kg) on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. Error bars indicate s.e. of mean from 7-8 experiments. Tachycardia to cathinone was significantly reduced by sympathectomy in both male and female rats (anova and Bonferroni test; * P<0.05).

Fig. 4.2. Effects of intravenous injection of MDMA (0.001-1 mg/kg) on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. Error bars indicate s.e. of mean from 5 experiments. Tachycardia to MDMA was significantly reduced by sympathectomy in both male and female rats (anova and Bonferroni test; * $P<0.05$).

Fig. 4.3. Effects of intravenous injection of tyramine (TYR) (0.001-1 mg/kg) on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. Error bars indicate s.e. of mean from 7-8 experiments. Heart rate responses to tyramine were significantly reduced by sympathectomy in both male and female rats. Tachycardia to tyramine was significantly greater in male than female vehicle treated rats (anova and Bonferroni test; * P<0.05).

Table 4.1. Relative potencies of tyramine, cathinone, and MDMA at producing tachycardia in anaesthetized male and female rats. Values are pED_{50} (-log μg/kg) ± s.e. of mean, with n, the number of experiments, in brackets. Statistical analysis was carried out using log data. For simplicity, values of ED_{50} (μg/kg), converted from pED_{50}, together with the ratio of agonist potency to that of cathinone (x/cath) are also shown for both genders. The ratio of potency of agonist in male to potency in female (M/F) are also shown.

agent	Male	x/cath	Female	x/cath	M/F
cathinone	1.36±0.11 (7)		1.51±0.13 (8)		
	22.9 μg/kg	*1.0*	*32.4 μg/kg*	*1.0*	*0.71*
MDMA	2.20±0.06 (5)**		2.19±0.13 (5)**		
	158 μg/kg	*6.9*	*155 μg/kg*	*4.7*	*1.02*
Tyramine	1.59±0.0.18 (8)		2.08±0.14 (8)**+		
	□□□□□μg/kg	*1.7*	*120 μg/kg*	*3.7*	
	0.32				

Asterisks denote potency of agonist significantly different from potency of cathinone in same gender (anova and Bonferroni test: ** $P<0.01$). Crosses indicate potency of agonist in female significantly different from potency in male (anova and Bonferroni test: + $P<0.05$).

4.3. Vehicle treated rats: pressor responses

In anaesthetised vehicle treated rats, cumulative injections of saline vehicle produced cumulative increases in DBP of 4.0±1.2, 2.9±1.4, 4±1.3 & 1.9±1.0 mmHg (n=7) in male rats, and 1.2±0.6, 0.9±0.6, 1.0±0.6 & 0.5 ±0.5 mmHg (n=7) in female rats. In anaesthetised vehicle treated rats, cathinone (1mg/kg) produced biphasic blood pressure responses: small pressor followed by small depressor, so that both are plotted in Fig.4.4. MDMA (1 mg/kg) produced a small pressor response but cathinone produced no significant pressor response (Fig. 4.5). Tyramine produced marked pressor responses that for the highest dose of 1mg/kg reversed to small depressor responses within 5 min (as responses were cumulative at 2 min intervals, a 5 min response was only obtained for the highest dose of tyramine) (Fig 4.6).

Potency of agonists at producing changes in DBP could only be calculated for tyramine in both male and female rats, and only in vehicle-treated animals. Responses to MDMA, although significant at a dose of 1 mg/kg, were too small to allow meaningful potency calculations.   Tyramine produced rises in DBP in vehicle treated animals, and a pED_{50} value (-log μg/kg) of 2.35±0.17 (n=8), or 224 μg/kg, was calculated for male rats.

4.4. Sympathectomised rats

In anaesthetised sympathectomised rats, the tachycardia to cathinone **(**Fig. 4.1), MDMA (Fig. 4.2), and tyramine (Fig. 4.3) was significantly reduced in both male and female from responses in vehicle animals.   The maximum tachycardia to cathinone (1 mg/kg) was significantly reduced to 18±6 bpm and 18±5 bpm (n=7 each) in male and female rats, respectively by sympathectomy (P<0.05). The maximum tachycardia to MDMA (1 mg/kg) was significantly reduced to 11±4 bpm and 16±5 bpm (n=5 each), in male and female, respectively, by sympathectomy (P<0.05). The maximum tachycardia to cathinone and MDMA following sympathectomy was still significantly greater than the response to vehicle injection in both male and

female rats (P<0.05). The tachycardia to tyramine (1 mg/kg), although significantly reduced to 38±6 bpm (n=7) and 30±5 bpm (n=8) in male and female rats, respectively, were still significant but not significantly different between males and females (Fig. 4.3). Hence, there was no gender difference in the tachycardia to tyramine following sympathectomy.

In anaesthetised sympathectomised rats, the large pressor response to tyramine, the late depressor response to tyramine, and the small pressor response to MDMA (1 mg/kg) were virtually abolished (Fig. 4.4-4.6). However, the maximum pressor response to tyramine, but not to cathinone or MDMA, in sympathectomised animals was still significantly greater than the response to vehicle injection in both male and female (P<0.01).

4.5. Comparison between results obtained for male rat in Chapters 3 & 4.

Comparison of Figures 3.2-3.4 with Figures 4.1-4.6 shows that the actions of cathinone, MDMA and tyramine   were similar between control untreated animals and vehicle-treated animals. Hence, male untreated animals and vehicle-treated animals did not differ significantly in their responsiveness in terms of HR or blood pressure. Hence, it would be reasonable to use untreated animals as controls for sympathectomised animals in cardiovascular studies, at least for male rats.
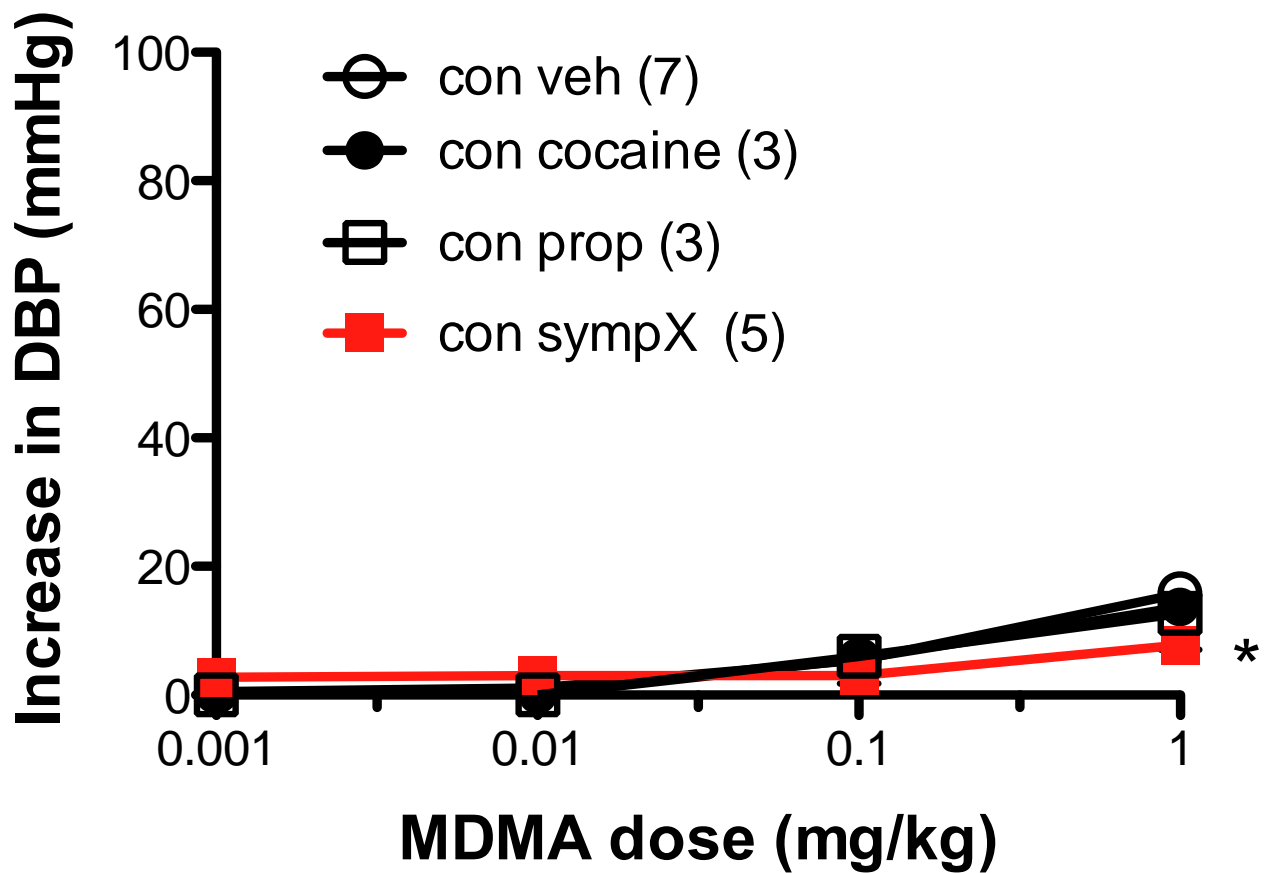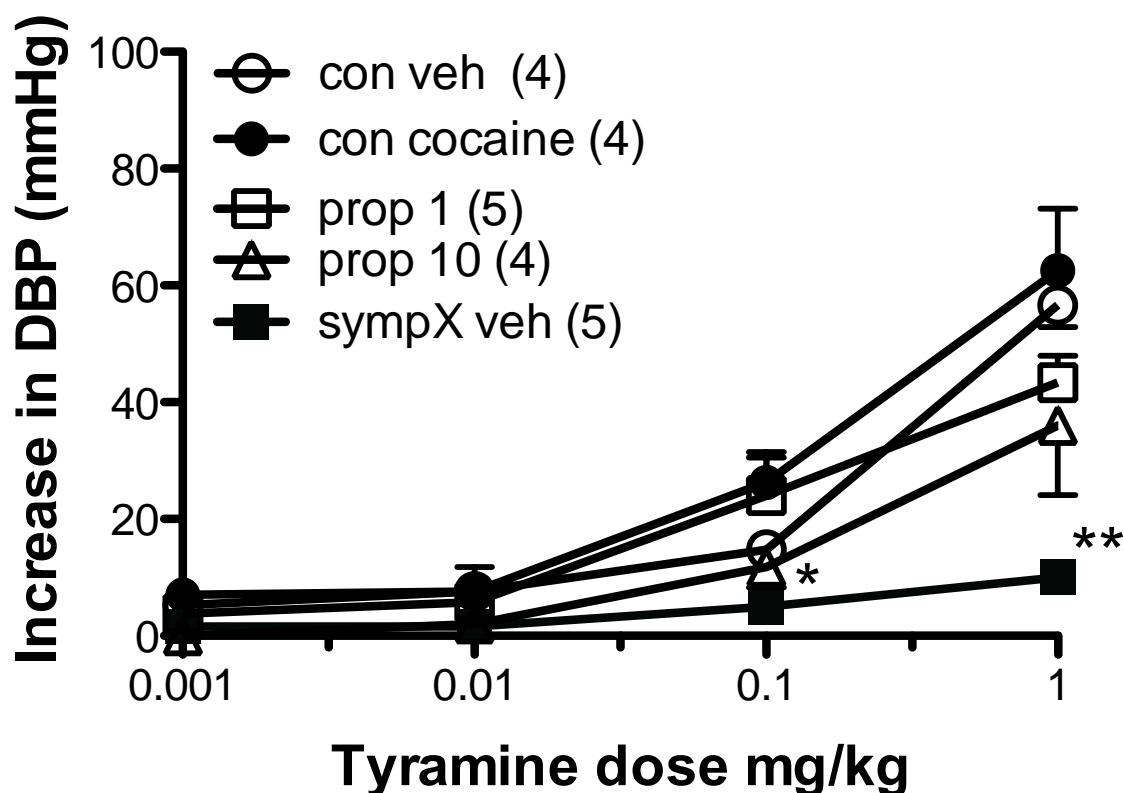
Fig. 4.4. Effects of intravenous injection of cathinone (cath) (0.001-1 mg/kg) on diastolic blood pressure (DBP) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. The late depressor response to cathinone (1 mg/kg) is shown by dashed lines. Error bars indicate s.e. of mean from 7-8 experiments. There were no significant differences.
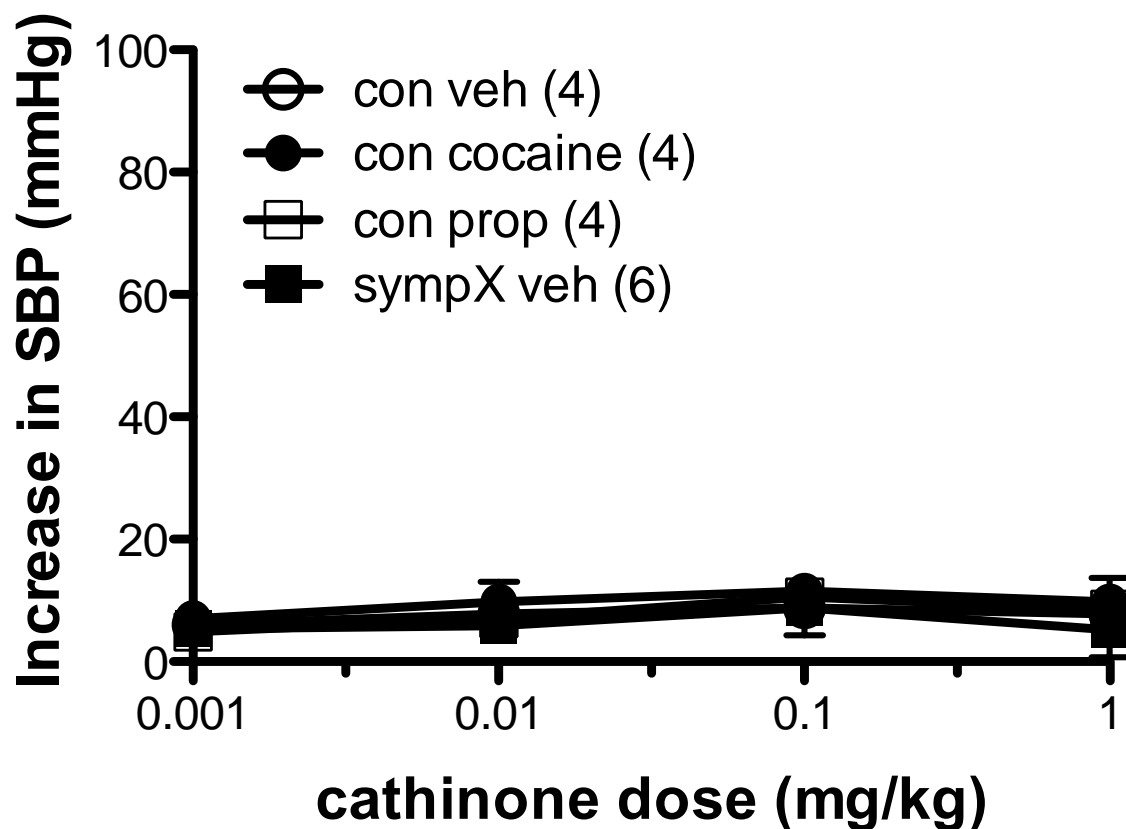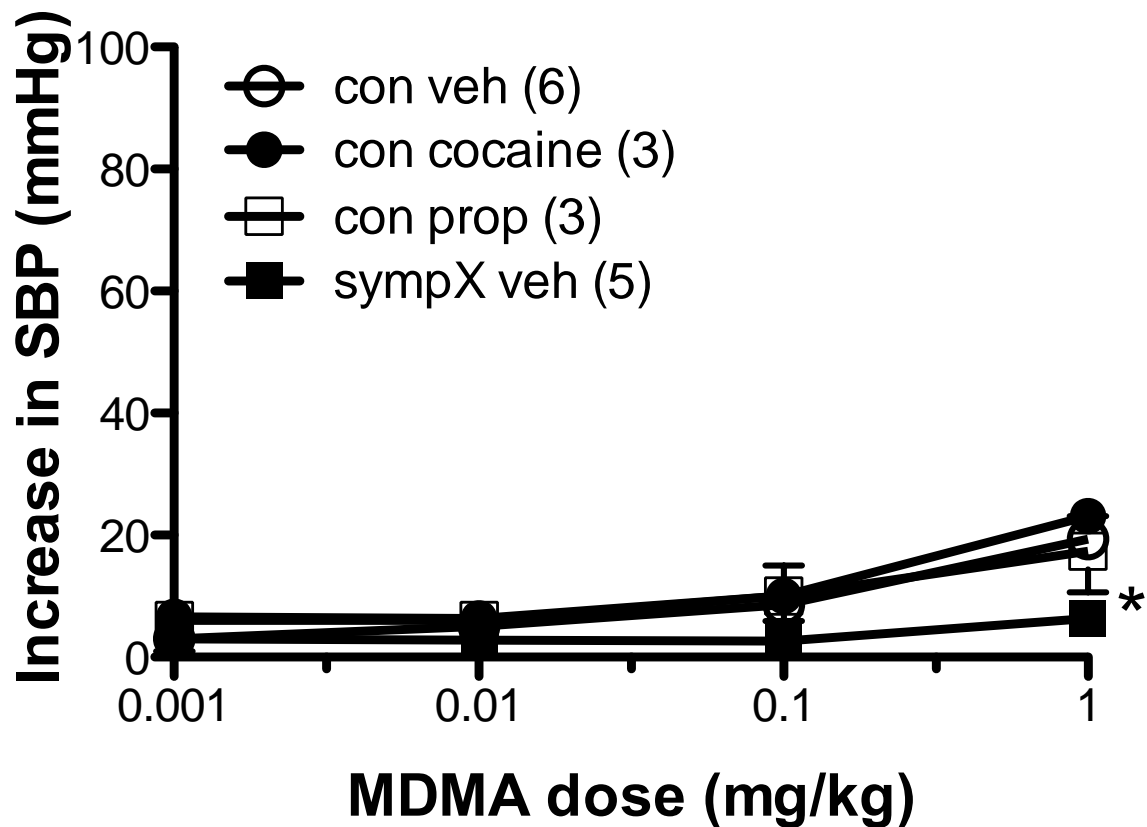
Fig. 4.5. Effects of intravenous injection of MDMA (0.001-1 mg/kg) on diastolic blood pressure (DBP) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. Error bars indicate s.e. of mean from 5 experiments. DBP responses to MDMA were significantly reduced by sympathectomy in both male and female rats (anova and Bonferroni test; * P<0.05).

Fig. 4.6. Effects of intravenous injection of tyramine (TYR) (0.001-1 mg/kg) on diastolic blood pressure (DBP) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. The late depressor response to tyramine (1mg/kg) is shown by dashed lines. Error bars indicate s.e. of mean from 7-8 experiments. DBP (both pressor responses and depressor responses) to tyramine were significantly reduced by sympathectomy in both male and female rats (anova and Bonferroni test; * P<0.05).

4.6. Summary

1. Gender differences in the direct and indirect sympathomimetic cardiovascular effects of the stimulant cathinone (and for comparison MDMA) and the archetypal indirect sympathomimetic agent tyramine, have been studied employing male and female Wistar rats.

2. Animals were sympathectomised by treatment with 6-OHDA or treated with vehicle.

3. In male and female vehicle treated pentobarbitone anaesthetised rats, all three agonists (0.001-1 mg/kg) produced significant tachycardia, tyramine produced large pressor, and in high doses small depressor responses, MDMA produced small but significant pressor responses, and cathinone produced no significant pressor effects.

4. In sympathectomised rats, pressor responses, even those to tyramine, were virtually abolished, and depressor responses to tyramine were abolished.

5. In vehicle treated rats, the tachycardia to tyramine, but not the tachycardia to cathinone or MDMA, was significantly greater in male than female rats. This may suggest that the mechanism of the tachycardia to tyramine differs from those of the stimulants cathinone and MDMA. Following sympathectomy, there were no differences between male and female rats in the tachycardia to any agent.

6. Hence, there were gender differences in the response for tyramine, in that the tachycardia was significantly greater in male animals, but no gender differences in the cardiovascular responses to the widely used recreational stimulants cathinone and MDMA. Cardiac stimulant actions of cathinone and MDMA were similar in male and female rats.

Chapter 5.

Effects of ephedrine on HR and blood pressure in vehicle-treated and sympathectomised male and females rats.

Chapter 5. Effects of ephedrine on HR and blood pressure in vehicle-treated and sympathectomised male and females rats.

In Chapter 4 it was shown that, in vehicle-treated anaesthetised male and female rats, cathinone, MDMA and tyramine all produced marked tachycardia but only tyramine produced marked pressor responses. The tachycardia to tyramine but not to cathinone or MDMA was significantly greater in male than in female rats. However, in sympathectomised rats, the tachycardia to cathinone or MDMA was markedly attenuated, but the tachycardia to tyramine was only partially reduced, but there were no gender differences. This suggested differences in mode of action between tyramine and cathinone.

This chapter looks at another widely used sympathomimetic, ephedrine, that is reported variously to have both direct and indirect actions to cause tachycardia and pressor responses. The mode of action of ephedrine was examined, including the action of both (±)- and (-)-ephedrine, as this is still not fully clarified, and possible gender differences were investigated.

Results

5.1. Anaesthetized rat: basal DBP and HR

In anaesthetized rats, resting DBP was 116±4 mmHg (n=13) and 113±5 mmHg (n=9) in vehicle treated male and female rats, respectively, and 101±3 mmHg (n=9) and 92±6 mmHg (n=7) in sympathectomised male and female rats, respectively. DBP was significantly reduced by sympathectomy in female rats ($P<0.05$), but there were no significant differences between male and female animals. However, number of animals studied was much less than in Chapter 4. DBP was subjectively more stable in sympathectomised rats of either gender.
Resting HR was 349±9 bpm (n=13) and 338±15 bpm (n=9), respectively in vehicle treated male and female rats, and 326±10 bpm (n=9) and 318±6 bpm (n=7), respectively in sympathectomised male and female rats. HR was not significantly reduced by sympathectomy and there were no significant differences between male

and female animals in resting HR in either vehicle treated or sympathectomised rats. However, number of animals studied was much less than in Chapter 4.

5.2. Effects of ephedrine on HR

Both (-) and (±)-ephedrine produced dose dependent increases in HR in anaesthetised vehicle-treated male and female rats (Figures 5.1 & 5.2). There was no significant difference between effects of (±)-and (-)-ephedrine in male rats, and although responses tended to be smaller to (±)-ephedrine in female rats, this did not reach significance.

Sympathectomy significantly reduced the tachycardia to (±)-ephedrine (1 and 10 mg/kg) in male and to 1 mg/kg in female animals (Figures 5.1 & 5.2). This clearly demonstrates that a significant component of the tachycardia to (±)-ephedrine is indirectly mediated. However, sympathectomy did not significantly reduce the tachycardia to (-)-ephedrine in male or female animals (Figure 5.1), although there was admittedly a low group size in female animals (Figure 5.2). Hence, sympathectomy had less effect on responses to (-)-ephedrine than (±)-ephedrine, or at least did not reach significance for (-)-ephedrine.

Figures 5.3. and 5.4 compare data from male and female animals. The tachycardia to (±)-ephedrine (10 mg/kg) was significantly greater in male than female animals, suggesting that the maximum response to (±)-ephedrine differed between male and female (Figure 5.3). This was not found for (-)-ephedrine where responses were similar in male and female animals (Fig. 5.4).

The direct β-adrenoceptor agonist isoprenaline produced a dose dependent tachycardia that was not affected by sympathectomy, and effects were not different between male and females (Figure 5.5). Hence, sympathectomy did not affect responses to a direct agonist: there was no evidence of hypersensitivity in terms of HR.

Fig. 5.1. Effects of intravenous injection of (±)-ephedrine (eph) or (-)-ephedrine ((-)-eph) (0.001-10 mg/kg), on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) male rats. Error bars indicate s.e. of mean from 6-8 experiments. Tachycardia to (±)-ephedrine was significantly reduced by sympathectomy at 1 mg/kg and 10 mg/kg.   Asterisks denote responses in sympathectomised rats significantly difference from responses in vehicle-treated animals (* P<0.05, anova and Bonferroni test).

Fig. 5.2. Effects of intravenous injection of (±)-ephedrine (eph) or (-)-ephedrine ((-)-eph) (0.001-10 mg/kg), on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) female rats. Error bars indicate s.e. of mean from 4-8 experiments. Tachycardia to (±)-ephedrine was significantly reduced by sympathectomy at 1 mg/kg. Asterisks denote responses in sympathectomised rats significantly difference from responses in vehicle-treated animals (* $p < 0.05$, anova and Bonferroni test).
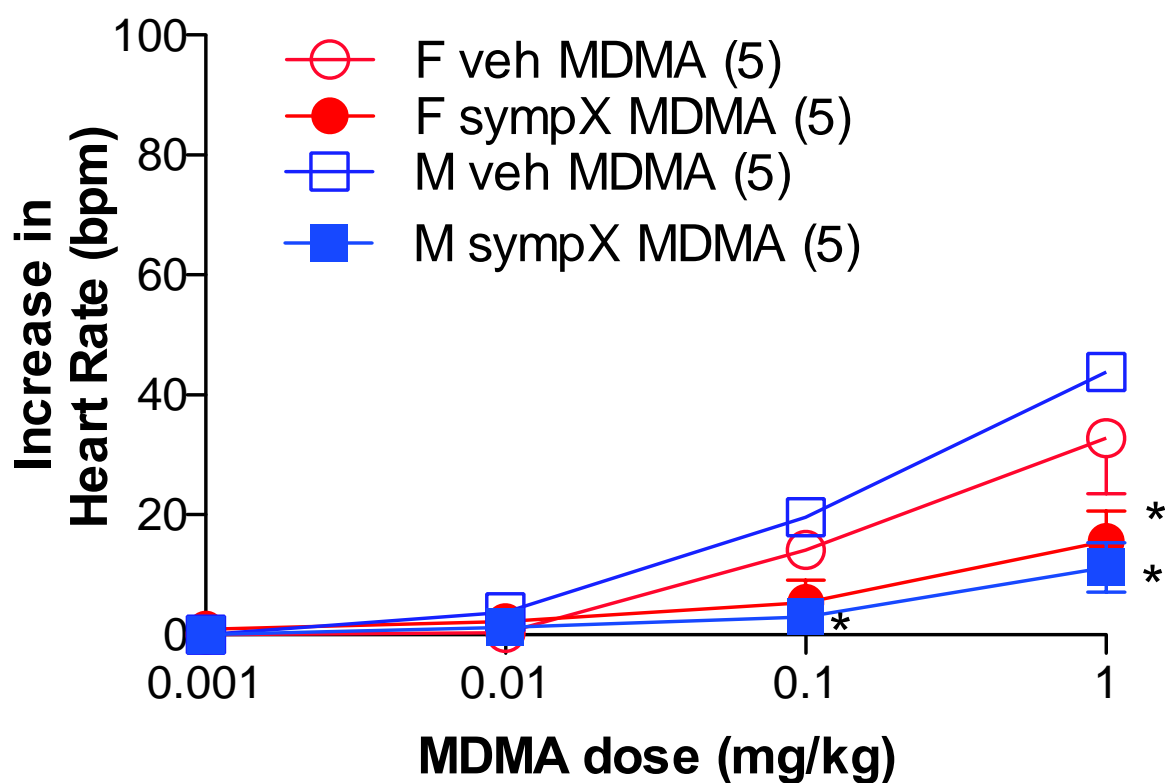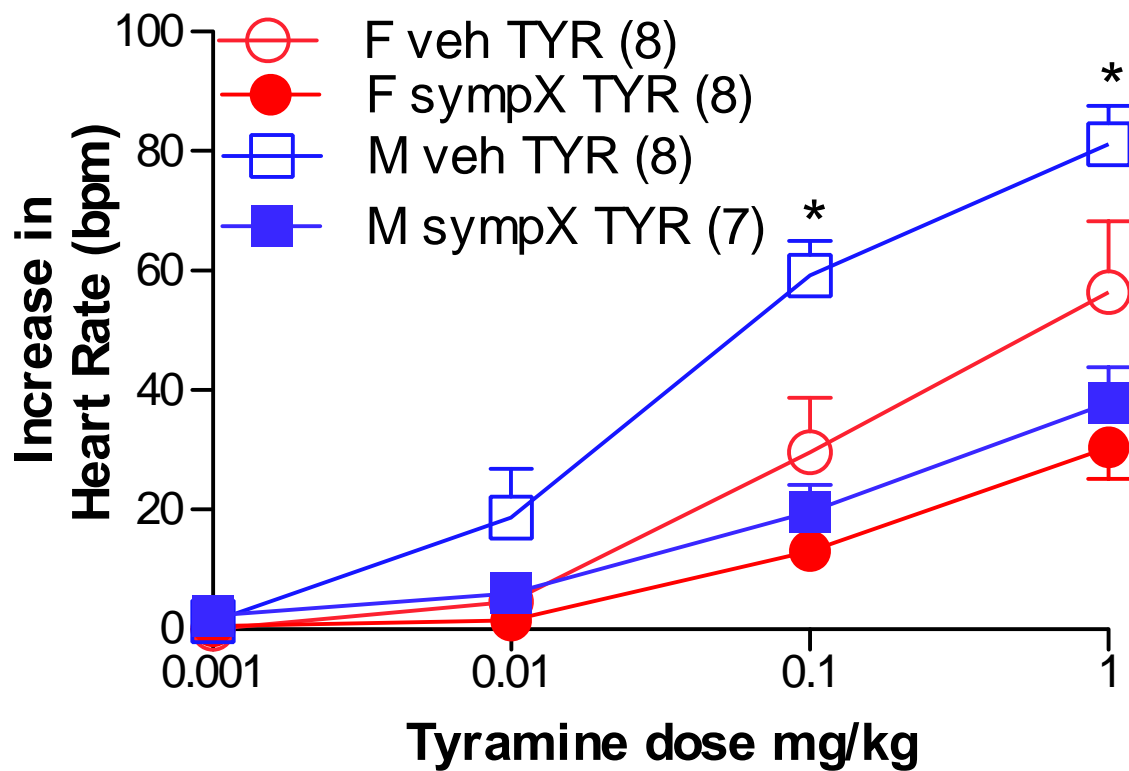
Fig. 5.3. Effects of intravenous injection of (±)-ephedrine (0.001-10 mg/kg), on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) male and female rats. Error bars indicate s.e. of mean from 7-8 experiments. Tachycardia to 10 mg/kg in vehicle treated animals was significantly greater in males than females. Asterisks denote responses in male rats significantly difference from responses in female rats (* P<0.05, anova and Bonferroni test).

Fig. 5.4. Effects of intravenous injection of (-)-ephedrine (0.001-10 mg/kg), on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) male and female rats. Error bars indicate s.e. of mean from 4-8 experiments. There were no significant differences (anova and Bonferroni test; * $P<0.05$).

Fig. 5.5. Effects of intravenous injection of isoprenaline (0.0001-0.1 mg/kg), on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) male and female rats. Error bars indicate s.e. of mean from 7 experiments. Tachycardia was unaffected by sympathectomy (anova and Bonferroni test).

5.3. Effects of ephedrine on blood pressure

Ephedrine produced biphasic responses, particularly at the dose of 10 mg/kg (see Figure 5.6), with increases in diastolic blood pressure reversing to small depressor responses in vehicle-treated male and female rats, and effects were not significantly different between male and female animals (compare Figures 5.7-5.10).

The isomers of ephedrine clearly differed in their blood pressure actions. (-)-ephedrine produced significantly greater pressor responses than ($\pm$)-ephedrine at a dose of 10 mg/kg in both vehicle-treated and sympathectomised tissues from males and females (Figures 5.7 & 5.8).

Sympathectomy had no effect on the peak pressor effect in both male and female, but increased the depressor response especially in males (Figures 5.9 & 5.10), and the time course was altered: the response reversed to a depressor response more rapidly, so that at 1 min for both male and female and for (-)- and ($\pm$)-ephedrine there was a depressor response significantly greater in sympathectomised than in vehicle treated animals (see Figure 5.6. and Table 5.1). Hence the depressor response was not inhibited by sympathectomy (but indeed potentiated) but the pressor response was reduced in terms of time course of response but not in terms of peak response.

The direct β-adrenoceptor agonist isoprenaline produced a dose dependent depressor response that was not affected by sympathectomy, and effects were not different between male and females (Figure 5.11). Hence, sympathectomy did not affect responses to a direct agonist: there was no evidence of hypersensitivity in terms of pressor responses.

Figure 5.6. Blood pressure (BP) recordings of the effects of injection of (-)-ephedrine (10 mg/kg, i.v.) in an anaesthetized vehicle treated (top trace) and a sympathectomised (bottom trace) male rat. BP calibration (mmHg) is shown together with time scale. At the arrow, (-)-ephedrine was injected. (-)-Ephedrine produced an initial pressor response that was similar in magnitude in vehicle and sympathectomised rats, followed by a depressor response that was faster in onset and more marked in sympathectomised rats (see Table 5.1). Heart rate responses are not shown as they are already maximal at this dose of (-)-ephedrine (see Figure 5.4).

Fig. 5.7. Pressor responses to intravenous injection of (±)-ephedrine (eph) or (-)-ephedrine ((-)-eph) (0.001-10 mg/kg) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) male rats. Values shown are rises in diastolic blood pressure (DBP). Error bars indicate s.e. of mean from 7-9 experiments. Pressor responses were significantly less for (±)-ephedrine (anova and Bonferroni test; * P<0.05).

Fig. 5.8. Pressor responses to intravenous injection of (±)-ephedrine (eph) or (-)-ephedrine ((-)-eph) (0.001-10 mg/kg) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) female rats. Values shown are rises in diastolic blood pressure (DBP). Error bars indicate s.e. of mean from 4-8 experiments. Pressor responses were significantly less for (±)-ephedrine (anova and Bonferroni test; * P<0.05).

Fig. 5.9. Depressor responses to intravenous injection of (±)-ephedrine (eph) or (-)-ephedrine ((-)-eph) (0.001-10 mg/kg) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) male rats. Values shown are falls in diastolic blood pressure (DBP). Error bars indicate s.e. of mean from 7-9 experiments. Depressor responses were significantly increased by sympathectomy (anova and Bonferroni test; * P<0.05).

Fig. 5.10. Depressor responses to intravenous injection of (±)-ephedrine (eph) or (-)-ephedrine ((-)-eph) (0.001-10 mg/kg) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) female rats. Values shown are falls in diastolic blood pressure (DBP). Error bars indicate s.e. of mean from 4-8 experiments. Depressor responses were significantly increased by sympathectomy (anova and Bonferroni test; * P<0.05).

These results suggest that the actions of ephedrine in the rat are a mixture of direct and indirect actions, both in terms of HR and blood pressure responses. For blood pressure, the initial peak pressure response appears to be mainly directly mediated, but the more rapid decline of the pressor response and the greater depressor response in sympathectomised animals indicates that the time course of the pressor response is greatly reduced in sympathectomised rats. Since depressor responses to isoprenaline were unaffected by sympathectomy, the increased depressor response to ephedrine following sympathectomy can only be due to loss of a pressor component.

Table 5.1. Pressor (positive values) and depressor (negative values) effects of (-)-ephedrine ((-)-eph) (top) and (±)-ephedrine ((±)-eph) (bottom) in vehicle treated and sympathectomised male and female rats. Responses shown are peak pressor response (positive value), response at 1 min (positive or negative value) and peak depressor response (negative value), all in mmHg. Error bars indicate s.e. mean from 4-9 experiments.

	Peak pressor	1 min response	Peak depressor
(-)-ephedrine			
Male			
veh (-)eph (7)	+24.3±1.9 mmHg	-4.4±2.2 mmHg	-4.4±2.0 mmHg
sympX (-)eph (7)	+30.3±3.7 mmHg	-16.7±1.5 mmHg*	-22.4±2.6 mmHg**
Female			
veh (-)eph (7)	+36.4±5.5 mmHg	+8.0±5.8 mmHg	-0.1±2.4 mmHg
sympX (-)eph (4)	+31.5±4.0 mmHg	-10.8±1.8 mmHg**	-12.5±0.9 mmHg
(±)-ephedrine			
Male			
veh (±)eph (9)	+15.3±2.1 mmHg	-7.8±2.9 mmHg	-10.6±2.3 mmHg
sympX (±)eph (9)	+10.1±2.4 mmHg	-17.7±2.4 mmHg*	-21.1±2.7 mmHg*
Female			
Veh (±)eph (8)	+15.2±2.1 mmHg	-8.9±2.8 mmHg	-8.8±4.0 mmHg
sympX (±)eph (7)	+13.8±1.6 mmHg	-17.8±1.5 mmHg*	-19.5±1.2 mmHg*

Asterisks denote significant difference from equivalent response in vehicle treated animals of the same gender (anova and Bonferroni test: * P<0.05; ** P<0.01).

Fig. 5.11. Depressor responses to intravenous injection of isoprenaline (0.0001-0.1 mg/kg), on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) male and female rats. Error bars indicate s.e. of mean from 7 experiments. Depressor responses were unaffected by sympathectomy.

5.4. Summary

1. Gender differences in the cardiovascular effects of the stimulant (±)-ephedrine have been studied, and for comparison (-)-ephedrine, employing vehicle-treated and sympathectomised anaesthetised male and female Wistar rats.

2. (±)-Ephedrine had a greater component of indirect actions for cardiac responses as compared to (-)-ephedrine in both male and females.

3. For (±)- but not (-)-ephedrine, the maximum HR response in vehicle treated animals was significantly greater in male than in female rats.

4. Peak pressor responses to (-)-ephedrine were significantly greater than those to (±)-ephedrine in both males and females.

5. Peak pressor responses to both (±)- and (-)-ephedrine were resistant to sympathectomy.

6. Depressor responses to both (±)- and (-)-ephedrine were increased by sympathectomy, but since responses to isoprenaline were unchanged, this suggests loss of a later maintained pressor response.

Chapter 6.

Effects of cathine and MHA on HR and blood pressure in vehicle-treated and sympathectomised male and female rats

Chapter 6. Effects of cathine and MHA on HR and blood pressure in vehicle-treated and sympathectomised male and female rats

In Chapters 4 and 5, pressor and tachycardia responses to cathinone, MDMA, tyramine and ephedrine were obtained in anaesthetized male and female rats, and the mode of action was investigated by examining the effects of sympathectomy. Two further stimulants are examined in this chapter: cathine, both a constituent of khat and a metabolite of cathinone, and MHA, a widely used stimulant. This chapter looks at the mode of action of these two stimulants in male and female rats.

6.1. Anaesthetized rat: basal DBP and HR

In anaesthetized rats, resting diastolic blood pressure (DBP) was 115±2 mmHg (n=14) and 108±2 mmHg (n=12) in vehicle treated male and female rats, respectively, and 99±5 mmHg (n=11) and 99±3 mmHg (n=11) in sympathectomised male and female rats, respectively. DBP was significantly reduced by sympathectomy in male rats (P<0.05), but there were no significant differences between male and female animals. However, the number of animals studied was much less than in Chapter 4. DBP was subjectively more stable in sympathectomised rats of either gender.

Resting HR was 348±6 bpm (n=14) and 310±13 bpm (n=12), respectively in vehicle treated male and female rats, and 320±8 bpm (n=11) and 316±15 bpm (n=11), respectively in sympathectomised male and female rats. HR was significantly reduced by sympathectomy in male rats, and HR was significantly greater in male than female vehicle-treated rats (P<0.05). However, the number of animals studied was much less than in Chapter 4.

6.2. Effects of stimulants on HR

Potency of agonists at producing tachycardia could be calculated for all agonists in vehicle experiments from both male and female rats. However, since clear maximum responses were obtained in few experiments, differences in potency

between male and female could reflect differences in maximum responses. With this made clear, potency is discussed below.

Cathine produced dose dependent increases in HR in vehicle-treated male and female rats, and effects were not significantly different between male and female animals. Sympathectomy virtually abolished the tachycardia in both male and female (Figure 6.1). Hence, cathine effects on HR were largely indirect and there were no gender differences.

MHA produced dose dependent increases in HR in vehicle-treated male and female rats, and effects were not significantly different between male and female animals. Sympathectomy virtually abolished the tachycardia in both male and female (Figure 6.2). Hence, effects of MHA on HR were largely indirect and there were no gender differences.

There was a significant tachycardia following sympathectomy only for cathine as compared to the effects of vehicle and only in male rats (P<0.5).

6.3. Effects of stimulants on blood pressure

Cathine produced very small increases in DBP in vehicle-treated male and female rats, and effects were not significantly different between male and female animals (Figure 6.3). However, sympathectomy virtually abolished this small pressor effect in female (P<0.01) but not male rats (Figure 6.3). Admittedly, these are very small pressure responses, and it is difficult to comment further on these results.

MHA produced small increases in DBP in vehicle-treated male and female rats, and effects were not significantly different between male and female animals (Figure 6.4). Sympathectomy virtually abolished this small pressor effect in female rats, but this did not reach significance in male rats (Figure 6.4).

Figure 6.1. Effects of intravenous injection of cathine (0.001-1 mg/kg) on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. Error bars indicate s.e. of mean from 7 experiments. The tachycardia to cathine was significantly reduced by sympathectomy in both male and female rats (anova and Bonferroni test; * P<0.05).
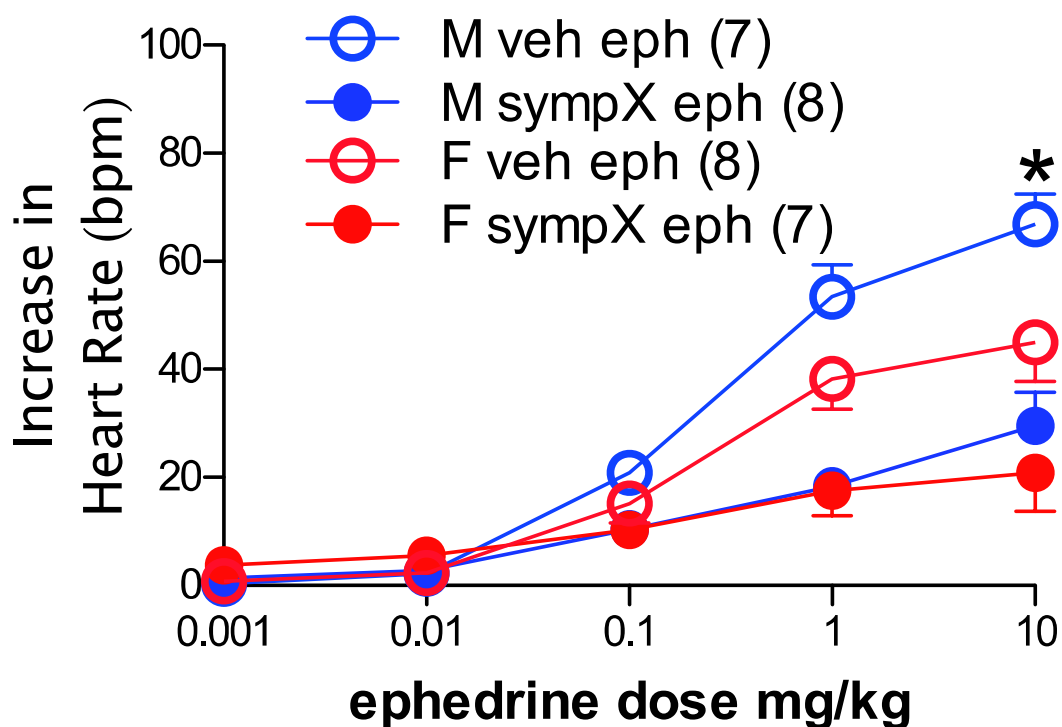
Figure 6.2. Effects of intravenous injection of MHA (0.001-1 mg/kg) on heart rate in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. Error bars indicate s.e. of mean from 6-8 experiments. The tachycardia to MHA was significantly reduced by sympathectomy in both male and female rats (anova and Bonferroni test; * P<0.05).

Figure 6.3. Effects of intravenous injection of cathine (0.001-1 mg/kg) on diastolic blood pressure (DBP) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. Error bars indicate s.e. of mean from 7 experiments. DBP was significantly reduced by sympathectomy in female rats (anova and Bonferroni test; * P<0.05).

Figure 6.4. Effects of intravenous injection of MHA (0.001-1 mg/kg) on diastolic blood pressure (DBP) in pentobarbitone anaesthetized vehicle (veh) or chemically sympathectomised (sympX) rats. Error bars indicate s.e. of mean from 6-8 experiments. DBP was significantly reduced by sympathectomy in female rats (anova and Bonferroni test; * P<0.05).

The maximum pressor response to both cathine and MHA in sympathectomised animals was significantly greater than the pressor response to vehicle injection in male but not female rats (P<0.01).

6.4. Anaesthetised rat: basal DBP and HR (all studies combined).

Since baseline DBP and HR values were measured prior to administration of stimulant, all baseline data from Chapters 4, 5 & 6 can be combined to give overall mean values for DBP and HR.

In anaesthetized rats, resting DBP was 116±2 mmHg (n=62) and 114±2 mmHg (n=51) in vehicle treated male and female rats, respectively, and 102±2 mmHg (n=41) and 98±3 mmHg (n=39) in sympathectomised male and female rats, respectively. DBP was significantly reduced by sympathectomy in both male and female rats (P<0.001), and there were no significant differences between male and female animals.

Resting HR was 346±3 bpm (n=62) and 328±5 bpm (n=51), respectively in vehicle treated male and female rats, and 319±6 bpm (n=41) and 319±7 bpm (n=39), respectively in sympathectomised male and female rats.
HR was significantly reduced by sympathectomy in male rats (P<0.01), and HR was significantly greater in male than female vehicle-treated rats (P<0.05). Hence, there was a significantly lower resting HR in female vehicle-treated rats, although the difference was small.

6.5. Summary

1. Gender differences in the direct and indirect sympathomimetic cardiovascular effects of the stimulants cathine and MHA have been studied, employing male and female Wistar rats.

2. Animals were sympathectomised by treatment with 6-OHDA or treated with vehicle.

3. In male and female vehicle treated pentobarbitone anaesthetised rats, cathine and MHA (0.001-1 mg/kg) produced significant tachycardia and small pressor responses.

4. In sympathectomised rats, the tachycardia to both cathine and MHA were significantly reduced, demonstrating that cardiac actions are mainly indirect for these agonists.   There were no differences between male and female in cardiac responses.

5. In sympathectomised rats, the pressor response to MHA was significantly reduced by sympathectomy in female rats. However, the small pressor response to cathine was resistant to sympathectomy only in male rats.

6. Hence, there were no gender differences in the tachycardia response to either agonist, or in the pressor response to MHA, but minor gender differences in the pressor response to cathine.

Chapter 7.

Effects of sympathectomy on responses of rat vas deferens to direct and indirect adrenergic stimulants

Chapter 7. Effects of sympathectomy on responses of rat vas deferens to direct and indirect adrenergic stimulants.

In this Chapter the effects of direct and indirect sympathomimetics on contractile responses of vas deferens from control and sympathectomised rats have been investigated. The vas deferens is a tissue in which stimulants can cause contractions both by direct and by indirect actions, due to the dense innervation. Hence, actions of indirect sympathomimetics can be easily identified. Initial studies investigated both phasic and tonic responses, but it became clear that tonic responses were more meaningful. Only direct agonists should cause marked tonic contractions in sympathectomised animals.

7.1. Effects of sympathectomy on isometric contractions to electrical stimulation.

The degree of sympathectomy was assessed quantitatively by examining the response to a single pulse electrical stimulus in rat whole vas deferens. In control animals, the contraction to a single stimulus was 1.40 ± 0.16 g (first phase) and 0.97 ± 0.11 g (n=10) (second phase), but in sympathectomised animals the maximum contractions were 0.84 ± 0.24 g and 0.48 ± 0.15 g (n=7), respectively . Hence, the second adrenergic phase was significantly reduced to 49% of control by sympathectomy ($P<0.05$).

In further studies, trains of pulses at 1Hz were examined. However, only the first pulse in the train (effectively a single pulse) was significantly reduced by sympathectomy (Figure 7.1). This graph plots the peak response, which is usually the first, purinergic component of the biphasic response. Since this purinergic response is resistant to sympathectomy, the graph underestimates the degree of reduction of the adrenergic component. The adrenergic phase becomes less important with each subsequent pulse.

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Figure 7.1.

Figure 7.1. Isometric contractions of rat vas deferens produced by stimulation with 10 pulses at 1 Hz in tissues from vehicle-treated and sympathectomised rats. Value are mean and s.e. of mean from 6 experiments. The response to the first stimulus was significantly reduced by sympathectomy (anova, $P<0.05$).

7.2. Prejunctional inhibition of nerve-evoked contractions by stimulants in rat vas deferens.

The inhibition by agonists of the contraction of rat vas deferens produced by single pulse electrical stimulation was examined in the presence of the calcium entry blocker nifedipine (10 μM) to eliminate postjunctional actions of agonists. Stimulants may produce postjunctional contractions involving calcium entry that would affect the nerve stimulation evoked contraction, complicating interpretation of possible prejunctional actions. Hence, nifedipine was present.

Potency at producing inhibition of this nerve-evoked contraction was calculated and compared between agonists and a rank order of potency compiled for actions presumably at α_{2A}-adrenoceptors on nerve terminals.

The selective α_2-adrenoceptor agonist xylazine produced concentration-dependent inhibition of stimulation-evoked contractions with a pIC_{50} of 7.25 (Figure 7.2 & Table 7.1). The stimulants cathine, ephedrine, norephedrine and MHA produced concentration-dependent inhibition of stimulation-evoked contractions with pIC_{50} values ranging from of 5.20 to 5.62 (Figure 7.2 & Table 7.1). All agonists tested were significantly less potent than xylazine.

Also show for comparison are published data from our laboratory for MDMA, MDEA, MDA and cathinone (Bexis & Docherty, 2006). All stimulants examined except MDEA had inhibitory potency in the range 5.20 to 6.08. MDEA had lowest potency (see Table 7.1).

The rank order of potency for the compounds most of interest in this thesis was (most potent first): cathinone>MDMA>cathine=MHA>($\pm$)-ephedrine=norephedrine (see Table 7.1) Hence, of the compounds of interest, cathinone had highest potency at prejunctional α_2-adrenoceptors.

Figure 7.2. Prejunctional actions of stimulants at inhibiting the contractile response to a single stimulus in rat vas deferens. Responses in the presence of agonist were expressed as a percentage of the control contraction. For comparison, the effects of the α_2-adrenoceptor agonist xylazine are also shown. Also shown are the effects of distilled water vehicle. Values are mean and s.e. of mean, with n, the number of experiments, in brackets.

Table 7.1. Prejunctional potency of stimulants at inhibiting the contractile response to a single stimulus in rat vas deferens. For comparison, the potency of the selective α_2-adrenoceptor agonist xylazine is also shown. Values are pIC_{50} (- logM) and s.e. of mean, with n, the number of experiments, in brackets.

Stimulant	pIC_{50}
xylazine	7.25±0.04 (5)
cathine	**5.62±0.10 (5)**
(±)-ephedrine	5.20±0.15 (8)
norephedrine	5.22±0.14 (9)
MHA	5.59±0.06 (5)
MDMA	5.88±0.16 (4)[1]
MDA	5.46±0.11 (8)[1]
MDEA	4.91±0.20 (4)[1]
cathinone	**6.08±0.11 (4)[1]**

[1]Values taken from Bexis & Docherty (2006).

Figure 7.3. Original recording showing tonic and spikey contractions of a vehicle-treated rat vas deferens produced by NA in the absence of cocaine. NA was added cumulatively in 0.5 log unit increments beginning with 10^{-8}M. Values shown are concentration (logM). Arrows indicate time of addition of NA concentration. Total contractions are measured as tonic contractions plus spikes. Tension and time calibrations are also shown.

rat vas total

Figure 7.4. Total contractions (grams tension) to NA in rat vas deferens from vehicle-treated (first veh) or sympathectomised (sympX) rats. Responses to increasing concentrations of NA (log M) were obtained in the absence (second veh) or presence of cocaine (3 μM) (CO). Values are mean values and vertical bars indicate s.e of mean from 6-8 experiments. Potency of NA was significantly increased by cocaine in vehicle-treated rats, and sympathectomy significantly increased the potency of NA in the absence of cocaine (anova and post test).

7.3. Effects of sympathectomy on contractions to NA

7.3.1. Total contractions to NA

In rat vas deferens from vehicle treated animals, NA produced contractions consisting of phasic contractions superimposed on tonic contractions. Total contractions were measured as the maximum height of tonic contractions combined with intermittent spikes.   A typical original isometric tension recording of vas deferens contractions is shown in Figure 7.3, demonstrating tonic and phasic components. These responses were obtained in a vas deferens from a vehicle treated animal in the absence of NA re-uptake blockade.

NA produced total contractions in the absence of cocaine in rat vas deferens with a similar maximum response of 0.81±0.14g (n=8) in vehicle treated rats, and 0.99±0.13 g (n=6) in sympathectomised rats (see Figure 7.4) (no significant difference). NA potency (pEC$_{50}$, -log M) at producing total contractions in rat vas deferens in absence of cocaine was 4.80±0.15 (n=8) in vehicle treated rats, and 5.61±0.18 (n=6) in sympathectomised rats (P<0.05) (Figure 7.4). In the presence of cocaine (3 μM), spontaneous contractions occurred in the absence of NA especially in sympathectomised rats, and contributed to the large contraction to 0.1 μM NA seen in Figure 7.4.

The potency of NA at producing total contractions was significantly increased by cocaine (3 μM) in vehicle treated animals (5.80±0.26, n=8; P<0.05) but did not reach significance in sympathectomised animals (6.39±0.30, n=6) (Figure 7.4). In vehicle treated animals, cocaine increased the maximum response to NA (1.41±0.16g, n=8, P<0.05) but in sympathectomised rats in the presence of cocaine (1.28±0.25, n=6) there was no significant difference in maximum response (Figure 7.4).

Sympathectomy greatly increased the height and frequency of spontaneous contractions (P<0.05) especially in the presence of cocaine, as demonstrated by the apparent large contraction to NA (0.1 μM), and this may explain the increased

rat vas tonic

Legend:
- ○ veh veh (8)
- ● sympX veh (6)
- □ veh CO (8)
- ■ sympX CO (6)

Y-axis: Tension (g), values 0.0, 0.5, 1.0, 1.5
X-axis: noradrenaline concentration, values -7, -6, -5, -4

Figure 7.5. Tonic contractions (grams tension) to NA of rat vas deferens from vehicle-treated (first veh) or sympathectomised (sympX) rats. Responses to increasing concentrations of NA (log M) were obtained in the absence (second veh) or presence of cocaine (3 μM) (CO). Values are mean values and vertical bars indicate s.e of mean from 6-8 experiments. Potency of NA was significantly increased by cocaine in vehicle-treated and sympathectomised rats (* $P<0.05$, anova and post test). Asterisks indicate significant differences in contractile response between vehicle-treated and sympathectomised rats (* $P<0.05$, anova and post test).

Table 7.2. Maximum contraction to agonists in producing tonic contractions in rat vas deferens. Agonists producing marked contractions in both vehicle-treated and sympathectomised rats are shown.

agonist	Vehicle treated	sympathectomised
NA (vehicle)	0.69±0.11g (8)	0.81±0.10g (6)
NA (cocaine 3 μM)	1.08±0.17g (8)	0.99±0.16g (6)
norephedrine	0.32±0.06g (6)	0.99±0.30g (6)**
tyramine	0.85±0.10g (6)	0.55±0.11g (6)

All agonists were investigated in concentrations up to 1000 μM.

Asterisks denote agonist responses in sympathectomised animals significantly different from responses in vehicle-treated animals (anova: ** $P < 0.01$).

potency and maximum total contraction of NA in the presence of cocaine in vehicle treated but not sympathectomised rats (Figure 7.4).

7.3.2. Tonic contractions to NA

NA produced tonic contractions in rat vas deferens with a similar maximum response of 0.69±0.11g (n=8) in vehicle treated rats, and 0.81±0.10g (n=6) in sympathectomised rats (no significant difference) (see Table 7.2 & Figure 7.5). NA potency (pEC_{50}, -log M) at producing tonic contractions in rat vas deferens was 4.86±0.17 (n=8) in vehicle treated rats, and 5.15±0.20 (n=6) in sympathectomised rats (no significant difference) (see Table 7.4 & Figure 7.5). The potency of NA at producing tonic contractions was significantly increased by cocaine (3 μM) in vehicle treated animals (5.74±0.27, n=8) but did not reach significance in sympathectomised animals (5.75±0.16, n=6) (Table 7.4 & Figure 7.5). The differences in maximum responses did not reach significance (Figure 7.5. and Table 7.2).

However, tonic responses to low concentrations of NA were significantly increased by sympathectomy in vas deferens in the absence but not the presence of cocaine (Figure 7.5). For example, the response to NA (10 μM) in the absence of cocaine was increased from 0.27±0.06 g (n=8) to 0.63±0.13 g (n=6) by sympathectomy (P<0.05), whereas in the presence of cocaine the response was 0.99±0.20 g (n=8) in vehicle treated animals and 0.99±0.16 g (n=6)(non-significant) in sympathectomised animals. Hence, sympathectomy significantly increased the tonic effects of low concentrations of NA in the absence of cocaine.

7.3.3. Effects of sympathectomy on contractions to the stimulants cathinone and MDMA.

In rat vas deferens, stimulants produced isometric contractions with two components: tonic contractions and phasic contractions superimposed on these.

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Rat Vas deferens: total

Figure 7.6. Total contractions (grams tension) to cathinone (cath) and MDMA in rat vas deferens from vehicle-treated (veh) or sympathectomised (sympX) rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments. There were no significant differences.

Total contractions to cathinone were not significantly reduced in terms of maximum response by sympathectomy (Figure 7.6). Total contractions to MDMA were unaffected by sympathectomy (Figure 7.6).

Cathinone produced no significant tonic contraction in vehicle-treated or sympathectomised rats (Figure 7.7). MDMA produced tonic contractions at higher concentrations, but tonic contractions to MDMA were virtually abolished by sympathectomy (Figure 7.7.). Cathine produced very small tonic contractions in tissues from vehicle-treated animals, but statistical analysis in comparison with sympathectomised was not carried out due to low n values for sympathectomised animals. All of these difficult to obtain agonists were used at a maximum concentration of 100 μM. Table 7.3 shows maximum contractions to agents producing relatively small contractions.

7.3.4. Effects of sympathectomy on contractions to other stimulants

The maximum tonic contractions obtained to stimulant agonists (100 μM or 1000 μM) are shown in Table 7.2 (agonists producing large contractions following sympathectomy) and Table 7.3 (agonists producing small contractions following sympathectomy). All agonists examined produced phasic contractions, but only cathinone failed to produce tonic contractions.

The potency of tyramine at producing total contractions in rat vas deferens was significantly increased by sympathectomy (P<0.05) (Figure 7.8 and Table 7.4), and the maximum response to tyramine in producing tonic contractions was not significantly reduced by sympathectomy (Figure 7.9 & Table 7.2). Prazosin (10^{-7}M) virtually abolished contractions to tyramine (Figure 7.8), demonstrating that the response is α_1-adrenoceptor mediated. Even in sympathectomised tissues, tyramine still produced marked tonic contractions. Contractions to tyramine, both total and tonic, were markedly reduced by prazosin (Figure 7.8)

Rat Vas deferens: tonic

Figure 7.7. Tonic contractions (grams tension) to cathinone, cathine and MDMA in vas deferens from vehicle-treated (veh) or sympathectomised (sympX) male rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments, except for cathine in sympathectomised tissues. Responses to cathine are shown as dashed lines, and no statistical test was carried out for cathine due to the low n value for sympathectomised tissues.   Maximum response to MDMA was significantly reduced in sympathectomised rats (P<0.05, anova and Bonferroni post test).

Table 7.3.

Maximum contraction to agonists in producing tonic contractions in rat vas deferens.
Only agonists producing small contractions in sympathectomised rats are shown.

agonist	Vehicle treated	sympathectomised
MDMA+	0.38±0.05g (6)	0.10±0.10g (6)*
cathinone+	0.07±0.03g (6)	0.02±001g (6)
MDA+	0.33±0.09g (6)	0.36±0.08g (6)
MDEA+	0.13±0.03 (6)	0.14±0.05g (6)
(±)-ephedrine	0.44±0.06g (6)	0.05±0.03g (6)*
MHA+	0.33±0.12g (6)	0.08±0.06g (6)*
cathine+	0.16±0.03g (6)	/

+ denotes agonist maximum concentration of 100 μM. For unmarked agonists
maximum concentration was 1000 μM. Asterisks denote agonist responses in
sympathectomised animals significantly different from responses in vehicle-treated
animals (anova: * $P < 0.05$).

Rat vas deferens: total

Figure 7.8. Total contractions (grams tension) to tyramine (TYR) in rat vas deferens from vehicle-treated (veh) or sympathectomised (sympX) rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments. Asterisks indicate significant differences between vehicle-treated and sympathectomised rats, and between vehicle treated and vehicle-treated in the presence of prazosin (10^{-7}M) (* $P<0.05$, anova and Bonferroni post-test).

Table 7.4. Potency of agonists in producing tonic contractions in rat vas deferens from vehicle-treated and sympathectomised animals. Only agonists producing marked contraction in both vehicle-treated and sympathectomised rats are included.

	Vehicle treated	sympathectomised
NA (vehicle)	4.86±0.17 (8)	5.15±0.20 (6)
NA (cocaine 3 μM)	5.74±0.27 (8)*	5.75±0.16 (6)
Tyramine	4.17±0.16 (6)	4.00±0.15 (6)
MDA+	4.06±0.15 (6)	3.67±0.35 (6)
Norephedrine	3.35±0.33 (6)	3.46±0.32 (6)

+ denotes agonist maximum concentration of 100 μM. For unmarked agonists maximum concentration was 1000 μM. Asterisks denote significant differences between responses to NA in presence and absence of cocaine (anova * $P<0.05$). Sympathectomy did not significantly change potencies.

Rat Vas deferens: tonic

Figure 7.9. Tonic contractions (grams tension) to tyramine (TYR) in rat vas deferens from vehicle-treated (veh) or sympathectomised (sympX) rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments. There were no significant differences between vehicle-treated and sympathectomised rats.

Figure 7.10. Total contractions (grams tension) to (±)-ephedrine (eph) and norephedrine (noreph) in rat vas deferens from vehicle-treated (veh) or sympathectomised (sympX) rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments. Asterisks indicate significant differences between vehicle-treated and sympathectomised rats in maximum response obtained (* $P<0.05$, anova and post test).

Total contractions to MHA and (±)-ephedrine were not significantly affected by sympathectomy, but contractions to norephedrine were significantly increased by sympathectomy (Figure 7.10).

Tonic contractions to MHA and (±)-ephedrine were virtually abolished by sympathectomy (Table 7.3), but contractions to norephedrine were significantly increased (Figure 7.11 & Table 7.2). Although most studies of ephedrine on rat vas deferens were carried out with (±)-ephedrine, a small number of experiments were carried out with (-)-ephedrine, as shown in Figure 7.12 for tonic contractions. In terms of tonic contractions, (-)-ephedrine behave identically to (±)-ephedrine, producing small contractions in control tissues, abolished by sympathectomy (Figure 7.12).

MDA was more potent than MDEA at producing total contractions, but total contractions to both were unchanged by sympathectomy (Fig. 7.13). Tonic contractions to both MDA and MDEA were unaffected by sympathectomy (Figure 7.14).

The phasic response to MDMA, ephedrine and MHA were largely unaffected by sympathectomy, but for NA, norephedrine and tyramine, phasic contractions were significantly increased by sympathectomy.

Figure 7.11. Tonic contractions (grams tension) to (±)-ephedrine (eph) and norephedrine (noreph) in rat vas deferens from vehicle-treated (veh) or sympathectomised (sympX) rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments. Asterisks indicate significant differences between vehicle-treated and sympathectomised rats (* P<0.05, anova and post test).

Rat Vas deferens: tonic

Figure 7.12. Tonic contractions (grams tension) to (±)-ephedrine (eph) and (-)-ephedrine ((-)-eph) in rat vas deferens from vehicle-treated (veh) or sympathectomised (sympX) rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments for (±)-ephedrine, but only 2-4 experiments for (-)-ephedrine. Statistical tests were not carried out due to the small n values for (-)-ephedrine. Statistical test for (±)-ephedrine is shown in Figure 7.11.

Rat Vas deferens: total

Figure 7.13. Total contractions (grams tension) to MDA and MDEA in rat vas deferens from vehicle-treated (veh) or sympathectomised (sympX) rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments. There were no significant differences between vehicle-treated and sympathectomised.

Rat Vas deferens: tonic
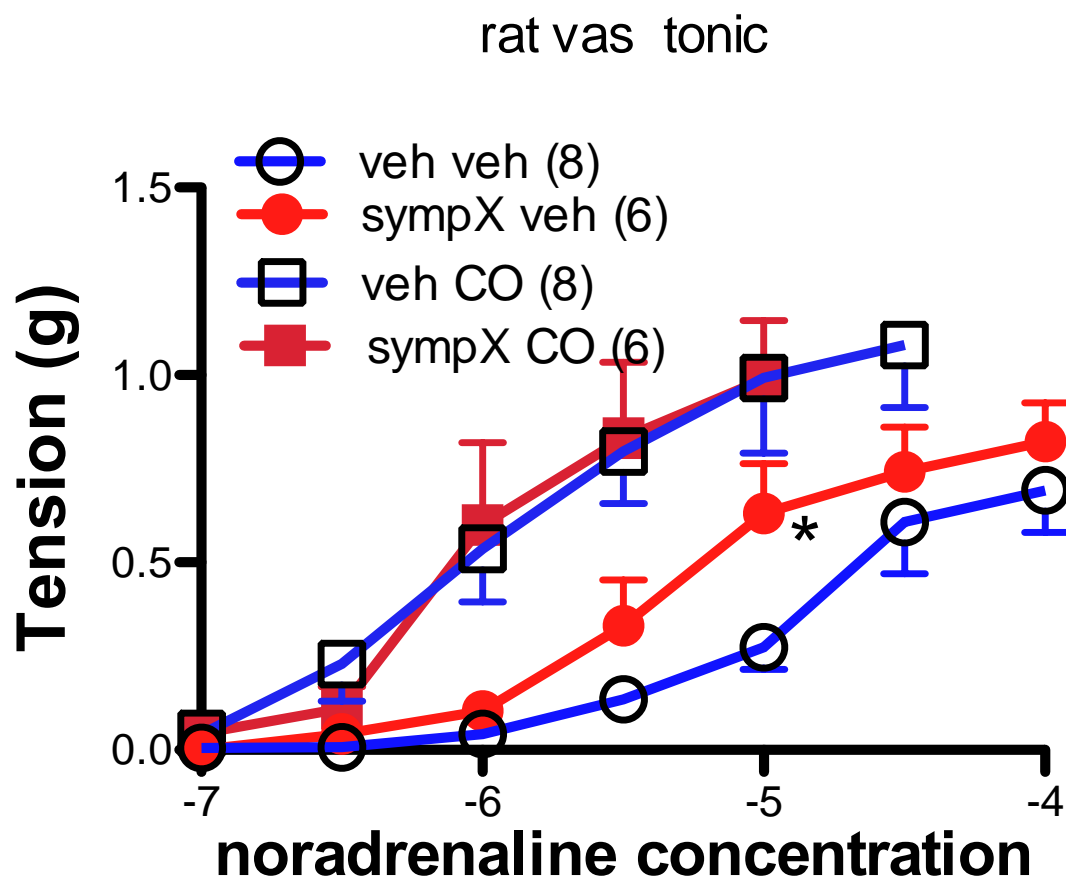
Figure 7.14. Tonic contractions (grams tension) to MDA and MDEA in rat vas deferens from vehicle-treated (veh) or sympathectomised (sympX) rats. Values are mean values and vertical bars indicate s.e of mean from 6 experiments. There were no significant differences between vehicle-treated and sympathectomised.

7.4. Summary

1.   The effects of direct and indirect sympathomimetics on contractile responses of vas deferens from control and sympathectomised rats have been investigated. Contractions of rat vas deferens to agonists consist of two components: tonic and phasic. All agonists produced phasic contractions of vas deferens from control rats.

2. Phasic contractions were more resistant to sympathectomy. The phasic contraction to cathinone was not significantly reduced by sympathectomy, the phasic contraction to NA, norephedrine and tyramine were significantly increased by sympathectomy, and for the other agonists there was no effect of sympathectomy.

3. Cathinone failed to produce tonic contractions in vehicle treated animals. Tonic contractions to MDMA, ephedrine and MHA were virtually abolished by sympathectomy, and tonic contractions to tyramine were reduced by sympathectomy. Only for NA and norephedrine were tonic contractions significantly increased by sympathectomy, suggesting that the responses to these agonists, as substrates for NET, are potentiated by loss of this disposal mechanism.

4. Sympathectomy significantly increased the tonic response to low concentrations of NA and to norephedrine, suggesting both are direct agonists subject to marked uptake by NET.

5. Tonic contractions are more useful than phasic contractions of the rat vas deferens for the study of the direct and indirect actions of stimulants.

Chapter 8.

Gender differences in the effects of sympathectomy on responses of rat aorta to stimulants

Chapter 8. Gender differences in the effects of sympathectomy on responses of rat aorta to stimulants

In this Chapter, contractions to NA and a number of stimulants have been examined in aorta from vehicle-treated and sympathectomised male and female rats. The rat aorta is a tissue in which direct actions of stimulants can be examined in the absence of indirect actions, due to the sparse innervation.

8.1. Contractions to NA

In rat aorta, NA produced concentration-dependent isometric contractions (see Figure 8.1) with pEC_{50} values (-log M) of 7.02±0.08 (n=6) and 6.76±0.10 (n=6), and maximum contractions of 0.42±0.05g and 0.54±0.08g, in tissues from male and female rats, respectively in the absence of cocaine (no significant differences) (see Figure 8.2 and Table 8.1).

Cocaine (3 μM) did not significantly affect potency or maximum contraction to NA in male or female vehicle treated rats (Table 8.1 and 8.2; compare Figures 8.2 and 8.3). This suggests that NET is largely absent in rat aorta.

Cocaine (3 μM) did not significantly affect potency or maximum contraction to NA in male or female sympathectomised rats (Table 8.1 and 8.2; compare Figures 8.2 and 8.3). However, sympathectomy tended to increase the maximum response to NA, but this effect was not significant (Figures 8.2, 8.3 & Table 8.1). Sympathectomy also tended to increase the potency of NA in aorta from both male and female, but this effect did not reach significance, perhaps due to variability in EC_{50} values obtained (Figure 8.3. & Table 8.2).

8.2. Contractions to other stimulants

In aorta from male or female rats, in concentrations up to 100 μM (10^{-4}M), cathinone,

Figure 8.1. Original recording of the increase in isometric tension produced by increasing concentrations of NA in the absence of cocaine in an aorta from a male vehicle-treated rat. Tension and time scales are indicated. At the arrows, NA was added in increasing concentrations (log M) in 0.5 log unit increments, beginning with 10^{-8}M (-8) (effects of lowest doses added are not shown).

Figure 8.2. Contractions of aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats produced by the agonist NA in the absence of cocaine.   Values are tension (g) and s.e. of mean, with n, number of experiments, in brackets. There were no significant differences in potency or maximum response (anova and post-test).

Table 8.1. Contractions produced by agonists in aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats. Abbreviations: NA (veh), NA in the absence of cocaine; NA (Co), NA in the presence of cocaine 3 μM; Noreph, norephedrine; eph, ($\pm$)-ephedrine; MHA, methylhexanamine.   For all agonists, except NA and norephedrine, a clear maximum contraction was not obtained so that values shown are the response obtained to the highest effective concentration investigated ([1] Maximum response, [2]100 μM, [3]1000 μM). Values are tension (g) and s.e. of mean, with n, number of experiments, in brackets.

	M veh	M sympX	F veh	F sympX
NA (veh)[1]	0.49±0.11	0.74±0.13	0.51±0.11	0.82±0.17
	(6)	(6)	(6)	(6)
NA (Co)[1]	0.44±0.06	0.71±0.09	0.53±0.07	0.48±0.10
	(6)	(6)	(6)	(6)
noreph[1]	0.45±0.08	0.54±0.09	0.26±0.04	0.59±0.11*
	(10)	(6)	(6)	(7)
($\pm$)-eph[3]	0.22±0.08	0.34±0.13	0.06±0.02+	0.26±0.06*
	(10)	(7)	(7)	(6)
tyramine[3]	0.19±0.02	0.34±0.03*	0.17±0.02	0.20±0.05+
	(8)	(5)	(9)	(7)
MDA[2]	0.14±0.06	0.29±0.06	0.14±0.08	0.17±0.08
	(7)	(8)	(6)	(6)
MDEA [2]	0.05±0.05	-0.00±0.04	-0.01±0.04	-0.01±0.02
	(6)	(7)	(7)	(6)
MDMA[2]	0.05±0.05	0.01±0.02	-0.02±0.02	-0.03±0.02
	(6)	(5)	(8)	(6)

	M veh	M sympX	F veh	F sympX
cathinone[2]	0.01±0.03 (12)	0.01±0.03 (7)	-0.03±0.03 (9)	-0.09±0.04 (9)
cathine[2]	0.03±0.05 (7)	ND	0.01±0.01 (6)	ND
MHA[2]	0.04±0.02 (9)	ND	0.00±0.00 (6)	0.00±0.02 (4)
KCl	0.29±0.04 (7)	0.42±0.03 (4)	0.34±0.05 (10)	0.44±0.1 (5)

Asterisks denote significant difference between sympathectomised tissue and relevant vehicle (* P<0.05, anova and post test). Crosses denote significant difference between male and female (+ P<0.05, anova and post test).

Table 8.2. Potency of agonists at producing contraction in aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats. Abbreviations: NA (veh), NA in the absence of cocaine; NA (Co), NA in the presence of cocaine 3 μM; noreph, norephedrine. Values are pEC50 (-log M) and s.e. of mean, with n, number of experiments, in brackets. Only for NA and norephedrine was a clear maximum contraction obtained in all groups. For other agonists, potency may be overestimated.

	M veh	M sympX	F veh	F sympX
NA (veh)	7.02±0.08 (6)	7.38±0.22 (6)	6.76±0.10 (6)	7.20±0.29 (6)
NA (Co)	6.74±0.19 (6)	7.45±0.40 (6)	6.68±0.07 (6)	7.18±0.24 (6)
noreph	4.76±0.12 (10)	5.39±0.17* (6)	4.47±0.10 (6)	4.62±0.19++ (7)
ephedrine	3.54±0.35 (9)	5.18±0.31* (6)	/	3.42±0.31+ (6)
tyramine	3.40±0.312 (8)	5.07±0.41* (5)	3.12±0.20 (7)	3.12±0.40++ (7)

Asterisks denote significant difference between sympathectomised tissue and relevant vehicle (* $P<0.05$, anova and post test). Crosses denote significant difference between male and female (+ $P<0.05$, ++ $P<0.01$, anova and post test).

Figure 8.3. Contractions of aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats produced by the agonist NA in the presence of cocaine (3 μM). Values are tension (g) and s.e. of mean, with n, number of experiments, in brackets. There were no significant differences in potency or maximum response (anova and post-test).

cathine, MDMA, MDEA and MHA produced no significant contractions (Table 8.1). Sympathectomy did not increase the contractile response to any of these agonists.

Norephedrine, ephedrine, tyramine and to a lesser extent MDA, produced significant contractions in aorta from male and female animals (Table 8.1). Contractions to norephedrine were larger in tissues from male than from female vehicle-treated rats, but this did not reach significance (Figure 8.4). However, sympathectomy significantly increased the responses to especially lower concentrations of norephedrine in both male and female rats. Maximum response was not altered in male rats, where a maximum response could be obtained in vehicle experiments. Since norephedrine produced clear maximum contractions, potency could be calculated. Potency of norephedrine was significantly greater in aorta from male that in female vehicle treated animals (Table 8.2). Potency of norephedrine was significantly increased by sympathectomy in male animals such that, following sympathectomy, potency was significantly greater in male than in female sympathectomised tissues (Figure 8.4 & Table 8.2).

(±)-Ephedrine produced relatively small contractions in vehicle-treated animals, especially in tissues from female animals, but sympathectomy significantly increased contractions to lower concentrations (10-100 μM) only in male animals (Figure 8.5 & Table 8.1.), although responses also tended to increase in female animals, and the response to the highest concentration was significantly increased by sympathectomy (Table 8.1). Potency of (±)-ephedrine was significantly increased in tissues from sympathectomised male animals as compared to male vehicle and female sympathectomised (Figure 8.5. & Table 8.2). Potency was not calculated for control tissues from female rats due to the very small response. However, (±)-ephedrine had similar potency in vehicle tissues from male and sympathectomised tissues from female animals (Table 8.2).

Although most studies employed (±)-ephedrine, a small number of studies employed (-)-ephedrine. (-)-Ephedrine also produced contractions of rat aorta which tended to be larger in males than in females (Figure 8.6). Interestingly, contractions to (-)-ephedrine

Figure 8.4.

Contractions of aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats produced by the agonist norephedrine. Values are tension (g) and s.e. of mean, with n, number of experiments, in brackets. Asterisks denote responses significantly different in sympathectomised aorta from the respective gender vehicle treated control (* $P<0.05$).

aorta

Figure 8.5. Contractions of aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats produced by the agonist (±)-ephedrine. Values are tension (g) and s.e. of mean, with n, number of experiments, in brackets. Asterisks denote responses significantly different in sympathectomised aorta from the respective gender vehicle treated control (* P<0.05).

Figure 8.6. Contractions of aorta from vehicle treated (veh) male (M) and female (F) Wistar rats produced by the agonists (±)-ephedrine and (-)-ephedrine. Values are tension (g) and s.e. of mean, with n, number of experiments, in brackets. Statistical comparison was not carried out due to low n values for female tissues.

tended to be larger than contractions to (±)-ephedrine in both male and female. Given the small number of experiments, statistical analysis was not carried out.

Contractions to tyramine were similar in tissues from male and female vehicle treated rats, but sympathectomy significantly increased the response to tyramine over a wide range of concentrations from 1 μM to 1000 μM in male but not female tissues (Figure 8.7 & Table 8.1). Potency was calculated and tyramine potency was significantly greater in male sympathectomised as compared to male vehicle and female sympathectomised tissues (Figure 8.7. and Table 8.2), although admittedly no clear maximum was obtained to tyramine in all except male sympathectomised animals (but taking submaximal contractions as maximum would tend to overestimate potency if responses were small).

MDA produced small contractions in both male and female vehicle-treated animals, and sympathectomy did not significantly affect responses (Figure 8.8). Potency was not calculated due to the small responses in all except tissues from male sympathectomised animals.

Hence, the contractions to norephedrine, ephedrine and tyramine were significantly increased by sympathectomy in tissues from male rats, but not in tissues from female rats.

8.3. Contractions to KCl

KCl produced isometric contractions, but there were no significant differences between male and female animals, and no significant effects of sympathectomy (Figure 8.9). However, again responses tended to be increased in sympathectomised animals.

Figure 8.7. Contractions of aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats produced by the agonist tyramine. Values are tension (g) and s.e. of mean, with n, number of experiments, in brackets. Asterisks denote responses significantly different in sympathectomised aorta from the respective gender vehicle treated control (* P<0.05). + indicates responses in tissues from female sympathectomised animals significantly different from the response in male animals from the same treatment group (+ P<0.05).

aorta

Figure 8.8. Contractions of aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats produced by the agonist MDA.   Values are tension (g) and s.e. of mean, with n, number of experiments, in brackets. There were no significant differences.

Figure 8.9. Contractions of aorta from vehicle treated (veh) and sympathectomised (sympX) male (M) and female (F) Wistar rats produced by KCl. Values are tension (g) and s.e. of mean, from 4-10 experiments (see Table 8.1). There were no significant differences.

Table 8.3. Comparison of actions of agonists at producing contractions in rat aorta from vehicle animals (aorta veh) with effects to cause tonic contractions in rat vas deferens from vehicle treated (vas veh) and from sympathectomised animals (vas sympX), and effects to increase DBP in anesthetized sympathectomised rats (approximate pressor response in mmHg) (rise in DBP sympX rats). For fair comparison, maximum concentration compared is 100 μM, and maximum dose is 1mg/kg. Note that ephedrine failed to produce tonic contractions in sympathectomised rat vas deferens, but contracted aorta and produced marked pressor actions.

	aorta veh	vas veh	vas sympX	rise in DBP sympX rats
noreph[1]	++(0.5)	++(0.55)	+++(1.0)	ND
eph[3]	**+ (0.3)**	**++(0.4)**	**0 (0.05)**	**+ (30)**
tyramine[3]	+ (0.2)	+++(0.85)	++(0.55)	0 (10)
MDA[2]	+ (0.25)	++ (0.5)	++(0.48)	ND
MDEA[2]	0	+ (0.13)	+ (0.14)	ND
MDMA[2]	0	++ (0.37)	+ (0.11)	0 (5)
cathinone[2]	0	0 (0.07)	0 (0.02)	0 (5)
cathine[2]	0	+(0.16)	ND	0 (10)
MHA[2]	0	++(0.33)	0 (0.08)	0 (5-10)

ND: not determined.

8.4. Comparison between contractile actions of stimulants in rat aorta and vas deferens and ability to increase DBP in anaesthetized rats.

Table 8.3 compares the ability of stimulants to produce contractions in rat aorta with actions in other tissues: tonic contraction of rat vas deferens and rise in DBP in anaesthetized rat. Since the rat aorta is probably uninnervated, responses found in aorta should be comparable with responses in vas deferens from sympathectomised rats.

Hence, directly acting agonists that contract the aorta should also contract the vas deferens from sympathectomised rats. This was true for norephedrine, tyramine and MDA (Table 8.3). However, (±)-ephedrine contracted rat aorta but not sympathectomised rat vas deferens. This suggests that (±)-ephedrine has direct agonist actions at α_{1D}-adrenoceptors of rat aorta, but not α_{1A}-adrenoceptors of rat vas deferens. This may explain the surprisingly large pressor response seen to ephedrine in sympathectomised rats (see Table 8.3).

8.5. Summary

1. The rat aorta, which is poorly innervated, is useful for the study of direct actions of stimulants at α_{1D}-adrenoceptors. Most stimulants examined produced no or small contractions. Sympathectomy did not reduce contractions to stimulants that produced contractions.

2. Contractions to NA and KCl were not significantly altered by sympathectomy.

3. Contractions to norephedrine, ephedrine and tyramine were significantly increased by sympathectomy in tissues from male rats, but not in tissues from female rats.

4. Hence, there were gender differences in direct actions of agonists norephedrine, ephedrine and tyramine following sympathectomy.

Chapter 9.

Interaction between caffeine and cathinone or MDMA on HR and blood pressure in anaesthetised male and female rats

Chapter 9.   Interaction between caffeine and cathinone or MDMA on HR and blood pressure in anaesthetised male and female rats

Khat chewers do not restrict their input of stimulants to the components of khat, but often chew khat in conjunction with coffee drinking in a social atmosphere. Hence, adverse actions of the khat chewing socialization may also include interaction between caffeine from coffee and the main constituents of khat, especially cathinone. For this reason, the next two chapters look at the interaction between caffeine and cathinone, starting with cardiovascular actions.

In this Chapter, the interaction between prior administration of caffeine (10 mg/kg) and the subsequent tachycardia and blood pressure responses to cathinone or MDMA have been investigated in anaesthetised male and female rats.

9.1. Effects of caffeine and interaction with cathinone and MDMA on HR.

9.1.1. Caffeine

Caffeine (1-10 mg/kg) produced dose dependent increases in HR.   Caffeine (3 mg/kg) produced peak transient increases in HR, increasing HR by 22±4 bpm (n=10) in male and 14±2 bpm (n=7) in female rats.   Caffeine (10 mg/kg) produced peak rises in HR, increasing HR by 44±5 bpm (n=10) in male and 38±3 bpm (n=7) in female rats, but caffeine (10 mg/kg) produced a more sustained increase so that HR was still elevated when cathinone or MDMA was administered (Figure 9.1). There was no difference between male and female rats.

9.1.2. Cathinone

In animals given vehicle (for caffeine) followed by cathinone, cathinone produced

Figure 9.1. Effects of intravenous injection of cathinone (0.001-1 mg/kg), on heart rate in the absence or presence of prior caffeine (10 mg/kg) in pentobarbitone anaesthetized male and female rats. Horizontal lines indicate level of heart rate after prior vehicle (continuous lines) or caffeine (dashed lines), with red for female and blue for male. Error bars indicate s.e. of mean from 6-7 experiments. There were no significant differences.

a dose dependent tachycardia that was similar in male and female animals (Figure 9.1), and was similar to responses obtained in our previous studies (without prior vehicle for caffeine) (see Chapters 3 & 4). Following caffeine, responses to cathinone were diminished, but if responses were plotted in terms of change from baseline prior to vehicle or caffeine, the effects of cathinone were unchanged by caffeine (Figure 9.1). Note that, following caffeine, HR was elevated and this can be seen as the dashed horizontal lines in Fig 9.1. There were no significant gender differences in the interaction between caffeine and cathinone.

9.1.3. MDMA

MDMA was studied only in male rats. In animals given vehicle (for caffeine) followed by cathinone, MDMA produced a dose dependent tachycardia (Figure 9.2), which was similar to responses obtained in our previous studies (without prior vehicle for caffeine) (see Chapters 3 & 4). Following caffeine, responses to MDMA were diminished, but if responses were plotted in terms of change from baseline prior to vehicle or caffeine, the effects of MDMA were unchanged by caffeine (Figure 9.2). Note that, following caffeine, HR was elevated and this can be seen as the dashed horizontal lines in Fig 9.2. There were no significant differences in the interaction between caffeine and MDMA in this study of male rats.

9.2. Effects of caffeine and interaction with cathinone and MDMA on DBP.

Caffeine had only minor effects on blood pressure. Caffeine (3 mg/kg) produced transient decreases in DBP: -6±5 mmHg (n=10) in male and -3±4 mmHg (n=7) in female rats (no significant differences). Caffeine (10 mg/kg) also produced

Figure 9.2. Effects of intravenous injection of MDMA (0.001-1 mg/kg), on heart rate in the absence or presence of prior caffeine (10 mg/kg) in pentobarbitone anaesthetized male rats. Horizontal lines indicate level of heart rate after prior vehicle (continuous lines) or caffeine (dashed lines) . Error bars indicate s.e. of mean from 4-5 experiments. There were no significant differences.

Figure 9.3. Effects of intravenous injection of cathinone (0.001-1 mg/kg), on diastolic blood pressure (DBP) in the absence or presence of prior caffeine (10 mg/kg) in pentobarbitone anaesthetized male and female rats. Please note that DBP was not significantly elevated after prior vehicle or caffeine, but before cathinone. Error bars indicate s.e. of mean from 6-7 experiments. There were no significant differences.

Figure 9.4. Effects of intravenous injection of MDMA (0.001-1 mg/kg), on diastolic blood pressure (DBP) in the absence or presence of prior caffeine (10 mg/kg) in pentobarbitone anaesthetized male and female rats. Please note that DBP was not significantly elevated after prior vehicle or caffeine, but before MDMA. Error bars indicate s.e. of mean from 4-5 experiments. There were no significant differences.

transient decreases in DBP: -15±6 mmHg (n=10) in male and -11±6mmHg (n=7) in female rats (no significant differences). Responses to caffeine had recovered to around baseline before cathinone or MDMA administration.

Caffeine (10 mg/kg) did not affect the small pressor responses produced by administration of cathinone (Figure 9.3). Cathinone responses in the absence of caffeine were similar to responses obtained in Chapters 3 & 4. There was no difference between male and female in the interaction of caffeine with cathinone.

Caffeine (10 mg/kg) did not affect the pressor responses produced by MDMA (Figure 9.4). MDMA responses in the absence of caffeine were similar to responses obtained in Chapters 3 & 4. MDMA was examined in male animals only.

9.3. Summary

1. We have studied gender differences in the interaction between caffeine and cathinone or MDMA in anaesthetized rats.

2. In male and female vehicle treated pentobarbitone anaesthetised rats, the combined effect of caffeine and cathinone on HR was the same as the total effect of cathinone alone.

3. In male vehicle treated pentobarbitone anaesthetised rats, the combined effect of caffeine and MDMA on HR was the same as the total effect of MDMA alone.

4. The pressor response to cathinone or MDMA was unaffected by caffeine.

5. Hence, there were no clear interactions between caffeine and cathinone or MDMA, and no gender differences in the interaction between caffeine and cathinone, in terms of cardiovascular responses.

Chapter 10.

Gender differences in the effects of cathinone and the interaction with caffeine on temperature and locomotor activity in the rat

Chapter 10. Gender differences in the effects of cathinone and the interaction with caffeine on temperature and locomotor activity in the rat

In this study, gender differences in the effects of cathinone and the interaction of cathinone with caffeine on temperature and locomotor activity monitored by radiotelemetry have been investigated in conscious Wistar rats. Animals were implanted with telemetry probes under isoflurane anaesthesia, and 7 days later, temperature and locomotor activity were recorded in conscious unrestrained animals. Caffeine (10 mg/lkg) or vehicle, and 30 min later, cathinone (5 mg/kg) or vehicle were injected subcutaneously.

Results obtained for activity and temperature will be considered separately, beginning with activity, starting with effects in each gender separately, then gender differences.

10.1. Activity: male rats.

All treatments significantly increased total activity (0-275 min) in male rats as compared with the effects of vehicle (one way anova and post-test, P<0.05) (see Table 10.1). Please note that the 5 min sample is a sample covering the period 0-5 min, so that 5-275 min samples means sampling from 0-275 min.

In Figure 10.1, and subsequent activity figures, activity is plotted as units per 5 min interval and in Table 10.2 activity is plotted in 30 min intervals. In terms of time course of changes in activity, activity was increased in male rats even in vehicle/vehicle treated animals by injection, as judged by the peaks in activity around the time of injection. In the subsequent Figures 10.2-10.6, the data from Figure 10.1 is replotted for individual pairs of treatment data, and statistical differences are shown by asterisks.

The next 3 Figures (Figures 10.2-10.4) compare treatments with the effects of vehicle in male rats. Figure 10.2 shows that cathinone significantly increased activity as compared

Table 10.1. Total locomotor activity recordings in conscious male and female rats given vehicle or caffeine (10 mg/kg) at 5 min and vehicle or cathinone (5 mg/kg) at 35 min, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as total activity (0-275 min). Values are mean±s.e. mean total activity from n=7 rats.   Four groups of animal were studied: vehicle/vehicle (veh veh); vehicle/cathinone (veh cath); caffeine/vehicle (caff veh) and caffeine/cathinone (caff cath).

	Interval (min)	veh cath	caff cath	caff veh	veh veh
Male	0-275	27410±4361*	28772±3675*	17851±2086*+	11458±1696
Female	0-275	44418±3231*$	36732±4774*+$	24949±2.885*+$	12942±1248

Symbols denote significance differences:

* $P<0.05$ from vehicle/vehicle of same gender;

+ $P<0.05$ from vehicle/cathinone of same gender;

$ $P<0.05$ from same treatment in male

(one way anova, and Dunnett post test).

Figure 10.1. Locomotor activity recordings in conscious male rats given vehicle or caffeine (10 mg/kg) at 5 min (indicated by first arrow) and vehicle or cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Four groups of animal were studied: vehicle/vehicle (veh veh); vehicle/cathinone (veh cath); caffeine/vehicle (caff veh) and caffeine/cathinone (caff cath). Statistical significance of differences are shown in the following Figures.

Figure 10.2. Locomotor activity recordings in conscious male rats given vehicle at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.3. Locomotor activity recordings in conscious male rats given caffeine at 5 min (indicated by first arrow) and vehicle at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences from veh/veh at any 5 min time point (Two way anova).

Figure 10.4. Locomotor activity recordings in conscious male rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Table 10.2. Locomotor activity recordings at 30 min intervals in conscious male rats given vehicle or caffeine (10 mg/kg) at 5 min and vehicle or cathinone (5 mg/kg) at 35 min, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/30 min). Values are mean±s.e. mean activity/30min from n=7 rats.   Four groups of animal were studied: vehicle/vehicle (veh veh); vehicle/cathinone (veh cath); caffeine/vehicle (caff veh) and caffeine/cathinone (caff cath).

Interval (min)	veh cath	caff cath	caff veh	veh veh
5-35	1031±166	2710±315+	1891±360	2145±398
35-65	3108±482	4914±543*	3015±388	1456±248
65-95	4415±823*	2501±694	2260±308+	991±228
95-125	3656±732*	2830±1088	2194±277	1449±342
125-155	4121±857*	1745±423+	1939±183+	1669±454
155-185	3549±649*	3140±533*	2094±268	1155±288
185-215	2876±583*	3937±814*	1833±233	393±151
215-245	2168±458	3574±627*	1712±309	777±276
245-275	2176±302	3110±671*	1257±259	1197±444

Symbols denote significance differences: * $P<0.05$ from vehicle/vehicle; + $P<0.05$ from vehicle/cathinone (Two way anova, and Bonferroni post test).

to vehicle over a wide range of 5 min time periods in male rats. The vehicle/cathinone group differed significantly from vehicle/vehicle from 65-95 min and 130, 180 and 195 min, and, taking 30 min intervals, at 65-215 min (two way anova and Bonferroni post-test) (Fig. 10.2 & Table 10.2).   The caffeine/vehicle group did not differ significantly from the vehicle/vehicle group at any time point (two way anova and Bonferroni post-test) (Fig. 10.3).

Figure 10.4 shows the complex effect of the combination of caffeine and cathinone in comparison with vehicle in terms of activity in male rats. The combination of caffeine and cathinone had a significantly greater peak response soon after cathinone injection, but caffeine thereafter decreased the effects of cathinone on activity in the time period 60-160 min, with a maintained increased activity after about 175 min (Figure 10.4). The caffeine/cathinone group differed significantly from vehicle/vehicle at 45-55 min and for most points between 180 and 230 min, and, taking 30 min intervals, at 35-65 min and 155-275 min (two way anova and Bonferroni post-test) (Fig. 10.4 & Table 10.2).

The next 2 Figures (Figures 10.5-10.6) compare single treatments with cathinone or caffeine with the combination caffeine/cathinone in male rats. Figure 10.5 shows that, as compared to cathinone alone, the combination of caffeine and cathinone produced a larger peak response on injection of cathinone in male rats, with inhibition of the response from about 60-160 min and a late recovery to the same response as cathinone alone after 175 min. The response to caffeine/cathinone was significantly increased above the response to vehicle/cathinone only at 50-55 min. The combination of caffeine/cathinone significantly increased the response beyond that of cathinone alone in the 30 min period 5-35 min (effects of injection of caffeine) (Fig. 10.5), but significantly decreased the response below that of cathinone alone at 125-155 min (compare data of Table 10.2). This suggests two components to the effects of caffeine on the peak response to cathinone: a direct effect of caffeine and a short-lived facilitation of the effects of cathinone followed by an long-lived inhibition of the response.

Figure 10.5. Locomotor activity recordings in conscious male rats given caffeine at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle and cathinone, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from veh/cath are shown by asterisks above the relevant time points (* $P<0.05$, Two way anova, and post test).

Figure 10.6. Locomotor activity recordings in conscious male rats given caffeine at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given caffeine and vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from caff/veh are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.6 shows that, as compared to caffeine alone, the combination of caffeine and cathinone produced a clear peak response on injection significantly greater than that to caffeine alone in the 10 min period after injection of cathinone in male rats.

10.2. Activity: female rats.

All treatments significantly increased total activity (5-275 min) in female as compared with the effects of vehicle (one way anova and post-test, $P<0.05$) (see Table 10.1).

In Figure 10.7, and subsequent activity figures, activity in female rats is plotted as units per 5 min interval and in Table 10.3, activity is plotted in 30 min intervals. In terms of time course of changes in activity, activity was increased in female rats even in vehicle/vehicle treated animals by injection. In the subsequent Figures 10.8-10.12, the data from Figure 10.7 is replotted for individual pairs of treatment data, and statistical differences are shown by asterisks.

The next 3 Figures (Figures 10.8-10.10) compare treatments with vehicle in female rats. Figure 10.8 shows that cathinone significantly increased activity as compared to vehicle over a wide time range of time periods in female rats. The vehicle/cathinone group differed significantly from vehicle/vehicle from 50-225 min and at 235 min (Figure 10.8) and, taking 30 min intervals, at 35-275 min (Table 10.3)(two way anova and Bonferroni post-test). The caffeine/vehicle group differed significantly from the vehicle/vehicle group at no time point except at the end of recording at 260-265min (Figure 10.9), but taking 30 min intervals, the caffeine/vehicle group was significantly higher at 65-95 min, 185-215 min and 245-275 min (Table 10.3) (two way anova and Bonferroni post-test).

Figure 10.10 shows the complex effect of the combination of caffeine and cathinone in comparison with vehicle in terms of activity in female rats. The combination of caffeine and cathinone had a significantly greater peak response soon after cathinone injection,

Figure 10.7. Locomotor activity recordings in conscious female rats given vehicle or caffeine (10 mg/kg) at 5 min (indicated by first arrow) and vehicle or cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Four groups of animal were studied: vehicle/vehicle (veh veh); vehicle/cathinone (veh cath); caffeine/vehicle (caff veh) and caffeine/cathinone (caff cath). Statistical significance of differences are shown in the following Figures.

Figure 10.8. Locomotor activity recordings in conscious female rats given vehicle at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min(indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.9. Locomotor activity recordings in conscious female rats given caffeine at 5 min (indicated by first arrow) and vehicle at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.10. Locomotor activity recordings in conscious female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

but caffeine thereafter decreased the effects of cathinone on activity to a lower maintained but still increased level of activity after about 100 min in female rats (Figure 10.10). The caffeine/cathinone group differed significantly from vehicle/vehicle at 55-65 min and after 230 min (Fig. 10.10) and, taking 30 min intervals, and most of the time between 35-275 min (Table 10.3) (two way anova and Bonferroni post-test). This is slightly different in shape from the effects in the male rat (compare Figure 10.4, and see below), but overall similar.

The next 2 Figures (Figures 10.11-10.12) compare single treatments with cathinone or caffeine with the combination caffeine/cathinone in female rats. Figure 10.11 shows that, as compared to cathinone alone, the combination of caffeine and cathinone produced a clear peak response on injection of cathinone in female rats, with inhibition of the response from about 60-160 min and a late recovery to the same response as cathinone alone after about 180 min. Caffeine/cathinone was significantly different from vehicle/cathinone at a number of time points between 55 and 165 min (Fig. 10.11) and at the 30 min intervals from 35-185 min (Table 10.3). The combination of caffeine/cathinone significantly increased the response beyond that of cathinone alone only soon after at the injection of cathinone, at 35-65 min, but thereafter caffeine significantly diminished the response to cathinone at 65-185 min (compare data of Table 10.3).

Figure 10.12 shows that, as compared to caffeine alone, the combination of caffeine and cathinone produced a clear peak response on injection of cathinone in female rats, and they were otherwise similar. Hence, in female rats the effects of caffeine/cathinone differed significantly from those of caffeine alone in a 25 min period a little after cathinone injection.

10.3. Activity: comparison of male and female rats.

Total activity (0-275min) was significantly increased in female rats as compared with male rats for all except vehicle groups (one way anova and post-test $P<0.05$) (see Table 10.1). Three way anova also showed a significant effect of gender ($F=13.6$, $P<0.001$) (see also below).

Table 10.3. Locomotor activity recordings at 30 min intervals in conscious female rats given vehicle or caffeine (10 mg/kg) at 5 min and vehicle or cathinone (5 mg/kg) at 35 min, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/30 min). Values are mean±s.e. mean activity/30min from n=7 rats. Four groups of animal were studied: vehicle/vehicle (veh veh); vehicle/cathinone (veh cath); caffeine/vehicle (caff veh) and caffeine/cathinone (caff cath).

Interval (min)	veh cath	caff cath	caff veh	veh veh
5-35	1967±125	2603±450	2909±522	2050±216
35-65	4695±532*	6639±1080*+	3252±485	1901±145
65-95	6768±665* ^{X}	4863±978*+ ^{X}	2822±546*+	1382±205
95-125	6585±500* ^{X}	3606±521+	2937±390+	1484±241
125-155	6425±587* ^{X}	3595±283*+	2452±316+	1528±335
155-185	5989±610* ^{X}	3805±572*+	2614±329+	1394±266
185-215	4842±526* ^{X}	4059±867*	2635±279*+	675±175
215-245	3826±339*	3738±682*	2479±254	1101±228
245-275	3075±285*	3507±552*	2654±171*	1017±263

Symbols denote significance differences:

* $P < 0.05$ from vehicle/vehicle;

+ $P < 0.05$ from vehicle/cathinone;

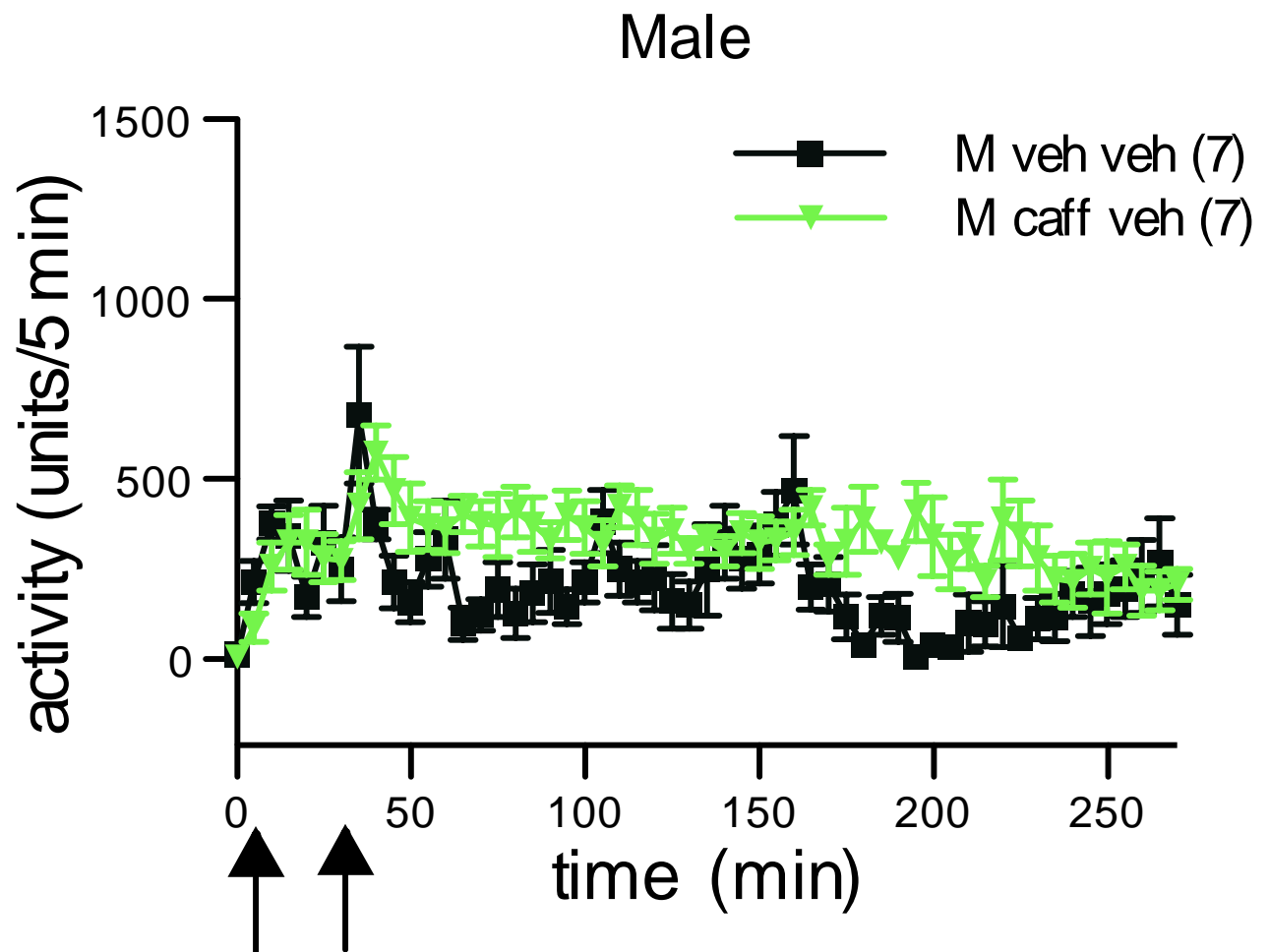^{X} $P < 0.05$ from equivalent treatment in male rats, comparing with data in Table 10.2 (Two way anova, and Bonferroni post test).
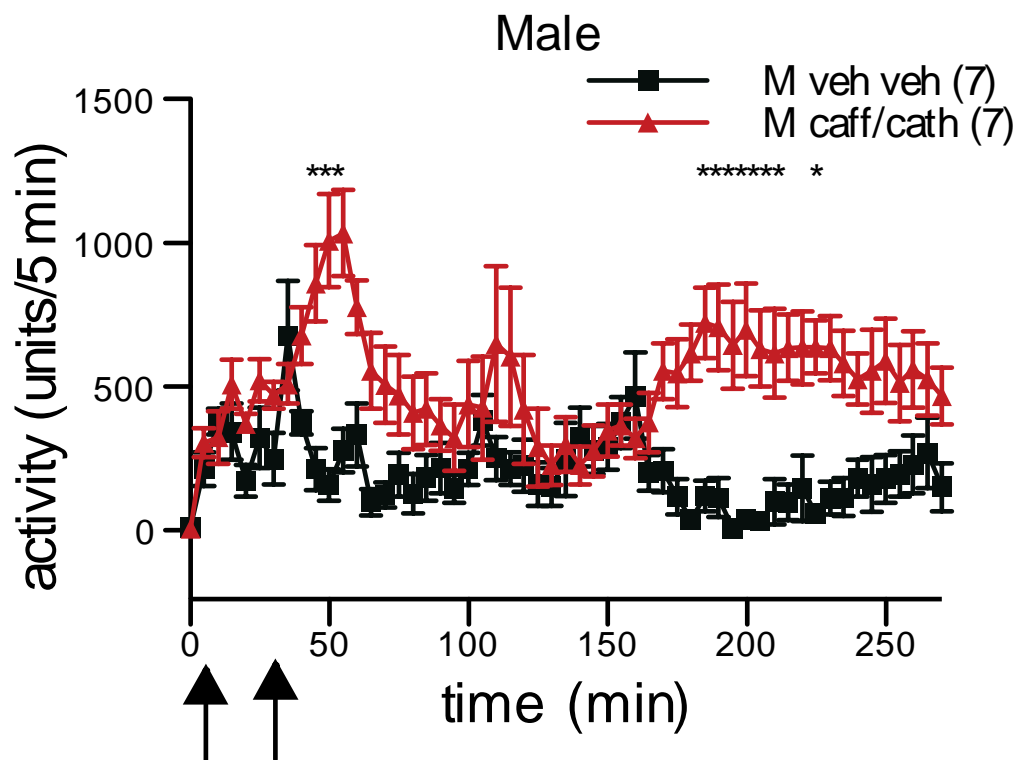
Figure 10.11. Locomotor activity recordings in conscious female rats given caffeine at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle and cathinone, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from veh/caff are shown by asterisks above the relevant time points (* $P < 0.05$, Two way anova, and post test).

Figure 10.12. Locomotor activity recordings in conscious female rats given caffeine at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given caffeine and vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from caff/veh are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Three way anova compared pretreatment (vehicle or caffeine), treatment (vehicle or cathinone) and gender. There was a significant interaction between caffeine and cathinone, pooling gender for the total sampling period 5-275min (F= 7.40, P<0.01) and this was also true for both sub-periods 5-140 min (F=6.03, P<0.05) and 140-275 min (F=5.63, P<0.05). This reflects the major actions of caffeine to inhibit the increase in activity produced by cathinone in both male and female rats. However, it should be remembered that three way anova compares, for example, all caffeine with, for example, all cathinone. Hence, 3 way anova produced significance for the most marked effect seen in the activity studies. Interestingly, interaction between gender and cathinone, pooling caffeine (P<0.05), did not quite reach significance for total sampling period 5-275 min (F=3.24, P=0.078), nor for the sub-period 5-140 min (F=3.72, P=0.059, not quite significant) or the sub-period 140-275 min (F=1.60, P=0.218) or 155-275 min (F=0.74, P=0.39). But this again is because all cathinone results are compared in 3 way anova, with or without caffeine.

Figures 10.13-10.16 show pairs of comparisons between male and female rats. Responses to vehicle or caffeine did not differ between male and female rats (Figure 10.13 & 10.14). The response to cathinone was qualitatively similar in male and female rats, but cathinone produced significantly greater total activity in female rats (Table 10.1, one way anova), but reached significance at only one 5 minute timepoint (Figure 10.15). In terms of 30 min intervals, vehicle/cathinone produced significantly greater activity in female rats than in male rats for the 30 min time intervals between 65-215 min (Table 10.3). The combination of caffeine and cathinone had similar effects in male and female rats: an initial peak on injection of cathinone, and a subsequent fall in activity, but the initial peak was significantly greater in females (Figure 10.16). Caffeine/cathinone produced a significantly greater activity in female rats than in male rats only for the time period 65-95 min (Table 10.3). There were no significant differences between males and females

Figure 10.13. Locomotor activity recordings comparing conscious male and female rats given vehicle at 5 min (indicated by first arrow) and vehicle at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences between male and female at any 5 min time point (Two way anova).

Figure 10.14. Locomotor activity recordings comparing conscious male and female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and vehicle at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences between male and female at any 5 min time point (Two way anova).

Figure 10.15. Locomotor activity recordings comparing conscious male and female rats given vehicle at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences between male and female are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.16. Locomotor activity recordings comparing conscious male and female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min). Vertical bars indicate the s.e. mean from 7 rats. Significant differences between male and female are shown by asterisks above the relevant time points (* $P<0.05$, Two way anova, and post test).

in terms of 30 min time intervals for vehicle/vehicle or caffeine/vehicle (compare Tables 10.2 and 10.3).

10.4. Baseline temperature

There were no significant differences between groups within gender in baseline body temperature at time zero prior to drug injection. Baseline body temperature was 37.96±0.16, 38.00±0.26, 38.02±0.09 & 37.81±0.30 $^{\circ}$C for male rats in the vehicle/cathinone, caffeine/cathinone, caffeine/vehicle and vehicle/vehicle groups, respectively (n=7). Baseline body temperature was 38.44±0.12, 38.49±0.12, 38.48±0.14 & 38.35±0.15 $^{\circ}$C for female rats in the vehicle/cathinone, caffeine/cathinone, caffeine/vehicle and vehicle/vehicle groups, respectively (n=7).

However, baseline body temperature was significantly higher in female (38.44±0.06 $^{\circ}$C, n=28) than in male (37.94±0.10 $^{\circ}$C, n=28) rats (P<0.05).

10.5. Temperature: male

Table 10.4 shows temperature results in male rats as total area under the curve (AUC) for the time period 0-275 min. A negative value indicates that the overall AUC response was a fall in temperature below baseline. The temperature total AUC response in male rats to caffeine/vehicle and caffeine/cathinone were significantly greater than the response to vehicle/vehicle (one way anova and Bonferroni test, P<0.05)(Table 10.4). The temperature AUC response to caffeine/cathinone and caffeine/vehicle were significantly greater than the response to cathinone alone (Table 10.4).

Temperature responses are expressed as change in temperature from baseline, time zero (Figure 10.17). The time course of temperature changes produced by vehicle/cathinone in male rats did not differ significantly from vehicle/vehicle (2

Table 10.4. Total area under the curve (AUC) for temperature obtained in conscious male and female rats given vehicle or caffeine (10 mg/kg) at 5 min and vehicle or cathinone (5 mg/kg) at 35 min, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as total AUC (0-275 min): a negative value indicates that the total response was a fall in temperature below baseline. Values are mean±s.e. mean from n=7 rats. Four groups of animal were studied: vehicle/vehicle (veh veh); vehicle/cathinone (veh cath); caffeine/vehicle (caff veh) and caffeine/cathinone (caff cath).

	Interval (min)	veh cath	caff cath	caff veh	veh veh
Male	0-275	-66.5±44	91.9±20.9*+	66.2±23.5*+	-100.3±28.3
Female	0-275	44.4±34.3*$	73.6±18.7*	36.3±36.2*	-71.3±21.4

Symbols denote significance differences:

* $P<0.05$ from vehicle/vehicle of same gender;

+ $P<0.05$ from vehicle/cathinone of same gender,

$ $P<0.05$ from same treatment in male

(one way anova, and Bonferroni or Dunnett post test).

Figure 10.17. Core body temperature recordings in conscious male rats given vehicle or caffeine (10 mg/kg) at 5 min (indicated by first arrow) and vehicle or cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Four groups of animal were studied: vehicle/vehicle (veh veh); vehicle/cathinone (veh cath); caffeine/vehicle (caff veh) and caffeine/cathinone (caff cath). Statistical significances of differences are shown in the following Figures.

way anova, NS) (Fig. 10.18). Caffeine/vehicle differed significantly from the vehicle/vehicle group only towards the end of the sampling at 210-220 min and 235 min (Figure 10.19) (two way anova and Bonferroni test, P<0.05). In contrast, caffeine/cathinone produced a large increase in temperature soon after cathinone injection, falling back to a lower level after about 120 min in male rats, and this was significantly different from vehicle/vehicle at 45-125 min and again at 275 min (2 way anova, P<0.05) (Fig. 10.20). Hence, the combination of caffeine and cathinone were additive and produced a significant rise in temperature in the 90 min after cathinone injection.

Figure 10.21 shows that the combination of caffeine and cathinone produced a marked effect on temperature in male rats in the hour after cathinone injection, so that prior caffeine then cathinone caused a significant rise in temperature that was not seen in the absence of caffeine (Figure 10.21). Figure 10.22 shows that, as compared to cathinone alone, the combination of caffeine and cathinone produced a marked rise in temperature that was significant over the 5 min intervals between 30-125 min. Hence, Cathinone alone had no effect on temperature in male rats, but the combination with caffeine produced a marked rise in temperature that was significant especially after injection of cathinone (Figure 10.22), and had two components: effects of caffeine and potentiation of cathinone by caffeine.

10.6. Temperature: female

Table 10.4 shows temperature results in female rats as total area under the curve (AUC) for the time period 5-275 min. A negative value indicates that the overall AUC response was a fall in temperature below baseline. The temperature AUC responses to all treatments in female rats were significantly different from the response to

Figure 10.18. Core body temperature recordings in conscious male rats given vehicle at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences from vehicle/vehicle at any 5 min time point (Two way anova).

Figure 10.19. Core body temperature recordings in conscious male rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and vehicle at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.20. Core body temperature recordings in conscious male rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* $P<0.05$, Two way anova, and post test).

Figure 10.21. Core body temperature recordings in conscious male rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given caffeine and vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Significant differences from caff/veh are shown by asterisks above the relevant time points (* $P<0.05$, Two way anova, and post test).
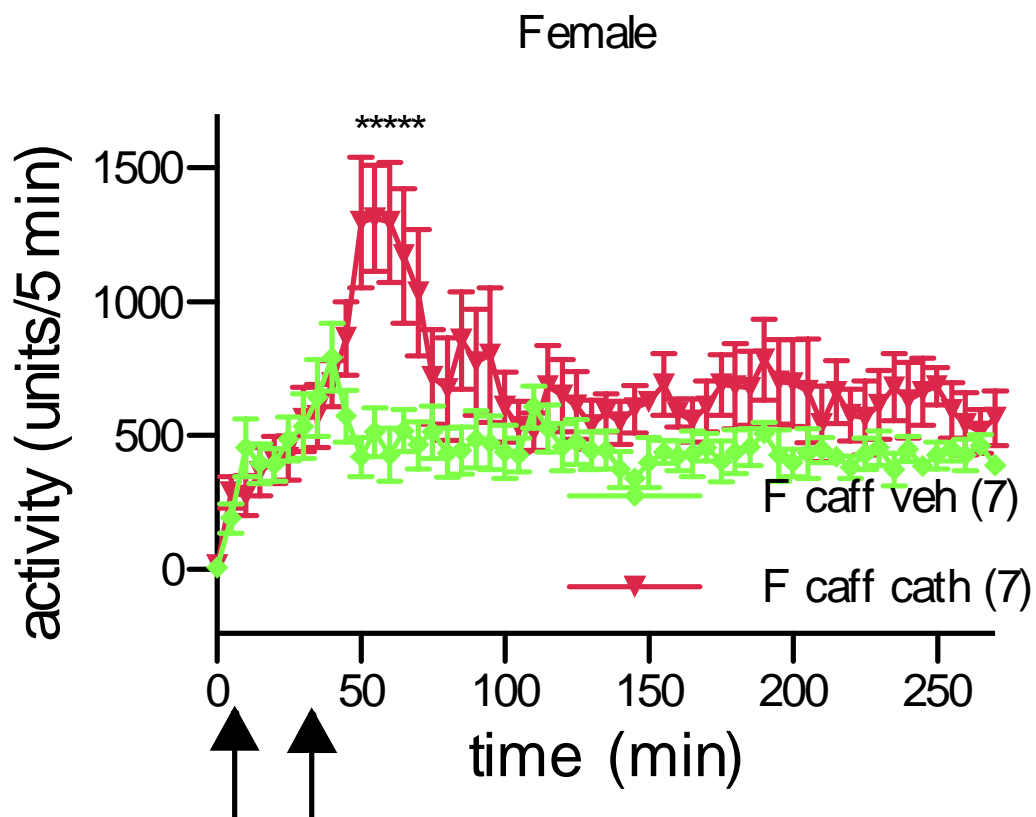
Figure 10.22. Core body temperature recordings in conscious male rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle and cathinone, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Significant differences from veh/cath are shown by asterisks above the relevant time points (* $P<0.05$, Two way anova, and post test).

vehicle/vehicle (one way anova and Dunnett test, P<0.05) (Table 10.4). The temperature AUC response to caffeine/vehicle or caffeine/cathinone were not significantly different from the response to cathinone alone.

Temperature responses are expressed as change in temperature from baseline, time zero (Figure 10.23). The time course of temperature changes produced by vehicle/cathinone was significantly different from vehicle/vehicle only towards the end of the sampling period, at 165-190 and 205-225 min (2 way anova, P<0.05) (Fig. 10.24) and was significantly different in one way anova (Table 10.4). However, although it tended to raise temperature, caffeine alone did not significantly increase temperature at any 5 min time point (Figure 10.25). Likewise caffeine/cathinone produced rises in temperature that were significantly different from vehicle/vehicle only towards the end of the sampling period, at 245-275min (2 way anova, P<0.05) (Fig. 10.26).

Figure 10.27 shows that the combination of caffeine and cathinone produced a similar small effect on temperature in female rats as caffeine alone, and in fact the traces were almost superimposable, with no significant differences for any 5 min time period (2 way anova). Figure 10.28 shows that, as compared to cathinone alone, the combination of caffeine and cathinone produced a similar time course of response in female rats except for an early peak response to caffeine injection in the combined group, but this did not reach significance in 5 min sample periods.

10.7. Temperature: comparison of male and female rats.

Table 10.4 shows temperature results in male and female rats as total area under the curve (AUC) for the time period 5-275 min. The temperature AUC response in female rats to cathinone alone was significantly greater than the response in male rats (one way anova and Bonferroni test, P<0.05) (Table 10.4; see also Figure 10.31 below).

Figure 10.23. Core body temperature recordings in conscious female rats given vehicle or caffeine (10 mg/kg) at 5 min (indicated by first arrow) and vehicle or cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Four groups of animal were studied: vehicle/vehicle (veh veh); vehicle/cathinone (veh cath); caffeine/vehicle (caff veh) and caffeine/cathinone (caff cath). Statistical significance of differences are shown in the following Figures.

Figure 10.24. Core body temperature recordings in conscious female rats given vehicle at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats.   Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).
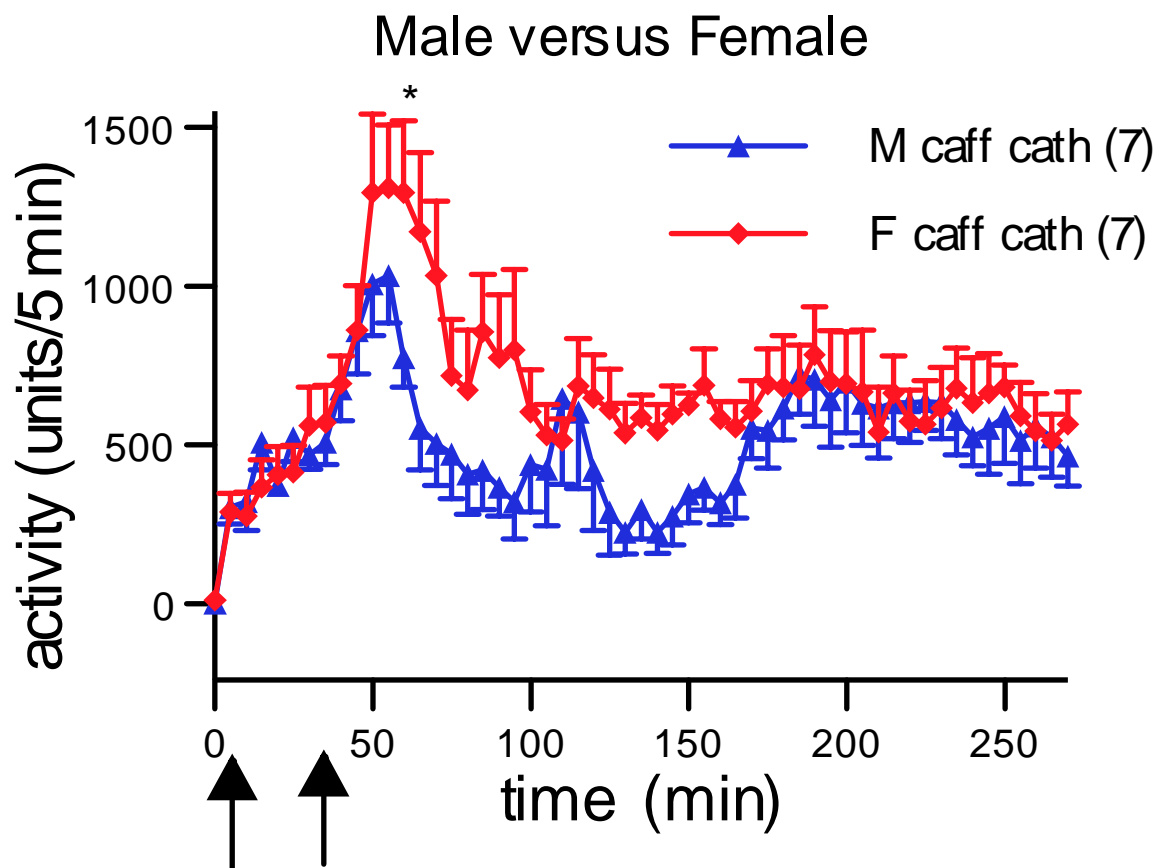
Figure 10.25. Core body temperature recordings in conscious female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and vehicle at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences from vehicle/vehicle at any 5 min time point (Two way anova).

Figure 10.26. Core body temperature recordings in conscious female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle/vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.27. Core body temperature recordings in conscious female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given caffeine and vehicle, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences between caff/veh and caff/cath at any 5 min time point (Two way anova).

Female

Figure 10.28. Core body temperature recordings in conscious female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared to animals given vehicle and cathinone, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences between veh/cath and caff/cath at any 5 min time point (Two way anova).

Three way anova comparing pretreatment (vehicle or caffeine), treatment (vehicle or cathinone) and gender showed a significant interaction between gender and caffeine, pooling cathinone, for the time period 0-275 min (F=5.04, P<0.05). This interaction occurred in the first 140 min of recording, since there was a significant interaction between gender and caffeine, pooling cathinone, for the time period 0-140 min (F=8.92, P<0.01), but not for the period 140-275 min (F=1.67, P=0.20). This significant interaction is not particularly helpful, since, looking ahead to Figures 10.30 and 10.32 (as combined in Figure 10.33), it is clear that the male/female difference occurs in the caffeine/cathinone groups and not in the caffeine/vehicle groups. Hence, Figure 10.33 explains more than the 3 way anova results.

There was also a significant interaction between caffeine and cathinone, pooling gender, for the period 140-275 min (F=4.58, P<0.05) but not for the earlier time period or total recording time. However, it should be remembered that Three way anova compares, for example, all caffeine with, for example, all cathinone. Caffeine has consistent effects in all groups of animal, but cathinone had inconsistent effects. Cathinone only increased temperature acutely in one group (male caffeine/cathinone)(see below). Hence, yet again the interaction occurred in the caffeine/cathinone group in males, and this is more clear in Figure 10.33.

Figures 10.29-10.32 show pairs of comparisons in terms of temperature actions between male and female rats. Responses to vehicle or caffeine alone did not differ significantly between male and female rats, and were virtually superimposable (Figure 10.29 & 10.30). The response to cathinone was qualitatively similar in male and female rats with almost no effect in males, but cathinone produced a significant rise in temperature in female as compared to male rats soon after cathinone injection (Figure 10.31). However, the total response (0-275 min) to injection of cathinone was significantly greater in female than male rats, and it should be remembered that this response was significantly different from the respective vehicle controls only in female rats (Table 10.4).

Figure 10.29. Core body temperature recordings comparing conscious male and female rats given vehicle at 5 min (indicated by first arrow) and vehicle at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences between male and female at any 5 min time point (Two way anova).

Figure 10.30. Core body temperature recordings comparing conscious male and female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and vehicle at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. There were no significant differences between male and female at any 5 min time point (Two way anova).
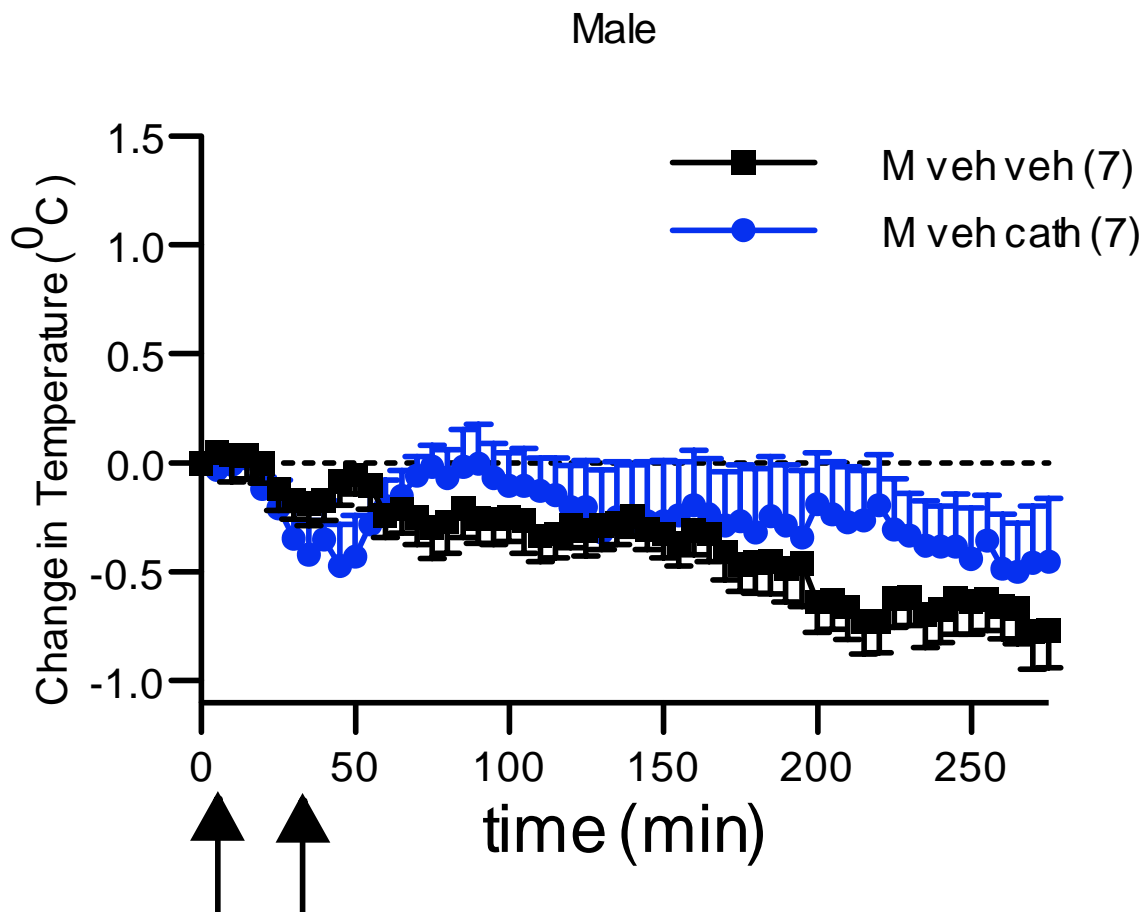
Male versus Female

Figure 10.31. Core body temperature recordings comparing conscious male and female rats given vehicle at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Significant differences between male and female are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

The combination of caffeine and cathinone had similar overall effects in male and female rats, except there was a clear short-lived rise in temperature on injection of cathinone only in male rats from about 60-120 min that was significant at the 5 min interval at 65 min, and thereafter responses were similar in male and female animals (Figure 10.32). Combining Figure 10.30 with Figure 10.32 shows that the effects of caffeine alone was similar in male and female animals, the effects of caffeine with cathinone were the same as caffeine alone for female rats, but the combination in male rats was different from the other three responses (see Figure 10.33). Hence, there was an interaction between caffeine and cathinone to cause hyperthermia in male rats that did not occur in female rats.

10.8. Comparison between time course of change in locomotor activity with time course of change in temperature

Vehicle/cathinone produced marked increases in locomotor activity in both male and female rats. For female rats, locomotor activity was significantly increased by cathinone (injected at 35 min) from 50 min until virtually the end of sampling, but temperature was only significantly increased in the second half of the sampling period (Figure 10.34). The increase in temperature occurs subsequent to the increase in activity in female rats, and may be as a result of it.

For male rats, the increase in activity produced by vehicle/cathinone (injected at 35 min) was significantly less that that for female rats, and was only continually significant between 60-90 min. There was no significant rise in temperature (Figure 10.35). Hence, in male rats, an increased activity to cathinone was not matched by an increased temperature, although admittedly the increase in activity was less than that for female rats.
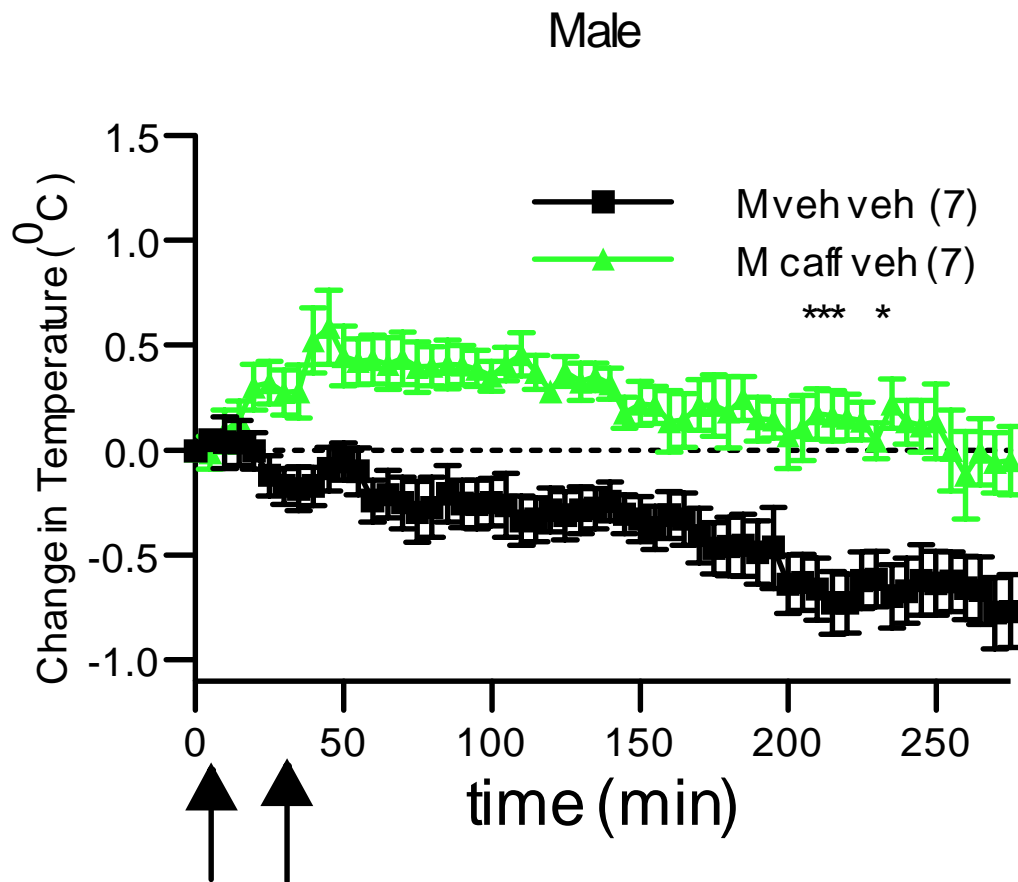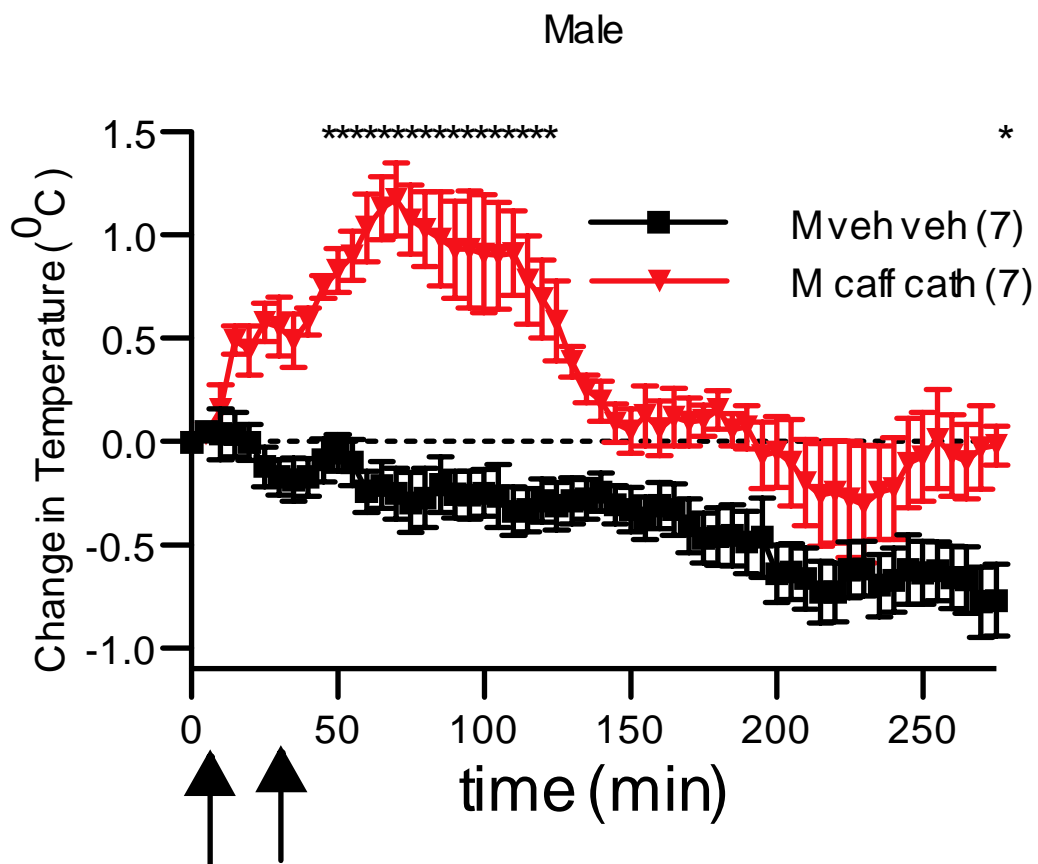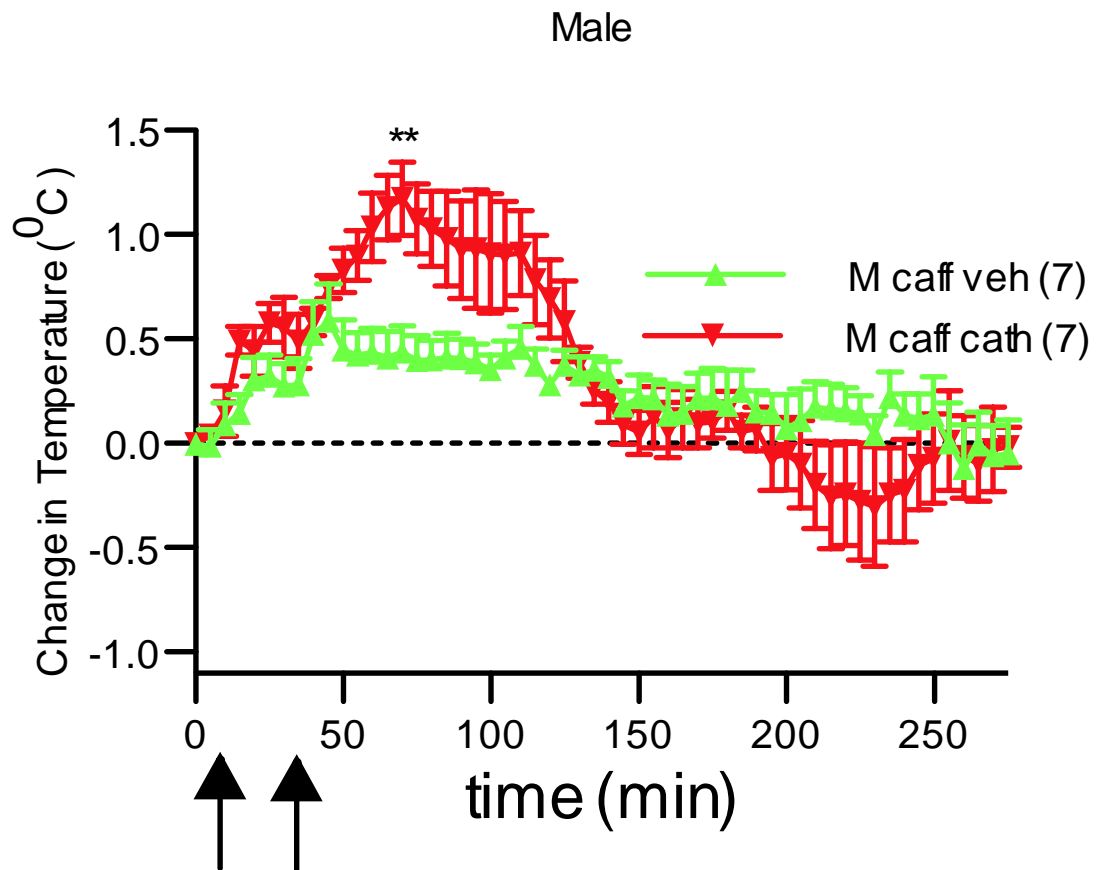
Figure 10.32. Core body temperature recordings comparing conscious male and female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Significant differences between male and female are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).
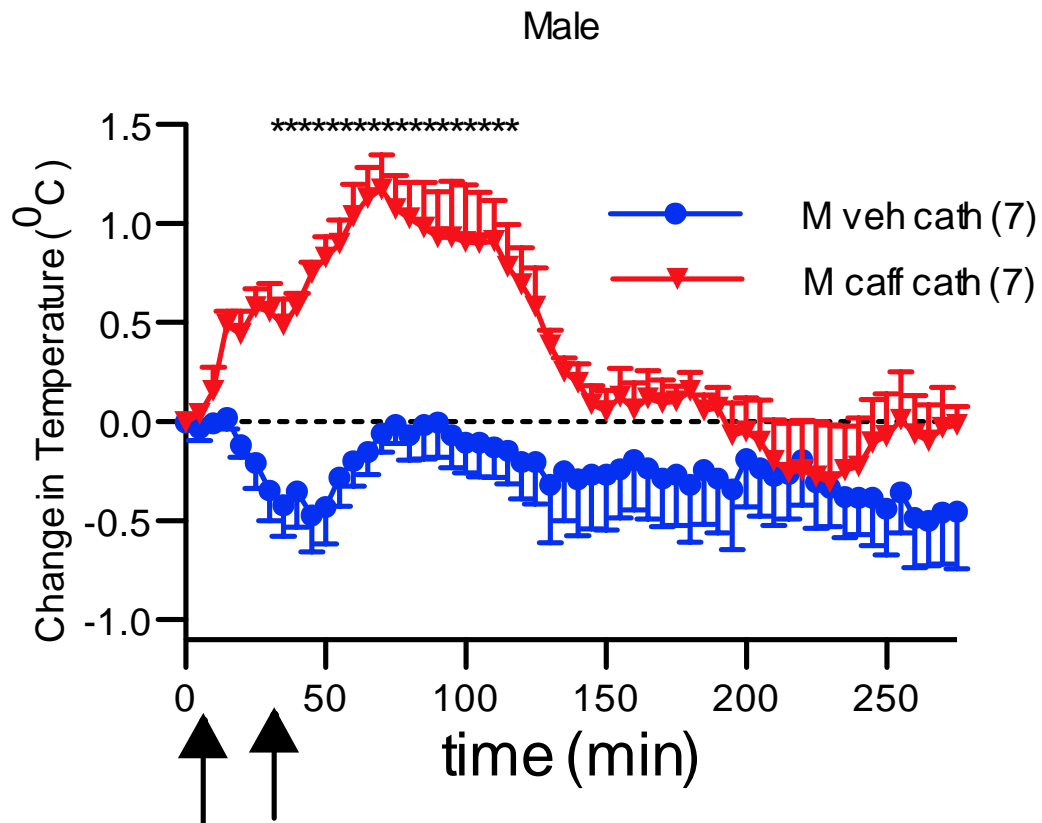
Figure 10.33. Core body temperature recordings comparing conscious male and female rats given caffeine (10 mg/kg) at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), compared with animals given caffeine alone, at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as change in body temperature from body temperature at time zero. Vertical bars indicate the s.e. mean from 7 rats. Significant differences from caff/vehicle of the relevant gender are shown by asterisks above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.34. Locomotor activity and core body temperature recordings in female rats given vehicle at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min) (left hand axis) and change in body temperature from body temperature at time zero (right hand axis). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by colour-coded asterisks (red: activity; green: temperature) above the relevant time points (* P<0.05, Two way anova, and post test).

Figure 10.35. Locomotor activity and core body temperature recordings in male rats given vehicle at 5 min (indicated by first arrow) and cathinone (5 mg/kg) at 35 min (indicated by second arrow), at room temperature of 22°C. All drugs or vehicles were administered s.c. Responses are expressed as activity (units/5 min) (left hand axis) and change in body temperature from body temperature at time zero (right hand axis). Vertical bars indicate the s.e. mean from 7 rats. Significant differences from vehicle/vehicle are shown by colour-coded asterisks (blue: activity) above the relevant time points (* P<0.05, Two way anova, and post test).

For other treatments, the comparison of time courses between locomotor activity and temperature changes were less clear, and can be seen by comparing the relevant activity and temperature figures.

Hence, only for female rats following vehicle/cathinone is there evidence for a delayed rise in temperature following markedly increased locomotor activity.

10.9. Summary

1. Gender differences have been shown to occur in the effects of stimulants, including khat, in humans, but female animal models have been rarely studied.

2. Gender differences in the effects of cathinone (from khat) and the interaction with caffeine on temperature and locomotor activity have been investigated in Wistar rats.

3. Caffeine alone produced a minor increase in locomotor activity and produced small but significant increases in temperature over the full time course. Cathinone produced significant and marked increases in locomotor activity, and the response to cathinone was significantly greater in female animals. The combination of caffeine and cathinone caused a short lived potentiation followed by a prolonged inhibition of the locomotor response to cathinone.

4. Cathinone alone did not significantly affect temperature in male animals, and produced a small slow prolonged rise in female animals. However, the combination of caffeine and cathinone produced a significant acute rise in temperature in male rats from 10 min until 95 min after cathinone injection. The effect was not seen in female rats.

5. Hence, cathinone causes greater increases in locomotor activity in female than in male rats. Secondly, although cathinone alone had slow effects on temperature in female rats, in combination with caffeine it significantly raised temperature acutely in male but not female rats.

6. These differences highlight the need to carry out gender studies of the actions of stimulants.

Chapter 11.

Discussion

Chapter 11. Discussion

The various aspects of the findings of this thesis will be discussed before entering a final round up of the implications of these findings. This will include a justification of the methodology and drugs and drug doses employed. Cardiovascular studies of the various amphetamine-like stimulants will be discussed further, followed by isolated tissues studies in aorta and vas deferens, and finally interaction between caffeine and cathinone in cardovascular and telemetry studies. This discussion will begin by looking at the mode of action of stimulants and at the effectiveness of sympathectomy in these studies.

11.1. Mode of action of stimulants

Stimulants such as cocaine and amphetamine derivatives have a variety of actions but these actions tend to overlap through usually multiple actions on the monoaminergic systems including: (1) block of the NA, serotonin or dopamine transporter; (2) indirect actions to stimulate monoaminergic receptors through the release of the neurotransmitter; (3) direct stimulation of monoamine receptors; (4) stimulation of presynaptic, usually inhibitory, monoaminergic receptors. Monoamines can be released by indirectly acting agents by two mechanisms: 1) reverse transport and 2) increase in cytoplasmic NA by release from vesicles (Rudnick and Clark, 1993; Rudnick, 1997). A further thing to consider is that drugs may act as antagonist (blocking agent) or substrate (carried into the cell) at both cell membrane and vesicular transporters. A combination of the two actions (cell membrane and vesicular) may be necessary for maximum indirect actions by displacement of neurotransmitter (Trendelenburg, 1990).

Since this study examines largely peripheral actions, and since the sympathectomy dosing schedule acts mainly peripherally, discussion can be restricted to the

noradrenergic system.   These actions would be: (1) actions at the neuronal membrane transporter for noradrenaline (NET); (2) actions at the synaptic vesicular monoamine transporter (VMAT-2); (3) release of NA from nerve terminals to cause indirect stimulation of adrenoceptors, an action that may involve steps 1 or 2 above; (4) direct agonist stimulation at α- and/or β-adrenoceptors.   Levels of NA in the synaptic cleft can be increased by a) actions at NET and b) release from synaptic vesicles (Rudnick and Clark, 1993; Rudnick, 1998). One of the major objectives of this thesis is to identify the relative importance of direct and indirect actions of the stimulants including cathinone and MDMA in comparison with the classical indirect sympathomimetic tyramine.

11.2. Effectiveness of sympathectomy

Chemical sympathectomy is commonly carried out using 6-OHDA or reserpine. They have different modes of action: destruction of nerve terminals and depletion of vesicular neurotransmitter, respectively.   More recently, knock-out mice have been used as a further method of causing 'sympathectomy" (see Liles et al., 2007). In our laboratory, 6-OHDA has normally been used. However, there are results to suggest that 6-OHDA is the superior method. In rats, pretreatment with 6-OHDA, but not reserpine, decreased basal blood pressure (Furukawa et al., 1988). In conscious rats, NPY induced potentiation of pressor responses to NA was abolished by pretreatment with 6-OHDA, not reserpine (Lopez et al., 1989). In dog mesenteric artery, 6-OHDA, but not reserpine, abolished nerve-evoked contractions (Muramatsu et al., 1984) and in rat vas deferens, the potentiation of nerve-stimulation evoked contractions by capsaicin was abolished by 6-OHDA and not reserpine. In the studies of this thesis, 6-OHDA was employed to produce chemical sympathectomy.

Before considering the results with test agents, the effectiveness of sympathectomy has to be considered.   The treatment schedule with 6-OHDA employed to produce chemical sympathectomy in the present study did not produce obvious sedation, suggesting that central actions were minimal and effects were mainly peripheral (see

Kostrzewa & Jacobowitz, 1974). If depletion of NA by sympathectomy significantly reduced responses to an agonist, this should indicate involvement of release of NA in that response.   However, it cannot be concluded definitively that a response remaining following sympathectomy is due to direct actions as the degree of sympathectomy may not be 100% and may vary from animal to animal. However, comparison between agonists may suggest actions that are probably direct for some agonists. In the present study, the residual tachycardia to tyramine following sympathectomy was significantly greater than that to cathinone, suggesting possible direct actions of tyramine (see later).

An attempt was made to obtain a quantitative measure of the degree of sympathectomy in each individual animal, since biochemical measures may tell little about functional responses. An 80% or even 95% depletion of NA content in a tissue tells us not very much about the immediate stores available for release in nerve terminals. Nerve stimulation-evoked responses were initially employed to quantify the degree of sympathectomy. A previous study found that the contractile response of vas deferens from sympathectomised rats to single pulse nerve stimulation was reduced to 34.4% of that found in tissues from control animals (Cleary et al., 2004). In the present study, the response in vas deferens from sympathectomised rats to single stimulus was fairly similar at 49% of that of control animals. Trains of pulses at 1Hz were also examined, and perhaps surprisingly, the response to the second to tenth pulse at 1 Hz was not smaller with sympathectomy. The contractile responses obtained in rat vas deferens, suggest on average a partial sympathectomy, but the anaesthetized rat results suggest a high degree of sympathectomy. The discrepancy may be due to the dense innervation of the vas deferens, so that even a high degree of sympathectomy does not markedly reduce nerve responses, perhaps coupled to postjunctional supersensitivity where the postjunctional smooth muscle cells are more responsive to the neurotransmitter. In addition, the rat vas deferens is very complicated in that is has two major neurotransmitters: NA (adrenergic) and ATP (purinergic) (Brown et al.,1983). Nerve-mediated single pulse responses in rat vas deferens involve two components, an early non-adrenergic mainly prostatic component mediated by ATP and involving purinergic receptors (see Brown et al., 1983), and a later adrenergic mainly epididymal component involving α_{1D}-

adrenoceptors (see Aboud et al., 1993; Honner & Docherty, 1999; Bexis et al., 2008). The response to the first pulse in a train is partly adrenergic, but the response to the second and subsequent pulses became increasingly purinergic. However, nerve-evoked responses of vas deferens proved really surprisingly resistant to sympathectomy, with only the response to a single stimulus significantly reduced. It is possible that the presence of the purinergic co-transmitter also affects the response to sympathectomy. The results with trains of pulses may point at smaller adrenergic but unchanged purinergic response. It was not the purpose of this thesis to investigate the complex responses of rat vas deferens, it was to be used as a functional assay of degree of sympathectomy and it did not prove particularly useful so use of vas deferens nerve response was stopped.

Fortunately, evidence of sympathectomy could be found by examining responses to agonists both *in vivo* and *in vitro*, and the effects of the dosing regime to produce sympathectomised rats was relatively consistent. In anaesthetized rat studies, there were clear effects of sympathectomy, as follows. Very stable blood pressure, both systolic and diastolic, reduced resting blood pressure, and reduced resting HR (at least in males) were observed. Secondly, pressor responses to tyramine were very clearly altered from a large pressor response in control animals to virtually no pressor response in sympathectomised. Hence, in the sparsely innvervated vasculature, sympathectomy causes very clear and consistent effects, in both male and female rats, and this difference from control is sufficient to quantify sympathectomy.

In addition, as will be reported, in rat aorta, a tissue with sparse innervation, most stimulants, including cathinone, MDMA, cathine, MHA, and MDEA failed to produced contractions, and only norephedrine and to a lesser extent ephedrine, MDA and tyramine produced marked contractions, and these contractions were at the very least resistant to sympathectomy. Hence, the rat aorta will be used as a tissue to look at mostly direct actions of stimulants.

11.3. Cardiovascular actions of cathinone, MDMA and tyramine in anaesthetised rats

In the initial studies in this thesis of cardiovascular responses in anaesthetized rats, the modes of action of the stimulants cathinone and MDMA were established, comparing to tyramine in the cardiac and blood pressure actions. Anaesthetised animals were studied to minimize baroreflex bradycardia to pressor responses. Blood pressure and HR were measured, but DBP was chosen rather than SBP, as DBP is more dependent on vascular actions, whereas SBP reflects both vascular and cardiac actions. DBP is commonly measured to quantify vascular actions of drugs and has been widely used for this in our laboratory (see e.g. Seto et al., 2010). However, the use of a β-adrenoceptor antagonist gave results that would confirm the choice of DBP as the more reliable measure of vascular actions (Alsufyani & Docherty, 2015). This will be discussed at the appropriate point in discussion of results below (Section 11.3.1).

In this study, three strategies were used to assess cardiovascular responses to cathinone, MDMA and tyramine; propranolol to confirm β-adrenoceptor mediated actions, and cocaine and chemical sympathectomy to assess direct or indirect sympathomimetic actions. The results obtained confirmed β-adrenoceptor involvement in tachycardia, and demonstrated that chemical sympathectomy is a better strategy than uptake blockade with cocaine to assess direct or indirect effects. These and other points are discussed below.

11.3.1. Choice of cocaine and propranolol

Cocaine was chosen as a blocker of NET as work in our laboratory showed that the other very commonly used NA transporter blocker desipramine has actions as antagonist of $\alpha-$adrenoceptors in doses used to block uptake (Docherty, 2014). Cocaine was chosen at a dose of 1 mg/kg, because work in our laboratory has looked at cocaine in the anaesthetised rat (McDaid & Docherty, 2001). That study found that cocaine (1 mg/kg) did not affect resting DBP, but cocaine (10 mg/kg)

significantly reduced DBP and so has a depressant action on the cardiovascular system. Cocaine also caused a significant increase in resting HR, confusing effects of agonists following cocaine, especially for agonists like tyramine that cause marked tachycardia.

Propranolol was chosen as β-adrenoceptor antagonist for it is a commonly used nonselective β-adrenoceptor antagonist, and has been used for this in our laboratory (Al Zubair et al., 2008). Many β-adrenoceptor antagonists have actions as α_1-adrenoceptor antagonists that may complicate interpretation of actions (see Brahmadevara et al., 2004). For instance cyanopindolol has actions at α_1-adrenoceptors with a pK_i (-log M) of 6.33, and even propranolol has a pKi of 5.80 (Brahmadevara et al., 2004). To avoid actions at α_1-adrenoceptors propranolol (1 mg/kg) was used in most studies, but as said below, higher doses were used in some studies. In these studies, propranolol significantly reduced the pressor response to tyramine in terms of SBP but not DBP (Chapter 3; Alsufyani & Docherty, 2015). This demonstrates that part of the rise in SBP, but not DBP, to tyramine is due to cardiac β-adrenoceptor stimulation.

11.3.2. Cardiovascular responses to cathinone, MDMA and tyramine

Both cathinone and MDMA produced tachycardia in anaesthetized rats, but cathinone was approximately 10 times more potent than MDMA, and had 5 times higher potency than tyramine. The larger tachycardia seen to high doses of tyramine may reflect both indirect and direct actions, given that the response was only partially affected by sympathectomy. However, blood pressure actions suggest an apparent anomaly: cathinone and tyramine are sympathomimetics with similar potencies in terms of tachycardia, but only tyramine produced marked pressor responses as an indirect sympathomimetic and these were abolished by sympathectomy. However, sympathectomy had much more effect on the tachycardia to cathinone and MDMA than on that to tyramine. This suggests different overall modes of action for cathinone and tyramine, and perhaps that the tachycardia to tyramine involves both direct and indirect components.

The lower potency of MDMA (and perhaps cathinone) at the vesicular transporter may explain differences from tyramine: actions on NET may be more important than vesicular displacement in releasing or potentiating NA in the densely innervated heart, but the combination of both may be crucial in the sparsely innervated vasculature, and differences in actions as substrate and/or inhibitor at the transporters may contribute.   Tyramine also differs from cathinone and MDMA in that it poorly penetrates into the brain, but central actions are probably less important in terms of cardiovascular actions in the pentobarbitone anaesthetized rat.

Propranolol almost abolished the tachycardia to cathinone and MDMA, demonstrating that these actions are β-adrenoceptor mediated. There is evidence that propranolol blocks the effect of MDMA in rats (Schindler et al., 2014) and pindolol is effective in man (Hysek et al., 2010). Although a marked tachycardia remained to the highest dose of tyramine (10 mg/kg) following β-adrenoceptor blockade with propranolol (1 mg/kg), this is probably due to a competitive interaction by high doses of tyramine to overcome the antagonism.

11.3.3. Cardiovascular actions in relation to mechanism of action

Amphetamine-type agents are reported to act as substrates or even inhibitors of NET and cause reverse transport (Mandela & Ordway, 2006). Cathinone and MDMA may increase NA potency through action at NET in the rat right ventricle (Al Sahli et al., 2001), and are probably not substrates but inhibitors of NET (Cleary et al., 2003). Other studies suggest than MDMA may passively enter the nerve terminal to cause carrier mediated release of NA (Schmidt et al., 1987; Wang et al., 1987). Tyramine may both block the transporter, cause reverse transport (Berg & Jensen, 2013) and interact with the vesicular monoamine transporter (VMAT-2), causing release and increased cytoplasmic concentration of neurotransmitter (Partilla et al., 2006). Tyramine is approximately 30 times more potent than MDMA at inhibiting uptake into vesicles (Partilla et al., 2006), and MDMA is more potent at

blocking NA re-uptake (0.9 μM) (Cleary & Docherty, 2003) than at vesicular VMAT-2 (19.5 μM: Partilla et al., 2006). Cathinone had similar potency to MDMA at NET (0.9 μM) (Cleary & Docherty, 2003), and yet is more potent than MDMA in the present study of HR responses. Tyramine blocks catecholamine uptake at around 1 μM (Horn, 1973) and is therefore of similar potency to cathinone and MDMA as an inhibitor of NET. Another study found a similar potency for MDMA (0.36 μM) at NET (Rickli et al., 2015). The potencies of the stimulants in the above functional studies compare fairly well with their potencies in the anaesthetised rat in the present study (around 0.1 mg/kg) for tachycardia but not for pressor responses. Tyramine had similar potency at inhibiting uptake into vesicles (VMAT-2) (0.59 μM: Partilla et al., 2006) as at NET, whereas MDMA was much less potent at vesicular VMAT-2 (19.5 μM: Partilla et al., 2006; 36.3 μM: Yasumoto et al., 2009). Cathinone and MDMA probably act mainly at NET (Schmidt et al., 1987; Wang et al., 1987; Al-Sahli et al., 2001; Cleary & Docherty, 2003), but tyramine may act both on NET and the vesicular monoamine transporter (VMAT-2), in the doses used in the present study (Partilla et al., 2006; Berg & Jensen, 2013). In the heart with the densely innervated sino-atrial node, the two modes of indirect action may be important for a maximum tachycardia, and one possible site of gender differences could be at one of these sites (see later).

11.3.4. Cardiovascular actions of stimulants and effects of cocaine

Cocaine (1 mg/kg) had very little effect on pressor and tachycardiac responses to the 3 sympathomimetic agents in the anaesthetized rat, with only the tachycardia to tyramine significantly reduced (although cocaine also increased baseline HR), but this was not due to a failure of cocaine in this dose to block NET. Work from our laboratory has previously showed that cocaine (0.1 mg/kg), by blocking the transporter, significantly increases pressor responses to NA (Killian & Docherty, 2014), and cocaine (1 mg/kg) produces a marked potentiation. However, a high dose of desipramine (approximately 13 mg/kg) blocks the tachycardia and pressor response to tyramine (Berg & Jensen, 2013), and this may agree with the present

findings for the interaction of cocaine and tyramine. McDaid & Docherty (2001) found a larger pressor response to MDMA (1 mg/kg) than in the present study and that cocaine (10 mg/kg) reduces the pressor response to MDMA (5 mg/kg), but this dose of cocaine also significantly reduced blood pressure, implying possible depressant actions at this high dose. The only difference found in the current study of anaesthetised rats from that of McDaid & Docherty (2001) was the relatively weak pressor response to MDMA in doses of up to 1 mg/kg. Furthermore, cocaine (5 mg/kg) increases HR by about 50 bpm (see Chapter 3). Hence, effects of cocaine, especially in high doses, on baseline HR and blood pressure compromise interpretation of effects. Even so, cocaine did not have the effects of markedly blocking responses to stimulants. Perhaps, there are two opposing actions of cocaine in this situation: block of the transporter to reduce entry of the stimulant, but also block of the transporter to potentiate the actions of released NA. In addition, competition between cocaine and say cathinone for the transporter may have reduced the effectiveness of cocaine.

11.3.5. Cardiovascular actions in relation to human studies

This study examined cathinone and MDMA in doses of up to 1 mg/kg: similar doses produce cardiovascular effects in human studies. Cathinone is the main active component of khat leaves and has been studied both *in vivo* and *in vitro* to prove that the alkaloid acts as a weak amphetamine-like agent (Kalix, 1990; Brenneisen et al.,1990; Kalix et al.,1991). The typical duration of consuming khat is 3-4 hours with a consumption of almost 300g of leaves (Kalix,1994; Nencini et al.,1989). The dose of cathinone in khat leaves was compared to the dose of pure cathinone and showed similar effect with only a slower peak plasma concentration of cathinone from khat leaves due to its slow absorption (Widler et al., 1994). A study on 4 volunteers who chewed khat with a mean dose of 45 mg of cathinone (about 0.5 mg/kg) showed an increase in blood pressure after 3 hours of use (Toennes et al., 2003), and another study showed an increase in systolic and diastolic blood pressure and tachycardia that lasted for 4 hrs (Widler et al., 1994). The tachycardia to khat was blocked by the β_1-adrenoceptor antagonist atenolol while the pressor

response was resistant to the α_1-adrenoceptor antagonist indoramin (Hassan et al., 2005).

MDMA is reported to increase arterial blood pressure (Gouzoulis et al.,1993; Grob et al.,1996; Vollenweider et al., 1998) and it has been consistently shown that MDMA (1 mg/kg and above) can increase HR (Hayner and McKinney,1986; Bedford Russell et al., 1992; Mas et al.,1999; Kolbrich et al., 2008). MDMA has similar actions to cocaine in producing cardiovascular complications through the effect on NA (Al-Sahli et al., 2001) in addition to 5-HT (Liechi and Vollenweider, 2001; Tancer and Johanson, 2007).

11.3.6. Summary of cardiovascular actions in anaesthetized male rats

The results obtained confirmed β-adrenoceptor involvement in tachycardia, and demonstrated that chemical sympathectomy virtually abolished pressor responses to most agonists, and abolished depressor responses to tyramine, showing that these actions are largely indirect.

In conclusion, these studies have demonstrated, employing sympathectomy, that the tachycardia to cathinone and MDMA in the anaesthetized rat is mediated predominantly by release of NA. Cocaine proved ineffective as an agent to determine direct or indirect actions of a stimulant. Results obtained confirmed β-adrenoceptor involvement in tachycardia, and demonstrated that chemical sympathectomy virtually abolished all pressor responses showing that these actions are largely indirect. The tachycardia to tyramine was at least partly resistant to sympathectomy, suggesting some direct actions (Alsufyani and Docherty, 2015).

11.4. Cardiovascular actions: gender studies

The initial study of male rats employed control animals in comparison with sympathectomised. The gender study repeats this previous study in the case of the male rat data, with the difference that control rats were vehicle treated in the present study but untreated in the previous study. In the second study comparing male and female animals, vehicle-treated animals were compared with sympathectomised. It was first of all possible to examine whether the methodology of the initial studies and the methodology of the later studies produced similar results (control untreated animals or vehicle-treated animals). Fortunately, responses obtained in the initial study of untreated male rats (Chapter 3; Alsufyani and Docherty, 2015) were very similar to responses obtained in the second study in vehicle treated rats (Chapter 4; Alsufyani & Docherty, 2016). Results obtained in sympathectomised male rats were also very similar in the two studies (compare Figures in Chapter 3 & 4; see Alsufyani and Docherty 2015, 2016). However, the main object of the present study was to investigate male and female rats for possible gender differences in the actions of cathinone.

11.4.1. Gender differences in cardiovascular actions of cathinone. MDMA and tyramine

The second study of cathinone, MDMA and tyramine investigated male and female rats for possible gender differences. Too many pharmacological studies have examined male animals only, even though these studies may be of less relevance to the actions of stimulants in the general human population. Stimulants such as cathinone, in the form of khat, and MDMA are consumed by both males and females (see Nakajima et al., 2014), but there is little evidence from basic studies of possible gender differences in pharmacological effects.

In the present gender study, there were no differences between male and female in the tachycardia to cathinone or MDMA, or in the pressor reponses of any of the agonists, including tyramine. Nor was there any significant difference in the late depressor response to tyramine. The major findings concerned the tachycardia to

tyramine. The potency of tyramine at producing a tachycardia was significantly greater in male than in female rats. Since, relative to the potency of cathinone, tyramine was significantly less potent in female but not in male rats in terms of tachycardia, this suggests that the difference is that tyramine potency at producing a tachycardia is reduced in female rats.   However, it must be allowed that dose response curves to tyramine especially in female rats did not reach a clear maximum so that the difference between male and female rats in the tachycardia to tyramine could be a diminished maximum response. Hence, there were gender differences in the tachycardia to tyramine but not to cathinone and MDMA.

However, the EC_{50} for tyramine in producing tachycardia in male rats was 38.9 μg/kg but 245 μg/kg in producing pressor responses, and so tyramine was 6 times more potent in producing tachycardia. This may suggest that actions at NET, at which tyramine has lower potency, are more important in terms of indirectly mediated pressor and depressor responses than in terms of indirectly mediated cardiac responses.

Since sympathectomy affected the cardiac responses to cathinone and MDMA to the same degree in male and female rats, and both agonists had similar actions in vehicle treated rats, it can be argued that there were no gender differences in the effect of sympathectomy. Hence, in studies of tyramine, sympathectomy presumably had similar effects, but abolished a difference between males and females in the tachycardia to tyramine.

In conclusion, there were no gender differences in the pressor response to any agent, but gender differences were found in the tachycardia to tyramine, but not to cathinone or MDMA. These differences probably relate to the different modes of indirect actions between amphetamine like agents such as MDMA and cathinone, and other agents such as tyramine. However, the main conclusion is that for the widely used recreational drugs cathinone (in khat) and MDMA there was no evidence for gender differences in the cardiac actions.

11.4.2. Cardiovascular actions: gender studies of other stimulants: cathine, MHA, ephedrine

In addition to studies of cathinone, MDMA and tyramine, further studies were carried out comparing the cardiovascular actions of a number of other stimulants in male and female rats (Chapters 5 & 6). These stimulants were cathine, MHA and ephedrine.

Cathine was fairly potent at producing a tachycardia, with effects at 0.01 mg/kg, whereas MHA was about 10 times less potent. Sympathectomy virtually abolished the tachycardia to MHA, but a small component of the tachycardia to cathine was resistant to sympathectomy, at least in male rats. Pressor responses to both agents were small so that it is difficult to say much, although the pressor response to cathine were resistant to sympathectomy in male but not female rats. Otherwise, there were no gender differences in response to these agents.

For ephedrine both ($\pm$)-ephedrine and (-)-ephedrine were studied. There were differences between the two. Pressor responses to (-)-ephedrine were significantly larger that those to ($\pm$)-ephedrine in both male and female animals and this action was largely direct. Depressor responses were similar between (-)- and ($\pm$)-ephedrine. These comparisons suggest that the major direct pressor actions of ephedrine, acting as a direct agonist presumably on α_1-adrenoceptors in the vasculature, involve mainly the (-)-isomer of ephedrine, but that the depressor, presumed β_2-adrenoceptor mediated, actions involve both isomers. The gender studies with ephedrine found no difference in peak pressor responses nor peak depressor responses between males and females. However, depressor responses were significantly increased by sympathectomy for both (-)- and ($\pm$)-ephedrine and for both genders, but since sympathectomy did not increase the depressor response to the direct agonist isoprenaline, this difference must be due to loss of a pressor component. This was demonstrated in the time course of blood pressure response studies. Hence, the rapid peak pressor response to ephedrine is largely directly mediated, but the time course of the pressor response over the first minute has an indirect component. Since pressor responses in the rat involve both α_{1A}-

and α_{1D}-adrenoceptors (Docherty, 2012), the pressor actions of ephedrine could involve two subtypes or one subtype for both components, or a different subtype for the direct and indirect components. This was true for both (-)- and (±)-ephedrine. Results obtained in rat vas deferens and aorta (see later) would suggest that the direct component may be α_{1D}-adrenoceptor and indirect component α_{1A}-adrenoceptor mediated.

For cardiac β_1-adrenoceptor actions to cause tachycardia, the (-)-isomer had a largely direct effect as judged by resistance to sympathectomy, whereas presumably the (±)-ephedrine isomer has a partly indirect effect. Hence, sympathectomy significantly reduced the tachycardia to (±)- but not (-)-ephedrine, again suggesting that the actions of (+)-ephedrine are largely indirect, in this case on HR. In terms of gender differences, only the tachycardia to (±)-ephedrine was significantly reduced in female as compared to male rats. Interestingly, as was found for the mixed agonist tyramine, the tachycardia to the mixed agonist (±)-ephedrine was significantly reduced in female as compared to male animals, but this difference was abolished by sympathectomy. Hence, there are gender differences in a component of the indirect actions of these agonists, whereas indirect actions of cathinone or MDMA do not show gender differences.

Ephedrine is reported variously to be an indirectly acting or a mixed agonist. However, Liles et al., 2007) demonstrated that pressor responses to ephedrine were not impaired in dopamine β-hydroxylase knock out mice, mice that are unable to synthesis NA (Liles et al., 2007). Despite this, the present studies of ephedrine in vas deferens would suggest that it's actions, at least at α_{1A}-adrenoceptors is indirect by release of NA. Furthermore, the present *in vivo* blood pressure studies suggest there in an indirect component to the blood pressure effect. Ephedrine continues to stimulate interest in its actions in terms of the relationship between direct and indirect actions of this long used compound.

11.4.3. Actions of stimulants at NET and VMAT-2

Amphetamine-type agents are reported to act as substrates or even inhibitors of NET and cause reverse transport (i.e. release of NA: Mandela & Ordway, 2006). Cathinone and MDMA may increase NA potency through action at NET in the rat right ventricle (Al Sahli et al., 2001), and are probably not substrates but inhibitors of NET (Cleary & Docherty, 2003). Cathinone had similar potency to MDMA (0.9 μM: Cleary & Docherty, 2003) and tyramine (1 μM: Horn, 1973) at NET (0.9 μM) (Cleary & Docherty, 2003), and yet is more potent than MDMA in the present study of HR responses. Another study found a similar potency for MDMA (0.36 μM) at NET (Rickli et al., 2015). The potencies of the stimulants in the above functional studies compare fairly well with their potencies in the anaesthetised rat in the present study (around 0.1 mg/kg) for tachycardia but not for pressor responses. Tyramine had similar potency at inhibiting uptake into vesicles (VMAT-2) (0.59 μM: Partilla et al., 2006), whereas MDMA was much less potent at vesicular VMAT-2 (19.5 μM: Partilla et al., 2006; 36.3 μM: Yasumoto et al., 2009).

This study has demonstrated that cardiovascular actions of cathinone are similar in male and female rats, and the same was true for MDMA. Hence, agents like cathinone, with mechanisms of actions largely involving NET were not subject to gender differences in cardiovascular actions. However, tyramine, as an agent acting directly on the vesicular transporter, showed gender differences in tachycardia but not pressor responses, and these differences were abolished by sympathectomy. This suggests gender differences in responsiveness at the level of the vesicular transporter. These differences probably relate the different modes of indirect actions between amphetamine like agents such as MDMA and cathinone, and other agents such as tyramine and also (±)-ephedrine.
Although not directly relevant to cardiovascular actions, striatal dopamine release in response to methamphetamine was found to be greater in male than female mice (Dluzen & McDermott, 2008).

11.4.4. Reverse transport

The monoamine transporters NET, DAT and SERT are neurotransmitter/sodium symports, that is they carry sodium and a monamine molecule into the cell, and so cause a depolarization. Chloride follows passively, so that in the transport cycle it is thought that one cation monamine molecule, two sodium ions and one chloride ion may be transported (see Sitte & Freissmuth, 2015). Vesicles are docked by the synaptic vesicle protein synaptogyrin-3 at the plasma membrane monoamine transporter to ensure efficient uptake of the neurotransmitter into the nerve membrane straight into the synaptic vesicle (Egana et al., 2009).

Amphetamine induces efflux of monoamines by reverse transport, but also inhibits the transporter as a substrate. Since the entry of the monoamine causes entry of sodium ions, blockade of the Na^+/K^+ ATPase will result in increased intracellular levels of sodium ions and results in reverse transport. However, how amphetamine causes reverse transport is not fully understood: it requires amphetamine, elevated sodium ion levels, and sufficient levels of the monoamine transmitter inside the membrane (see Sitte & Freissmuth, 2015). Other stimulants such as MDMA and cathinone presumably have similar modes of action.

11.4.5. Actions of monamines at uptake$_2$ transporters

In addition to the transporters for monoamines in nerve terminals, often termed uptake$_1$ (the neuronal monoamine transporters NET, DAT, SERT), there are transporters in other tissues that may have a role in the removal of neurotransmitters, and these extraneuronal effects are often termed uptake$_2$. The plasma membrane monoamine transporter (PMAT) is an organic cation transporter that transports a number of biogenic amines, and is important as an uptake$_2$ transporter, especially in removing biogenic agents from the cerebral spinal fluid into the blood (Wang, 2016). The organic cation transporter OCT3 is present in the periphery, particularly in salivary glands to cause accumulation of biogenic amines in saliva, fortuitously of use in drug monitoring (Wagner et al., 2016). PMAT shows selectivity for 5-HT and dopamine, whereas OCT3 shows selectivity for NA (Wang,

2016).   Corticosterone, long used as an uptake$_2$ blocker (Trendelenburg, 1966), has high affinity for OCT3 but low affinity for PMAT (Wang, 2016).   These transporters, termed uptake$_2$ but involving multiple transporters including OCT3 and PMAT, have a role in inactivation of monoamines, but uptake$_1$ is probably more important in the periphery (see Docherty & McGrath, 1980).   In the CNS, PMAT is important in removing monoamines from the CSF and so controls the entry of monoamines into the brain (Wang, 2016).   It is not clear what role these uptake$_2$ transporters have in the actions of indirect sympathomimetics, and this was not investigated in this thesis.

11.5. Rat vas deferens

The main objectives of the rat vas deferens studies was first to ascertain that this model was suitable for the study of the direct and indirect effects of stimulants, and second to identify stimulants with differing modes of actions which could then be investigated in the anaesthetized rat model and third to correlate data on stimulants between the several models and at least be able to relate *in vitro* findings to *in vivo* findings.

11.5.1. Components of the contractile response of rat vas deferens

In rat vas deferens, agonists produce two types of contraction, phasic (or spikely intermittent) contractions which are superimposed on the tonic (maintained) contractions. Tonic responses involve mainly α_{1A}-adrenoceptors, whereas phasic contractions involve mainly α_{1D}-adrenoceptors (Docherty, 2014).   Phasic contractions to agonists were much more resistant to sympathectomy than tonic contractions (see also Cleary et al., 2004).   This may be because the α_{1D}-adrenoceptors are near nerve terminals so that even diminished amounts of displaced NA can activate these receptors.   Secondly, spontaneous phasic contractions occur more frequently in sympathectomised rats, suggesting spontaneous release of small amounts to NA to act on these α_{1D}-adrenoceptors

Since the rat vas deferens is densely innervated (Dahlstrom et al., 1966), it would be expected that an indirect sympathomimetic would produce contractions in vehicle treat animals. A direct agonist at α_{1A}-adrenoceptors would also produce tonic contractions of the vas deferens. Hence, sympathectomy can identify components of the response which may be direct, or more correctly can demonstrate actions that are indirect. Responses remaining after sympathectomy should be largely direct.

For NA, it can be shown that the increase in the contractile effects of low concentrations of NA produced by sympathectomy in vas deferens from vehicle animals is mimicked by uptake blockade with cocaine. Sympathectomy significantly increased the response to low concentrations of NA in the absence of cocaine, but in the presence of cocaine, this did not occur. This suggests that in sympathectomised rats, the response to NA is increased due to lack of NET. Hence, the response to any direct agonist subject to marked uptake by NET should be increased: this was true for NA and presumably for norephedrine for the same reason.

Hence, the major effect of sympathectomy in rat vas deferens seems not to be postjunctional supersensitivity, but loss of NET. For NA, its high potency allowed full concentration response curve to a maximum be carried out so that shifts in potency can be quantified. However, for most of the agonists examined a maximum response could not be attained, so that changes in contractile response could be due to a change in potency, a change in shape of the curve, or a change in maximum response.

11.5.2. Phasic contractions of rat vas deferens

Phasic contractions to agonists were much more resistant to sympathectomy than tonic contractions. All agonists including cathinone produced phasic contractions in vehicle-treated animals as well as sympathectomised animals, suggesting that

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these were more easily elicited by agonists. This may be because the α_{1D}-adrenoceptors are near nerve terminals so that even diminished amounts of displaced NA can activate these receptors. Work from our laboratory has previously reported that cocaine increases prazosin potency in rat vas deferens by increasing the α_{1D}-adrenoceptor mediated component to the contraction (Docherty, 2013). Phasic contractions were little affected by sympathectomy, rather in the way that nerve-evoked contractions were little affected. Secondly, spontaneous contractions occur more frequently in sympathectomised rats. If spontaneous contractions occurred in control animals, these did not normally affect interpretation of responses, as these were usually much smaller than those produced by agonists. However, spontaneous contractions occurred very commonly in sympathectomised animals, and these were sometimes 25-50% or more of the total contraction produced by an agonist, making interpretation of results difficult. This was a major reason for choosing tonic contractions in these studies.

However, even using phasic contractions, there was evidence that phasic contractions to a direct agonist were increased by sympathectomy: this was true for NA (low concentrations) and for norephedrine and tyramine. For other agonists, the phasic response was largely unchanged by sympathectomy.   Hence, even when examining phasic contractions of rat vas deferens, the evidence was that NA, norephedrine and tyramine had mainly direct actions.

11.5.3. Tonic contractions of rat vas deferens

Tonic contractions are, based on the results presented above, clearly of more relevance to our studies of direct and indirect sympathomimetics. Hence, analysis will concentrate on tonic contractions.   In rat vas deferens, it would be expected that the effects of agonists with largely direct actions would be either unchanged or increased by sympathectomy, and for those with largely indirect actions, responses would be decreased by sympathectomy. This was most clear for tonic contractions in which only NA and norephedrine, and to a lesser extent tyramine had increased or at least maintained responses and so had mainly direct actions.

Cathinone (up to 100 μM) produced almost no tonic contraction of vas deferens from vehicle treated animals, suggesting both lack of indirect actions and lack of agonism at α_{1A}-adrenoceptors. However, in the anaesthetised rat, cathinone was also relatively weak at raising blood pressure but potent at producing tachycardia. This suggests that the very weak (if at all) α_1-adrenoceptor contractile effects seen in this study of rat vas deferens mimic the very weak α_1-adrenoceptor pressor actions in the anaesthetised rat.

MDMA produced similar tonic contractions to tyramine in rat vas deferens (compare effects of 100 μM) but whereas sympathectomy virtually abolished contractions to MDMA, it merely reduces tonic contractions to tyramine, suggesting that tyramine has additional direct actions. Tonic contractions to MDA and MDEA were largely unaffected by sympathectomy, suggesting small direct actions. MHA produced tonic contractions of rat vas deferens that were almost abolished by sympathectomy, suggesting largely indirect actions, and cathine only produced very small tonic contractions in vehicle tissues. Norephedrine produced tonic contractions that were increased by sympathectomy, suggesting direct actions, whereas ephedrine produced contractions that were abolished by sympathectomy, suggesting indirect actions. Of all agonist responses examined, only the tonic response to norephedrine (and low concentrations of NA) was actually significantly increased by sympathectomy. This may suggest not only largely direct actions but that norephedrine is a substrate for uptake so that sympathectomy increased the response to norephedrine by reducing its disposal by NET.   For NA this did not happen (or at least not significantly at the maximum response), but if responses to NA were compared at a submaximal level of 3 or 10 μM a significant difference was obtained.

Of the agents examined only cathinone failed to produce tonic contractions in vehicle treated animals. NA was most potent, then tyramine and MDMA were about equipotent. Tonic contractions to MDMA, ephedrine and MHA were virtually abolished by sympathectomy, and tonic contractions to tyramine were reduced by

sympathectomy. Only for NA and norephedrine, were tonic contractions significantly increased by sympathectomy.

Hence, only for NA and norephedrine were tonic contractions significantly increased by sympathectomy. This suggests that norephedrine is a direct agonist in vas deferens, and, like NA, may also be a substrate for uptake. Tonic contractions to MDA and MDEA were unaffected by sympathectomy and tonic contractions to tyramine were only partially reduced by sympathectomy, suggesting that these agents are also largely direct agonists.

Possible involvement of trace amine receptors in responses to stimulants can also be considered. In the present study of rat vas deferens, the large contractions to tyramine were virtually abolished by prazosin 10^{-7}M, and the relatively small contractions to MDMA were abolished by sympathectomy. These results provide no evidence for actions of these stimulants at trace amine receptors in rat vas deferens.

In conclusion, in rat vas deferens, the effects of agonists with largely direct actions were either unchanged or increased by sympathectomy, but for those with largely indirect actions responses were decreased by sympathectomy. This was most clear for tonic contractions in which only NA and norephedrine, MDA and MDEA, and to a lesser extent tyramine had mainly direct actions. Phasic contractions were more resistant to sympathectomy, suggesting that these were more easily elicited by agonists, and did not give such clear results. Since responses to norephedrine were increased in a manner similar to those of low concentrations of NA, norephedrine may additionally be a major substrate for NET.

11.6. Rat aorta

The responses of aorta were investigated from male and female Wistar rats to a series of stimulants with and without sympathectomy.

The rat aorta is reported to have a very sparse innervation, if any (see Patil et al., 1972). Cocaine (3 μM) did not increase the potency of NA. In contrast, in tissues such as the densely innervated rat vas deferens, cocaine increases potency and maximum contraction to NA in a concentration-dependent manner, with significant effects employing cocaine 3 μM (Docherty, 2014).

Previous studies have reported contractions of rat aorta to cathinone, but only at concentrations of 100 μM and above (Broadley et al., 2013). That same study reported contractions to MDMA in concentrations of 30 μM and above (see Broadley et al., 2013). Lemarre et al. (2012) reported that cocaine, especially in high concentrations potentiated contractions of rat aorta by a mechanism not involving NA transporter inhibition. In that study, responses to lower concentrations of agonists, rather than the maximum response were potentiated by cocaine. This action resembles that of sympathectomy in the present study in that the effect was clearly not due to NA transporter blockade and the effect tended to be greater at low agonists concentrations for all agonists except NA, although admittedly the maximum response to NA was increased.

In the present studies of rat aorta, cathinone, MDMA, cathine, MDEA and MHA all failed to produce significant contractions in vehicle treated aorta from male or female rats. This would suggest that these agents are predominantly indirect sympathomimetics, and that they have no clear action at α_{1D}-adrenoceptors. Pressor responses obtained in anaesthetised sympathectomised rats can be compared with contractions in vehicle-treated rat aorta (Table 8.3). For cathinone, MDMA, cathine and MHA, lack of contraction of rat aorta matched lack of pressor response in sympathectomised rats. For ephedrine, a large contraction of rat aorta matched a large pressor response resistant to sympathectomy. For tyramine there was inconsistency in rat aorta: aortic contraction was larger in males than would be expected from the blood pressure studies. Perhaps a weak direct α_{1D}-adrenoceptor mediated stimulation action in aorta is the reason for this, and that actions *in vivo* on blood pressure are largely indirect.

Sympathectomy tended to increase contractions to NA and KCl (both male and female) and caused significantly increased contractions to tyramine (male only), and potency of ephedrine and norephedrine (both male only). Hence, ephedrine, norephedrine and tyramine all produced contractions in vehicle treated aorta that were similar in tissues from male and female rats, but the contractions to norephedrine, ephedrine and tyramine were significantly increased by sympathectomy in tissues from male rats, but not in tissues from female rats.

The differences between aortas from male and female rats can possibly be explained by the effects of oestrogen. Oestrogen has both genomic and non-genomic actions. It has been shown that oestrogen receptor agonists can produce relaxations of rat and mouse arteries (Al Zubair et al., 2005). Oestrogen can be shown to inhibit agonist induced contractions dependent on calcium entry by a non-genomic action (Brown et al., 1999; Hill et al., 2010). It has been shown in human blood vessels that oestrogen relaxes mammary artery but not saphenous vein (Haas et al., 2007), although other studies report that oestrogen enhances relaxations in veins from female but not male rats (Raffetto et al., 2010), with possible implications for development of varicose veins. Overall, the evidence is that oestrogen reduces vascular contractions involving especially calcium entry. Such a difference would explain the difference between males and females in this study of rat aorta.

Possible involvement of trace amine receptors in responses to stimulants can also be considered. In the present study of rat aorta, the stimulants cathinone, cathine, MDMA, MDEA and MHA produced no significant contraction in concentrations of up to 100 μM, which was the maximum examined for these agonists. These results are consistent with those of Broadley et al. (2013), who found that cathinone and MDMA produced contractions of rat aorta at 100 μM and 30 μM and above, respectively, resistant to a cocktail including prazosin. We did not examine these agonists at such high concentrations and therefore did not see any actions that might be considered actions at trace amine receptors. Tyramine produced contractions only at concentrations of 100-1,000 μM in vehicle rat aorta, but 1 μM and above produced contractions in sympathectomised aorta from male rats.

Interestingly, in male rat aorta, Broadley et al. (2013) found that tyramine produced only very small contractions at about 10 μM and above, and these contractions were abolished by a cocktail of drugs including prazosin (Braodley et al., 2013). Hence, the contractions of rat aorta to tyramine involve α_1-adrenoceptors, and not trace amine receptors.

It is concluded, firstly, since cocaine did not increase the potency of NA, and since indirect sympathomimetics such as cathinone and MHA failed to produce contractions, it can be confirmed that the rat aorta has a very poor innervation. Secondly, sympathectomy tended to increase the potency or maximum contraction of agonists especially in male rats. This effect was much less marked in female aorta.

11.7. Comparison of results between rat vas deferens and aorta.

Vas deferens and aorta were used in this study to assess direct versus indirect actions of stimulants, to give a ranking of relative potencies, and to aid in planning interpretation of *in vivo* studies. How far these goals were achieved will be discussed here. Firstly, looking at comparisons between vas deferens and aorta, only data from male aorta will be used**.**

In rat vas deferens, NA and norephedrine were full direct agonists subject to NET. In rat aorta, NA and norephedrine produced the largest contractions and these were not reduced by sympathectomy. Hence they were full direct agonists in rat aorta and sympathectomy should not affect these responses even if nerves were present. Contractions to MDA were smaller in rat vas deferens, but also not affected by sympathectomy, and a moderate contraction was obtained in rat aorta.

In rat vas deferens, responses to tyramine were partly resistant to sympathectomy, suggesting a mixture of direct and indirect actions. Tyramine did produce contractions in rat aorta, but responses were much smaller than to NA or ephedrine.

Cathinone, cathine, MHA, MDMA and MDEA all failed to produce significant contractions in rat aorta, and all produced relatively small contractions in vas deferens from sympathectomised rats. Incidentally, MHA produced marked contractions in vehicle treated rat vas deferens that were abolished by sympathectomy, and produced no contraction in aorta.

Only for ephedrine was there an apparent anomaly. Like MHA, ephedrine produced marked contractions in vehicle treated rat vas deferens that were abolished by sympathectomy, and this was true for both (±)-ephedrine and (-)-ephedrine, yet it produced contractions of rat aorta. Interestingly, although most results in rat aorta were obtained with (±)-ephedrine, a small number of experiments were obtained with (-)-ephedrine, and this isomer tended to produce larger contractions, which may again be indicate of larger direct actions to the (-)-isomer. This may suggest that ephedrine is a direct agonist at α_{1D}-adrenoceptors (which mediate contractions of rat aorta) but not α_{1A}-adrenoceptors (which mediate tonic contractions in rat vas deferens). This is of interest in terms of the cardiovascular actions of ephedrine found in this study of anaesthetized rats. The initial peak pressor effect of both (±)- and (-)-ephedrine was unaffected by sympathectomy, and so is directly mediated. However, in sympathectomised animals, the pressor response to both (±)- and (-)-ephedrine fell more rapidly, suggesting an indirect component to the more sustained pressor response blocked by sympathectomy. It is known that pressor responses in the pithed rat involve both α_{1A}-and α_{1D}-adrenoceptors (Docherty, 2012) and hence it seems likely that ephedrine may act directly at α_{1D}-adrenoceptors, producing the peak response found, and indirectly at α_{1A}-adrenoceptors (and possibly also α_{1D}-adrenoceptors) by release of NA, contributing to the more maintained response found. There is some evidence that ephedrine has vascular actions involving α_{1D}-adrenoceptors, whether direct or indirect (Kaye et al., 2006). However, receptor subtype selective direct effects of ephedrine need to be clarified further.

11.8. Prejunctional actions in rat vas deferens and α_2-adrenoceptor actions of stimulants.

α_2-Adrenoceptors are involved in pre- and post-junctional/synaptic (junctional in the periphery, synaptic in the central nervous system) actions of NA both in the peripheral and central nervous systems. α_2-Adrenoceptors have been classed into three subtypes, α_{2A}-, α_{2B} and α_{2C} (Bylund et al., 1994; Docherty, 1998), with α_{2A} and α_{2C} the dominant subtypes in the central nervous system (Philipp et al., 2002).

α_2-Adrenoceptor agonists potently inhibit the isometric contraction of epididymal portions of rat vas deferens. All of the stimulants examined had moderate potency at α_2-adrenoceptors. α_2-Adrenoceptors are present both peripherally and centrally. In the periphery, they mediate prejunctional inhibition of sympathetic neurotransmission, and postjunctionally on vascular smooth muscle they produce vascular contractions in at least parts of the vasculature. The α_{2A}-adrenoceptor is probably the major pre- and postjunctional/ postsynaptic α_2-adrenoceptor.   Drug actions at peripheral nerve endings in the periphery may be hard to quantify in the total action on the cardiovascular system. MDMA users may have increased levels of plasma catecholamines, possibly due to increased sympathetic activity (Stuerenburg et al., 2002) or by indirect actions of MDMA to release NA. Hence, peripheral prejunctional inhibitory actions may be masked in studies by release of neurotransmitter.

Control of the vascular system by the sympathetic nervous system involves mainly vasoconstriction by α_1-adrenoceptor activation, but α_2-adrenoceptors, especially α_{2A}-adrenoceptors, and to a lesser extent α_{2C}-adrenoceptors, are also involved (Docherty, 1998; Duka et al., 2000). α_{2C}-Adrenoceptors have been demonstrated in peripheral veins (Gavin et al., 1997) and are involved in cold-induced vasoconstriction in cutaneous arteries (Chotani et al., 2000). Postjunctional vascular smooth muscle α_2-adrenoceptors may be involved in cutaneous vasoconstriction to MDMA or cathinone and be involved in control of temperature and in the temperature effects of MDMA and cathinone (see later).

Clonidine and other α_2-adrenoceptor agonists cause hypothermia in wild-type (WT) mice, which is absent in α_{2A}-adrenoceptor-KO mice (Hunter et al., 1997; Zarrindast et al., 2003; Bexis and Docherty, 2005), and probably involves activation of post-synaptic α_{2A}-adrenoceptors in the hypothalamic pre-optic area (Myers et al., 1987). However, the α_{2A}-adrenoceptor antagonist BRL44408, or knockout of the α_{2A}-adrenoceptor, changed the monophasic hyperthermic response to MDMA in WT mice to a biphasic response revealing an initial hypothermia not seen in the absence of BRL 44408 (Bexis and Docherty, 2005). This is perhaps surprising as clonidine produces hypothermia (see above). In the central nervous system pre-synaptic α_2-adrenoceptors can inhibit release of NA (autoreceptors) or other neurotransmitters, such as dopamine and 5-HT (heteroceptors), and MDMA can also act at the transporter to increase extracellular levels of these monoamines. Under these prevailing conditions, α_{2A}-adrenoceptor activation seems to cause hyperthermia, but under resting conditions may cause hypothermia. This demonstrates the complexity of central interactions.

In addition to the predominant α_{2A}-adrenoceptor, it has been shown that a smaller proportion of α_{2C}-adrenoceptors are present prejunctionally, both centrally and peripherally, but especially in peripheral neurones (Ho et al., 1998; Philipp et al., 2002). However, the evidence is that α_{2A} adrenoceptors have a major role in temperature regulation since clonidine had no significant effect on temperature in α_{2A}-adrenoceptor knock-out mice (Bexis and Docherty, 2005),

There is also evidence of central α_{2A}-adrenoceptor mediated inhibition of locomotor activity (Lähdesmäki et al., 2003), so that α_{2A}-adrenoceptor antagonists such as BRL 44408 increased the locomotor actions of of MDMA (Bexis and Docherty, 2006). Other actions of amphetamine-like agents may involve central α_2-adrenoceptor-activation: jaw clenching (Hayner and McKinney, 1986; McCann et al., 1996) perhaps due to inhibition of the jaw opening reflex (Arrue et al., 2004), as well as psychiatric actions including panic attacks (McCann et al., 1996).

11.9. Telemetry studies of locomotor activity and temperature

The final part of this thesis looked at temperature and locomotor actions of cathinone in telemetry studies of conscious male and female rats. In this study, a number of effects of cathinone are reported on locomotor activity and temperature and also the interaction with caffeine, comparing male and female rats. These actions will be discussed separately below, in terms of locomotor activity and temperature and in terms of actions of cathinone and interaction with caffeine. First, choice of statistical test and of drug doses are considered.

11.9.1.1. Choice of statistical test

In these studies, both of male and female rats, one, two and three way anova have been employed. The choice was made for the following reasons. One way anova looks at a global effect, the total activity in the whole sampling period, or the area under the curve for temperature. This may make fairly small effects become significant, and some may not be important, as will be seen from the examples below. Two way anova can look at the time course of events, but if multiple time points are chosen (e.g. 5 min time periods) then significance can sometimes be difficult to obtain. However, 5 min time periods are good at showing significance of important times such as soon after injection and a peak response. Hence, 30 min time periods were also considered, which sometimes revealed significance not reached in 5 min time periods. Three way anova seemed like a good idea, to test significance of two way interactions, pooling the third variable, but the format is limited to 2 groups for 2 of the variables, like a 2x2x2 structure (although the third variable can be more than two). The outcome was probably no more useful than simply combining one and two way anova especially in the case of temperature studies, as will be seen.

11.9.1.2. Choice of stimulant doses

In anaesthetized blood pressure recording studies, dose response curves were constructed to stimulants, for all stimulants in doses up to 1 mg/kg, and for more easily available stimulants, in doses up to 10 mg/kg. Likewise, in isolated tissue experiments, concentration response curves were carried out to stimulants up to 100 μM for all agonists, and up to 1000 μM for more easily available stimulants. However, telemetry studies of locomotor activity and temperature changes required a single dose. This dose for cathinone and caffeine was chosen based both on the anaesthetized rat studies and published results. Since cathinone had marked effects in the anaesthetized rat in doses of 1 mg/kg intravenously, and it was more potent than MDMA, a dose of 5 mg/kg s.c. was chosen for telemetry studies, the same dose as our laboratory has chosen for MDMA (Bexis & Docherty, 2006). It has been shown that administration of cathinone to rats in a dose of 1.6 mg/kg increased locomotor activity while a lower dose of cathinone (0.2 mg/kg) did not enhance locomotor activity (Calcagnetti et al., 1992; Schechter et al 1993; Schechter et al.,1985). For caffeine, doses used in anaesthetised animals were up to 10 mg/kg, and this matches published studies (e.g. Camasara et al., 2006), so this dose was chosen for telemetry.

11.9.2. Locomotor activity

Cathinone produced marked increases in locomotor activity in both male and female rats. These effects are similar to those reported for other stimulants including MDMA (Palenicek et al. 2005; Walker et al. 2007; Vanattou-Saïfoudine et al. 2010b; Rodsiri et al. 2011) and d-amphetamine (Garrett and Holtzmann 1994). Cathinone produced significantly greater locomotor actions in female rats, an effect also reported for MDMA (Palenicek et al. 2005; Walker et al. 2007) and d-amphetamine (Savageau and Beatty 1981). Hence, there is a clear gender difference in the potency and/or efficacy of cathinone, and indeed of other stimulants, in producing locomotor activity in the rat.

Caffeine alone had almost no acute effect on locomotor activity, but produced a late increase in locomotor activity in female rats. However, overall over the 275 min of recording, caffeine produced an increase in activity for both male and female. 3 way anova demonstrated significant effects in the first half of recording (5-140 min) and second half of recording (140-275 min) with significant interaction between caffeine and cathinine (caffeine reducing actions of cathinone). The interaction of gender and cathinone (cathinone actions greater in female) did not quite reach significance in 3 way anova. The combination of caffeine and cathinone produced a biphasic effect: an initial increased response to cathinone soon after injection that showed a clear peak, but, perhaps surprisingly, this was followed by a prolonged period of diminished activity until about 180 min, at which point the response became similar to that of cathinone alone. Hence, caffeine and cathinone were initially additive producing an increased level of activity, but for the next 2 hours the locomotor effects of cathinone were effectively inhibited by caffeine. These effects may be of relevance to the effects of the combination on behavior in humans: increased peak effects perhaps coupled to more frequent dosing due to diminished later response, resulting in increased initial stimulant action, resulting in increased risk of adverse event, and increased need for further dosing, also resulting in increased toxic effects.   Hence, there may be a double effect of the interaction between caffeine and cathinone in causing increased adverse effects.

Previous studies have investigated the interaction between caffeine and other stimulants, particularly MDMA. In rats, caffeine increases the locomotor response produced by cocaine (Schenk et al. 1990) but not by MDMA (Vanattou-Saïfoudine et al., 2010b).   Vanattou-Saïfoudine et al. (2010b) reported a small increase in locomotor activity to caffeine and Zancheta et al. (2012) found that habituation to environment could alter the response to caffeine from no effect to increased locomotor activity. In mice, MDMA (5 mg/kg) caused a small increase in locomotor activity, caffeine (10 mg/kg) caused a marked increase and the combination produced a larger increase than either alone (Camarasa et al., 2006), although in other studies, caffeine (5-50 mg/kg) did not increase locomotor activity (Szopa et al., 2016).

Hence, previous studies have shown that stimulants increase locomotor activity, but that the effects of caffeine alone, or in combination, are more variable. The present results show a novel complex interaction between caffeine and cathinone: an initial potentiation by caffeine of the response to cathinone followed by inhibition. The mechanism for this is unclear, but it has been previously demonstrated that caffeine (10-30 mg/kg) increased locomotor activity in rats, but higher doses (60-120 mg/kg) did not (Marin et al. 2011). Perhaps the combination of caffeine and cathinone has a similar supramaximal action, switching the effect from stimulation to inhibition of activity.

11.9.3. Locomotor activity and hyperthermia

Amphetamine-like agents such as cathinone and MDMA increase locomotor activity as well as having effects on temperature. Indeed, in this study, locomotor actions were more marked than temperature actions. It could be thought that this increased activity contributes to the temperature actions of the stimulants. My studies would tend to suggest that activity did not directly affect temperature actions, except perhaps in the case of cathinone alone in females. Cathinone alone produced very marked increases in activity in females and also produced a delayed rise in temperature that could be explained by the preceeding level of activity. Cathinone produced the highest level of locomotor activity in the absence of caffeine, and this effect was greater in females than males. Despite this, there was a significant acute rise in temperature only in male rats given cathinone subsequent to caffeine. However, Rodsiri et al. (2008) did not find a correlation between locomotor activity and core body temperature, in this case with MDMA. Even so, why does this increase in activity not produce a consistent effect on body temperature. Perhaps it is because this locomotor activity produced by cathinone is low level activity (but perhaps enough activity in females to affect temperature) and not strenuous exercise. Perhaps even the standing position increases effective surface area for heat loss as compared to animal curled up in corner.

11.9.4. Physiological regulation of temperature

The involvement of the autonomic nervous system in the control of temperature is largely in terms of control of cutaneous blood flow, effects on heat production and in terms of control of sweat glands (see Introduction).   Amphetamine-like agents might be expected to act particularly on cutaneous blood flow and on heat production, with both central and peripheral actions. The diagram of Figure 1.9. showed possible sites of action of cathinone in altering temperature control: central actions, thermogeneration, cutaneous vasoconstriction.

Since the present studies examine changes in temperature in rats, we have to consider some special features in which the rat differs from humans in temperature control. The rat tail is a major organ determining heat loss: an increase in tail blood flow and cutaneous dilatation results in increased heat loss.   Rat also differs in the extent of panting to increase heat loss and in the importance of brown fat to cause a much more marked increase in heat production in rat than man.   Furthermore, in rat piloerection reduces heat loss by trapping air for insulation. Differences in body mass and in body fat distribution between males and females must also be considered. In these studies of age-matched rats, males were always heavier (around 250g) than female (around 200g) rats. Even surface area to volume has to be considered.

11.9.5. Changes in temperature produced by MDMA and other amphetamine-like agents at ambient room temperature

In studies that involve behaviour, it must be considered that housing of animals may affect responses. When rats are housed at normal room temperature (20–22°C), some studies have reported that their body temperature is increased by MDMA administration (see Green et al., 2003). However, a decrease in temperature has also been reported to occur in rats housed at this normal room temperature (Malberg and Seiden, 1998; Malpass et al., 1999; Daws et al., 2000; Bexis and Docherty, 2006). In the study of Bexis and Docherty (2006), both MDMA and MDEA

produced only hypothermia in rats housed at 22°C, whereas MDA actually produced a transient hypothermia followed by hyperthermia.

11.9.6. Effect of different ambient temperatures on temperature response to stimulants

In studies where ambient temperature was changed 15 min before injection, MDMA (5-7.5 mg/kg) produced a small hyperthermia of about 1°C in rats at 11°C, a hyperthermia of about 2°C at 24°C and about 2.5°C at 30°C (Dafters, 1995). However, in a previous study Dafters (1994) reported a hypothermia to MDMA in animals maintained for 24 hours at 11°C, but only a diminished hyperthermia in animals held for 90 min at 11°C. The magnitude of the hyperthermia was increased by water deprivation only at 30°C (Dafter, 1995). In rats housed at 15°C for 60 min prior to injection, MDMA (5 mg/kg) produced a short lived hypothermia that recovered by 60 min, whereas at 30°C MDMA produced a hyperthermia (Green et al., 2005). Amphetamine is also reported to produce hypothermia in rats in cool (4-15°C), and hyperthermia in warm, (20-37°C) rooms (Yehuda and Wurtman, 1972). It seems that temperature effects of stimulants switch from hypo- to hyperthermia, or at least from a small or no hyperthermia to a larger hyperthermia, at room temperature of around 20–22°C or slightly above, which happens to be around the range met for normal room temperature in laboratories. Around 22°C, there is more chance that MDMA or cathinone, will produce a hyperthermia, but this is by no means certain.

Another factor to be considered in comparing temperature studies is whether temperature was recorded by rectal thermometer (especially older studies) or by telemetry probes. The former is likely to stress the animals and may have effect on the temperature response obtained. In fact it has been shown comparing two studies in the same laboratory that MDMA produced a small increase in temperature in rat in rectal temperature studies, but a decrease in temperature in telemetry studies (Vanattou-Saïfoudine et al., 2010a, 2010b). Hence, the difference between rectal temperature and telemetry studies may be a largely overlooked source of difference.

In studies in metabolic cages, it was found that MDMA caused a hyperthermia in cages with an acrylic base, but not in cages with a metal base (Gordon & Fogelson, 1994), but this was a large dose of 30 mg/kg. Ability of the animal to lose heat is obviously a factor in the actions of stimulants.

11.9.7. Effects of cathinone on body temperature and the interaction with caffeine

in the present study of male and female rats, cathinone (5 mg/kg) did not significantly increase body temperature in male rats, but produced a small slow prolonged rise in temperature in female rats. Previous studies reported that cathinone (4 mg/kg) did not affect rectal temperature and cathinone (10mg/kg) produced hyperthermia in rats (Shorthall et al., 2013). More studies have examined MDMA. MDMA increased rectal temperature In dark Agouti rats (Mechan et al., 2002) but decreased rectal temperature in Lister-hooded rats (Shorthall et al., 2013), and in telemetry studies, MDMA produced hypothermia in Wistar (Bexis and Docherty, 2006), and Lister-hooded rats (Rodsiri et al., 2011), although repeated dosing produced subsequent hyperthermia (Rodsiri et al., 2011). In Sprague-Dawley rats, caffeine did not affect temperature, MDMA produced a small increase in temperature in rectal temperature studies, but a decrease in temperature in telemetry studies, but the combination of caffeine and MDMA produced a hyperthermia in both studies (Vanattou-Saïfoudine et al., 2010a, 2010b). Clearly, effects of MDMA, and presumably other stimulants, on temperature in the rat may depend partly on genetics, on experimental protocol, ambient temperature, and differences between rectal and telemetry probe measurement. Stress may be an important factor in determining temperature effects of stimulants.

In the present study, caffeine had no major acute effect on temperature, but produced a small increase in temperature throughout and towards the end of recording, significant in one way anova, but significant towards the end of the time course in 5 min studies sample studies only in female rats. Of relevance to this discussion of time course of drug action, the plasma half life of intravenous caffeine

is reported to be about 4 h in human volunteers (Sánchez-Alcaraz et al., 1991) and in rats (Wang & Yeung, 2010). The combination of caffeine and cathinone produced a significant increase in body temperature on injection of cathinone only in male rats, beginning within 15 min of cathinone injection, reaching a peak at around 60 min and ending around 120 min. This was also seen in 3 way anova when there was a significant interaction between both gender and caffeine and caffeine and cathinone for the first half of sampling, caffeine having more effect in males, especially in interaction with cathinone. In the female, the small prolonged effects of cathinone were not significantly altered by caffeine. Admittedly, baseline temperature was significantly higher in female rats, but it is unclear whether this influenced responses.

11.9.8. Interaction between caffeine and cathinone in terms of cardiovascular responses

This thesis also examined, but this time in anaesthetized rats, the interaction between caffeine and cathinone or MDMA in terms of blood pressure and HR. These studies allowed the use of multiple doses of both caffeine and cathinone. Only caffeine (10 mg/kg) produced a maintained tachycardia, and this together with published information led to the choice of caffeine 10 mg/kg in the telemetry studies. Although caffeine produced a tachycardia, it did not potentiate the cardiac actions of cathinone or MDMA. There was no response to low doses of cathinone or MDMA after caffeine. The effects of high doses of cathinine or MDMA were only comparable to responses in the absence of caffeine, when the total response (caffeine plus stimulant) was measured. Hence, caffeine and stimulants were not additive in terms of producing tachycardia and this was even true at a lower HR. Caffeine (10 mg/kg) had only minor effects on blood pressure, and since cathinone also had minor effects, there was no evidence of any interaction.

11.9.9. Central Mechanisms of locomotor and temperature actions of cathinone, MDMA and caffeine

Locomotor actions of stimulants and caffeine may involve release of dopamine (see Docherty and Green, 2010) since the dopamine D_2 antagonist sulpiride blocked increased locomotor activity both to d-amphetamine and to caffeine (Garrett and Holtzman, 1994), and caffeine blocks presynaptic adenosine A_2 receptors to increase dopamine release (Kim and Palmiter, 2003) but 5-HT uptake inhibition by MDMA is not affected by caffeine (Downey et al., 2014). MDMA releases 5-HT to act at 5-HT$_{1B}$ receptors to increase activity (Rempel et al., 1993), and at 5-HT$_2$ receptors to release dopamine also to increase activity (Yamamoto et al., 1995). Hence, MDMA-induced hypermotility may involve activation of 5-HT receptors and interaction of 5-HT and dopamine (Bankson and Cunningham, 2002; Cole and Sumnall, 2003a). Stimulants like MDMA and cathinone may have the additional α_{2A}-adrenoceptor (Lahdesmaki et al., 2003) and 5-HT$_{2C}$ receptor (Bankson and Cunningham, 2002) agonist actions to cause inhibition of locomotion (Lahdesmaki et al. 2003), and block of these actions increases the locomotor response to MDMA (Bankson and Cunningham, 2002; Bexis and Docherty, 2006).

Many temperature studies have concentrated on dopamine and 5-HT involvement in effects of stimulants and largely ignoring adrenergic actions. There is also confusion caused by use of antagonist drugs that may have actions at α-adrenoceptors. Hyperthermic actions of MDMA were attenuated by dopamine D_1-receptor antagonism (Mechan et al., 2002) and abolished by depletion of catecholamines but not of 5-HT (Vanattou-Saïfoudine et al., 2010a). MDMA produces hyperthermia in mice (Bexis and Docherty, 2008), although a hypothermic response can be revealed by α_1- (Bexis a Docherty, 2008) or α_2-adrenoceptor blockade/knockout (Bexis and Docherty, 2005), and a component of the hyperthermia may involve β_3-adrenoceptors (Bexis and Docherty, 2009). Although the 5-HT$_2$ receptor antagonist ketanserin antagonized the hyperthermia to MDMA in mice, this is consistent with α_1-adrenoceptor blockade (Docherty and Bexis, 2013). The evidence available suggests that dopamine (centrally) and NA (peripherally) have a major involvement in the temperature effects of MDMA (and presumably

cathinone). As well as direct actions on the vasculature to cause cutaneous vasoconstriction, MDMA and other amphetamine-like agents may also cause central sympathetic activation to cause cutaneous vasoconstriction, reducing heat loss and so causing hyperthermia especially in a warm environment where this vasoconstriction reduces the ability to lose heat to cool down (Pedersen and Blessing, 2001). Although 5-HT may not be involved acutely in temperature actions, it may be involved in modulating the response (see Docherty and Green, 2010). Peripheral α_1-adrenoceptor mediated vasoconstriction produced by MDMA or cathinone may act to diminish heat loss; hence, there may be gender differences since oestrogen has been shown to diminish vascular contractile responses (see Browne et al., 1999).

11.10. Gender Differences in central actions of stimulants

Gender studies of MDMA use have shown that women are more likely to have psychological symptoms, while men are more likely to have cardiovascular symptoms (Ogeil et al., 2013; Liechti et al., 2001). The present results are consistent with these findings: increased behavioural effects coupled to decreased susceptibility to hyperthermia in females may result in diminished dosing resulting in decreased cardiovascular and temperature side effect. Basal serotonin (5-HT) levels are reported to be higher in female than in male rat brain (Carlsson and Carlsson, 1988). Hormonal status can influence response to stimulants. Women have a greater response to amphetamine (White et al., 2002) or cocaine when estrogen levels are high, and response was reduced giving progesterone (Evans and Foltin, 2006a; Evans et al., 2002). Female mice increase self- administration of cocaine when estradiol levels are high during estrous cycle (Martini et al., 2014) and ovariectomised female rats with estrogen implants had greater response to MDMA than those with no estrogen implants (Zhou et al., 2003). Body temperature shows small changes during the oestrus cycle in the rat, falling in the light phase from 36.9°C (Diestrus 1 + 2), to 36.7 °C (proestrus), recovering to 36.8 °C (estrus) (Rashotte et al., 2002).

In this thesis, employing anaesthetized rats, the interaction between caffeine and cathinone was examined in terms of blood pressure and HR, comparing male and female rats. There was no difference between males and females in the effects of caffeine nor in the interaction with cathinone. Hence, gender differences between males and females do not seem to involve peripheral cardiovascular changes.

In this study, findings of gender differences are reported in the actions of cathinone in that the locomotor response was greater in female rats, and of complex interaction between caffeine and cathinone. Caffeine potentiated the acute rapid increase in activity produced by cathinone, but thereafter the locomotor effects of cathinone were diminished by caffeine, and the combination produced hyperthermia in male rats only.   In anaesthetized rats, the interaction between caffeine and cathinone or MDMA was examined in terms of blood pressure and HR, but no interaction was found.   In terms of application to the human situation, these results suggest an adverse interaction between caffeine and cathinone resulting in acutely enhanced but short duration behavioural effects likely to result in more frequent dosage of cathinone consumption, resulting in negative symptoms in terms of producing hyperthermia and cardiac stimulation. These differences highlight the need to carry out gender studies of the actions of stimulants.

11.11. Conclusion

The main aims of these studies were to investigate gender differences in the actions of the main constituent of khat: cathinone, and to a lesser extent cathine. In these studies of cathinone, it has been shown that it has cardiovascular effects relatively similar to those of MDMA, and like MDMA, cathinone acts predominantly as an indirect sympathomimetic acting at NET to produce these effects. The indirect actions of cathinone and MDMA can be confirmed by the effects of sympathectomy on cardiovascular responses, and by the lack of tonic contractile actions in sympathectomised rat vas deferens, and by the very weak contractions in rat aorta. Like cathinone, cathine had actions that were mainly indirect.

Studies on rat vas deferens (control and sympathectomised tissues) and rat aorta (control tissues) proved useful to assess direct actions of stimulants at α_{1A}-adrenoceptors and α_{1D}-adrenoceptors, respectively. These studies, and a comparison between isolated tissue and *in vivo* studies, allowed the discovery of an interesting indirect component to the pressor actions of ephedrine.

No gender differences were found in the cardiovascular actions of cathinone or MDMA or most other indirect sympathomimetics, but differences were found for tyramine and ephedrine which may have different modes of action, but similar modes of action to each other, involving synaptic vesicular transport. For ephedrine, some interesting results were obtained. Ephedrine has been somewhat controversial as to whether it is a directly acting or indirectly acting agent. The current results may give a solution to this mystery, ephedrine is largely a direct agonist on HR and at α_{1D}-adrenoceptors but actions at α_{1A}-adrenoceptors may be indirect. Hence, pressor responses to ephedrine consist mainly of direct α_{1D}-adrenoceptor mediated actions but there may also be an indirect α_{1A}-adrenoceptor component. Complex effects of ephedrine on blood pressure and direct and indirect effects at multiple receptors may explain the controversy in the published literature as to the exact mode of action of ephedrine.

In this thesis actions of cathinone with major central components were also studied: effects on temperature and effects on locomotor activity. Cathinone caused marked increases in locomotor activity and these effects were greater in female than male. This has been previously reported for other amphetamine-like stimulants, and is likely to be of interest in considering behavoural actions in humans. The most surprising result was the interaction of cathinone with caffeine. Caffeine significantly increased the peak effect of cathinone on locomotor activity but therafter decreased the effects of cathinone for the next 90 min. This demonstrates a complex interaction between caffeine and cathinone: initial augmentation followed by inhibition. Such a complex interaction between caffeine and cathinone may both increase the toxic effects of a single dose of cathinone but also increase the frequency of multiple dosing of cathinone, a double action to increase risk.

In the studies of temperature actions, cathinone alone or caffeine alone had only minor effects on temperature in both male and female rats, with cathinone producing a small late prolonged rise only in females. In previous studies from our laboratory MDMA had minor effects on temperature in rats (but marked effects in mice) so a lack of major effect of cathinone on temperature was not surprising as conditions for obtaining hyperthermia can be complex. The major finding from the temperature study was the interaction between caffeine and cathinone only in male rats. Following caffeine, cathinone produced a significant acute hyperthermia only in male rats, demonstrating a gender difference in the ability of cathinone to cause a hyperthermia.

Too many pharmacological studies have examined male animals only, even though these studies may be of less relevance to the actions of stimulants in the general human population. The results presented in this thesis demonstrate the need to carry out studies in both males and females to demonstrate actions and interactions that may be relevant to the human situation.

Chapter 12.

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12. References

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Chapter 13

Appendices

13.1 Appendix 1

Published paper 1

Alsufyani HA, Docherty JR. Direct and indirect cardiovascular actions of cathinone and MDMA in the anaesthetized rat. Eur J Pharmacol. 2015 Apr 8; 758:142-146.

13.2. Appendix 2

Published paper 2

Alsufyani HA, Docherty JR. Investigation of gender differences in the cardiovascular actions of direct and indirect sympathomimetic stimulants including cathinone in the anaesthetized rat. Auton Autacoid Pharmacol. 2016 Jan;36(1-2):14-9.