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Chapter

Mineralocorticoid Receptor in Novel Target Tissues: A Closer Look at the Adipocyte

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Abstract

In addition to the well-documented role in the kidney, the mineralocorticoid receptor (MR) has been recently identified in different "non-classical" target tissues, such as the brain, the heart, vasculature, macrophages/monocytes, and adipose tissue. In this context, the MR is involved in adipocyte fundamental processes such as differentiation, autophagy, and adipokine secretion. Excessive activation of the MR contributes to metabolic derangements occurring in mice with obesity and metabolic syndrome. Interestingly, MR pharmacological blockade in murine models of obesity has led to protection from weight gain and adipocyte dysfunctions. Unfortunately, there is still a lack of knowledge on the metabolic effects of MR antagonists, and larger clinical studies are deemed necessary to clarify the metabolic role of MR blockade in humans. This review discusses the role of MR in adipose tissue, focusing on regulation by MR of key cellular processes occurring in the adipocyte. The molecular pathways affected by MR activation or blockade in adipose tissue have been investigated only in part. Hence, more studies are necessary to get more insights in the role of aldosterone/MR in this "non-classical" target tissue and to better understand its potential implications in obesity and metabolic syndrome.

Keywords: adipose tissue, autophagy, browning, insulin resistance, obesity

1. Introduction

Mineralocorticoid receptor (MR) activity in the distal nephron plays a well-known role in salt homeostasis and blood pressure regulation [1]. Importantly, studies performed since the late 1980s by different laboratories have revealed the presence of MR also in non renal tissues (i.e., heart, brain, adipose tissue (AT)) [2–4]. The discovery of expression of MR in adipocytes, cardiomyocytes, and vascular cells has promoted further research to investigate MR function in the pathophysiology of obesity [5–7], cardiovascular disease [8–10], and metabolic syndrome [11]. Both aldosterone and glucocorticoids are able to activate MR [12]. Expression of the enzyme 11b-hydroxysteroid dehydrogenase type 2 (11b-HSD2) favors MR activation by aldosterone in epithelial tissues, whereas in non-epithelial tissues (AT, cardiomyocyte), scarce expression of 11b-HSD2 suggests that MR is activated mainly by glucocorticoids [12].

However, it is still controversial whether increased circulating levels of aldosterone observed in obesity, metabolic syndrome, and primary aldosteronism (PA) can activate MR in non-epithelial tissues [13] and, in particular, in the adipocyte where this transcription factor regulates differentiation and modulates oxidative stress and adipokine expression [6, 14, 15]. Notably, preclinical studies in murine models of obesity suggest that MR blockade counteracts fat mass expansion and improves insulin sensitivity, indicating that pharmacological antagonism of MR may represent a valid approach to fight obesity [5, 6], even though human studies have not yet confirmed such anti-obesogenic effects for MR antagonists.

Although a deeper comprehension of MR function in the adipose cell, at a molecular level, requires further research, it appears clear right now that adipocyte-specific MR represents a topic of future research on AT dysfunctions and obesity.

This review examines the state of the art of research on adipocyte MR, describes AT function, and analyzes the contribution of altered function of MR in the pathophysiology of obesity and metabolic syndrome.

2. Adipose organ function

Adipose tissue is composed of two distinct types of fat: white adipose tissue (WAT) and brown adipose tissue (BAT) with distinct morphology and function. Both types of fat affect whole-body metabolism.

The adipose organ represents a multi-depot organ consisting of subcutaneous and visceral fat depots with a marked cellular heterogeneity, containing adipocytes, preadipocytes, endothelial cells, fibroblasts, and immune cells [16, 17]. The adipocyte is the most relevant cell type in the AT and mammals display at least two distinct types of adipocytes, characterized by diverse morphology and physiological function. White adipocytes are unilocular spherical cells with a peripheral flattened nucleus and a single large cytoplasmic lipid droplet. White adipocytes are cells specialized for storing energy in the form of triglycerides and display endocrine properties, being able to synthesize and release secretory proteins called "adipokines" involved in regulation of whole-body energy metabolism [16, 17]. On the other hand, brown adipocytes display a round central nucleus and a high number of cytoplasmic lipid droplets and mitochondria. Mitochondria in brown adipocytes are characterized by the expression of uncoupling protein 1 (UCP1) which is the hallmark of the brown adipocyte. UCP1 is a unique protein which allows uncoupling of oxidative phosphorylation from ATP synthesis, leading to dissipation of chemical energy as heat (non-shivering thermogenesis) [18]. WAT has storage and secretory function and contains mainly white adipocytes. In the brown adipose tissue (BAT), with thermogenic function, the prevalent type of adipocyte is the brown adipocyte. BAT function is regulated by the sympathetic nervous system, and its thermogenic activity maintains body temperature in the presence of cold exposure or during postnatal period [19]. Up until a few years ago, BAT function was considered relevant only in hibernating mammals and newborn humans, whereas adult humans were thought to lack BAT [20]. Notably, studies published in 2007 and 2009 performed using fluorodeoxyglucose positron-emission tomography (FDG PET) [21, 22] have revealed the presence of functional BAT also in healthy adult humans, detected as regions of increased tracer uptake [22, 23]. Human BAT has been detected in the supraclavicular and cervical regions, as well as in mediastinal, paravertebral, paraaortic, and suprarenal regions [21]. Cold exposure results in BAT activation both in mice and humans [24, 25] with parallel increase in energy expenditure and reduced fat mass, thus suggesting that BAT activity enhancement may be considered a valid approach to fight obesity also in humans [26].

3. Adipose tissue dysfunctions in obesity

Dysfunctional adipose tissue is characterized by enlarged size of the adipocyte, altered expression of adipokines, pro-inflammatory polarization of resident macrophages, and defective thermogenic capacity.

Altered metabolic regulation of AT leads to excessive WAT expansion through two possible mechanisms: increased cell number (hyperplasia) and/or increased cell size (hypertrophy) [27]. Deregulated enlargement of adipocyte size promotes macrophage recruitment within WAT, through production of chemokines such as MCP-1 and IL-8. Macrophage recruitment is also associated with changes in macrophage polarization toward a pro-inflammatory phenotype (M1), which contributes to insulin sensitivity alteration [28]. Increased content of immune cells such as $CD8^+$ T cells and IFN-γ^+T-helper type 1 cells, in the AT of obese subjects, may contribute to the low-grade chronic inflammation associated with obesity [29]. In the obese state, altered expansion of WAT is also accompanied by increased secretion of pro-inflammatory adipokines, such as leptin, TNF-α, and IL-6, paralleled by a reduced secretion of anti-inflammatory and insulin-sensitizing adipokines, thus promoting obesity-related complications. As discussed above, preclinical and human studies suggest that enhanced activity of brown adipocytes by stimulating thermogenic function can protect against obesity and associated alterations in glucose metabolism and lipid profile [24, 30]. Importantly, reduced amounts of BAT have been observed in overweight and obese subjects, indicating that impairment in brown adipocyte activity may favor AT dysfunctions [22]. Indeed, a recent study has shown that a specific single-nucleotide T to C variant in the FTO locus promotes obesity development through inactivation of genes involved in brown adipogenesis, further confirming the importance of the brown fat in counteracting AT metabolic alterations [31].

In the adipocyte, MR has a central role in signaling pathways regulating physiology and pathophysiology of AT. Research on MR function in AT has been summarized and discussed in the paragraphs below.

4. MR in adipogenesis

MR plays a key role in regulating adipogenesis. Overactivation of MR promotes adipose tissue dysfunction. On the other hand, MR antagonism counteracts white adipocyte differentiation and promotes brown adipogenesis.

The role of MR has been studied in adipocyte cultures and in murine models of obesity. MR activity has been shown to promote expression of adipocyte markers and stimulate differentiation of 3T3-L1 adipose cell cultures (**Figure 1**) [14], whereas MR antagonism counteracts adipogenesis in 3T3-L1 cells as well as in primary human adipocytes [32, 33]. In accordance with these data, primary adipocyte cultures obtained from mice knockout for MR have shown impaired adipogenesis [34], as well as knockdown of MR in cultures of human preadipocytes represses differentiation [32]. Altogether, these data show that MR impaired function represses white adipocyte differentiation. Notably, in vivo studies show increased expression of MR in adipose tissue of obese mice and humans, suggesting enhanced activity of this receptor in the obese state which may contribute to adipocyte dysfunctions [11]. Pharmacological MR antagonism in obese mice counteracts weight gain and excessive expansion of fat mass [5, 6] and, at a molecular level, prevents altered expression of adiponectin, PPAR-γ, and leptin [15] confirming that MR activity modulates expression of adipocyte marker genes and regulates AT function. Recent data by Feraco et al. have shown that adipocyte-specific MR-KO mice do not show

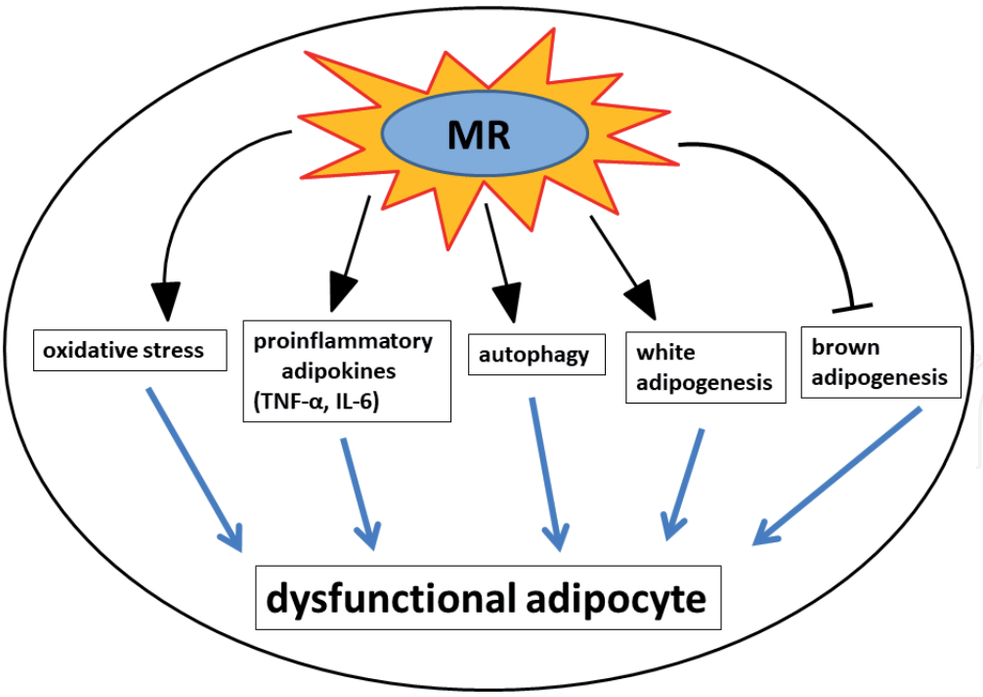

Figure 1.
Involvement of MR in the pathophysiology of adipose tissue. MR overactivation results in detrimental effects on adipocyte metabolism. Upregulated activity of adipocyte MR promotes expression of enzymes involved in reactive oxygen species (ROS) production and pro-inflammatory adipokines such as TNF-α and IL-6. In the adipocyte, increased function of MR stimulates the autophagic flux which, in turn, promotes white adipocyte differentiation. Altogether, these effects lead to alterations of adipose tissue metabolism. MR activation also represses brown adipogenesis, reducing thermogenic capacity and favoring adipose tissue dysfunctions.

changes in AT function or glucose tolerance, under both normal diet and high-fat diet (HFD), indicating a negligible role of MR in AT [35]. Nevertheless, these transgenic mice express a Cre-recombinase protein (adipoq-Cre) which removes MR only in mature adipose cells. This may indicate that MR activity is not required in mature adipose cells, suggesting that MR modulation may be crucial in regulating early stages of white adipogenesis.

Studies by Lombes and collaborators have shown that MR is expressed in brown adipocytes [36] where MR activation represses expression of UCP1 (**Figure 1**), which confers thermogenic function to BAT [37]. In accordance with these data, more recent studies by Caprio and colleagues revealed that MR blockade promotes brown adipogenesis in cultures of mouse primary preadipocytes derived from inguinal AT increasing expression of brown adipocyte markers such as PRDM16, CIDEA, and PPAR-γ coactivator 1α (PGC1-α) [5]. Moreover, mice upon pharmacological MR blockade showed upregulated expression of these markers in BAT, with a parallel increase in BAT activity, confirming the impact of MR function on brown adipocyte function also in vivo [5].

5. MR involvement in "browning" of adipose tissue

Appearance of brown-like (brite) adipocytes in murine WAT takes place upon cold exposure. In mice, treatment with MR antagonist has been shown to promote browning and protect against fat mass expansion.

White and brown adipocytes display distinct embryonic origin. White adipocytes are derived from myogenic factor 5 (myf5)-negative progenitors, whereas classical brown adipocytes (and skeletal muscle cells) are derived from myf5-positive precursors [38]. In addition, studies by Spiegelman and collaborators have identified a third

type of adipocyte, termed "beige" (or "brite"), localized in murine WAT depots [39]. These "brown-like" adipocytes display classical brown adipocyte morphological and biochemical features (several cytoplasmic lipid droplets and high number of mitochondria), even if they are derived from myf5-negative precursors [26]. Importantly, β-adrenergic stimulation results in increased levels in UCP1 and uncoupled respiration rate in beige adipocytes, leading to the acquisition of brown fat features by WAT [39], a process known as "browning," and several preclinical studies have shown that browning protects mice against glucose and AT dysfunctions. Transgenic mice with fat tissue-specific overexpression of PRDM16, a transcriptional regulator involved in brown adipocyte development, displayed brite adipocyte formation in inguinal WAT. These mice also showed increased energy expenditure and improved glucose tolerance with high-fat feeding and parallel reduction in weight gain and fat mass [40]. Accordingly, another study showed that transgenic mice lacking PRDM16 in AT display defective browning, obesity, and insulin resistance, strongly indicating that brite adipocyte function, at least in mice, affects AT metabolism and whole-body energy expenditure [41]. Treatment of mice fed with HFD with the MR antagonists spironolactone (spiro) or drospirenone resulted in browning of white fat depots, reduced weight gain, and decreased fat mass expansion, as well as improved glucose tolerance [5]. In this study, transcriptional induction of a thermogenic gene program by MR antagonism was observed both in mice and in primary cultures of mouse adipocytes treated with spiro and drospirenone, indicating that MR blockade-mediated browning is cell autonomous.

However, further studies are required to better understand the molecular mechanisms regulated by MR which affect the thermogenic gene program of brite adipocytes.

The negative causal link between MR signaling and browning of WAT has been confirmed by Pisani et al. with Task1 −/− mice lacking the TWIK-related acid-sensitive K+ channel. These mice displayed increased WAT mass and impaired browning, as well as reduced BAT activity upon adrenergic stimulation. In brown adipocytes from Task1 −/− mice, MR antagonist treatment was able to rescue defective expression of UCP1, suggesting that the absence of Task1 activity can result in enhancement of MR function and subsequent downregulated expression of thermogenic genes [42].

6. Regulation of autophagy by MR in adipose cells

The process of autophagy has been shown to regulate white and brown adipogenesis. Modulation of MR activity regulates the autophagic flux which, in turn, affects white and brown adipocyte differentiation.

In the eukaryotic cells, autophagy regulates organelle and protein turnover maintaining cellular homeostasis and function [43]. Increased autophagic flux has been observed during adipocyte differentiation [44, 45]. Recent research has identified genes involved in autophagy regulation (atg). In particular, the role of atg5 and atg7 has been analyzed both in transgenic mice and in adipocyte cultures [45, 46]. Impaired adipose differentiation, i.e., altered morphology and decreased lipid droplet accumulation, has been observed in autophagy-related 5 (atg5)−/− mouse embryonic fibroblasts (MEFs), and, accordingly, newborn mice lacking atg5 display reduced fat mass [45]. Likewise, both 3T3-L1 preadipocytes lacking atg7 and atg7−/− MEFs display impaired adipogenesis, showing that also atg7 affects adipocyte maturation [46, 47]. Interestingly, Singh et al. have analyzed the metabolic profile of atg7-knockout mice showing that these mice have reduced WAT amount, paralleled by increased interscapular BAT. Moreover, browning of WAT

has been also detected in atg7-KO mice. Indeed, WAT of atg7-KO mice displayed higher levels of UCP1 and PGC-1α, a master regulator of mitochondrial biogenesis, and higher levels of mitochondrial enzymes. Therefore, this study suggests that impaired autophagy leads to the formation of brite adipocytes in WAT. Recent data from Caprio and collaborators have shown that MR regulates the autophagic flux in murine adipocytes (**Figure 1**) [5]. Both in 3T3-L1 cells and primary murine adipocytes, aldosterone treatment increases autophagy, whereas MR blockade reduces the autophagic flux. In mice fed with HFD, pharmacological antagonism of MR leads to reduced AT autophagic flux inducing, in turn, browning of WAT. These data confirm the effects of impaired autophagy on browning of WAT observed in atg7-KO mice [46]. Evidence of a causal link between impairment in autophagy and induction of browning has been also demonstrated by treating murine adipocytes with either spiro or bafilomycin (autophagy inhibitor) or everolimus (autophagy activator) [5]. Both spiro and bafilomycin reduce autophagic flux with a concomitant increase in UCP1 levels, which indicates brown conversion of the adipose cell. Cotreatment with everolimus prevented UCP1 increase induced by spiro, indicating that decrease of autophagy is required for brown adipose conversion, whereas autophagy activation inhibits such process [5].

7. Metabolic effects of MR antagonism on adipose tissue metabolism

Overactivity of adipocyte MR contributes to the development of adipose tissue dysfunctions. Pharmacological blockade of MR counteracts adipocyte oxidative stress and adipocyte hypertrophy, improving insulin sensitivity and stimulating BAT activity.

Preclinical evidence suggests that increased expression of MR plays a role in AT dysfunctions. Increased levels of MR transcript have been detected in AT of obese humans and mice [11]. Data by Jaisser and collaborators has shown that adipocyte-specific MR overactivity leads to obesity and metabolic syndrome features in mice overexpressing MR in adipocytes (adipo-MROE mice) [11]. In particular, upregulated expression of MR in mouse adipocytes leads to increase in body weight and visceral AT hypertriglyceridemia, hypercholesterolemia, and impaired insulin response. Interestingly, in adipo-MROE mice, adipocyte area was increased and positively correlated with the MR expression in visceral AT. Hypertrophy of the adipocyte is a well-known feature of dysfunctional AT associated with local and systemic inflammation and impaired insulin sensitivity [48]. Accordingly, increased activity of MR promoted adipocyte hypertrophy associated with negative effects on glucose metabolism of adipo-MROE mice [11]. A recent study by Feraco et al. has investigated the effects of adipocyte-specific MR ablation on mouse AT and glucose metabolism. Mice lacking adipocyte MR (adipo-MRKO mice) do not show changes in fat mass, glucose, and lipid profile, suggesting that MR removal, at least in the mature adipocyte, does not alter AT function [35]. As discussed above, in adipo-MRKO mice the enzyme adipoq-Cre removes MR in mature adipocytes but does not alter MR function in preadipocytes, indicating that removal of MR in the early stages of adipogenesis may indeed affect AT and explain the metabolic effects of pharmacological blockade of MR observed in mice [5]. Accordingly with this hypothesis, treatment of preadipocyte cultures with MR antagonist represses differentiation [33].

Pharmacological antagonism of MR in obese mice protects against weight gain, fat mass expansion, and local inflammation [5, 6]. In the AT MR blockade counteracts adipocyte size enlargement, reduces the expression of pro-inflammatory adipokines (**Figure 1**), and promotes adiponectin production. In addition, MR antagonism reduces the expression of enzymes involved in reactive oxygen species

(ROS) production and, in parallel, increases the expression of ROS-eliminating enzymes, leading to decreased oxidative stress [6]. Increased oxidative stress has been detected in AT of murine models of obesity (mice fed with HFD or ob/ob mice), and mitochondrial dysfunction associated with obesity can contribute to production of ROS leading to carbonylation and impaired function of proteins such as IRS, contributing to AT dysfunction and insulin resistance [49]. In accordance with this, pharmacological blockade of MR reduces oxidative stress and improves insulin resistance in obese mice [6]. As mentioned above, treatment of mice fed with HFD with MR antagonists counteracts fat mass expansion and promotes also browning of WAT associated with improved glucose homeostasis. Therefore, preclinical studies with MR antagonists suggest that MR blockade counteracts AT dysfunctions dampening inflammation and oxidative stress and favoring brite adipocyte formation, at least in mice.

An increase in circulating levels of aldosterone is frequently observed in obese subjects, and recent data by Huby et al. suggest that leptin can contribute to the elevation of aldosterone [50]. This study reveals that the adipokine leptin can increase adrenal expression of the aldosterone synthase and plasma aldosterone levels, supporting the presence of a cross talk between AT and adrenal gland. In fact, increased secretion of leptin from the AT of obese subjects can lead to upregulated production of aldosterone which, in turn, may activate MR function in the adipocyte, further promoting leptin expression, fat expansion, and oxidative stress. Thus, in obesity, adipocyte MR might be overactivated by high plasma levels of aldosterone which further reinforces the dysregulated function of AT. There are very few studies that have investigated the role of adipocyte MR in humans. Karashima et al. have reported that treatment with MR antagonists for 12 months in subjects with primary aldosteronism (PA) led to reduction in blood pressure and visceral fat mass without changes in subcutaneous AT HOMA-IR, or in lipid profile [51]. To date, there are no other studies describing any effect of MR antagonism on human WAT. On the other hand, a recent study has shown that treatment with spiro in healthy adult subjects increases BAT volume and activity [52], indicating that MR blockade results in BAT function enhancement also in humans. These data suggest that the increase in thermogenic activity of BAT by MR antagonist-based therapies may represent a valuable approach to treat obesity.

8. Conclusion

A number of studies have shown that MR regulates AT physiology and can also contribute to the pathophysiology of obesity. In the adipocyte, MR has been shown to modulate transcript levels of adipogenic transcription factors, adipokines, and enzymes involved in ROS production (**Figure 1**) and scavenging. Notably, in murine models of obesity, treatment with MR antagonist is capable of counteracting excessive expansion, increased inflammation, and oxidative stress of AT. Moreover, in adipocyte cultures and obese mice, MR blockade can reduce the autophagic flux and promotes brown adipogenesis, upregulating BAT activity and inducing browning of WAT, a process that exerts favorable effects against glucose intolerance and AT dysfunction. Recent data have also shown that treatment with MR antagonist is able to enhance BAT activity in humans, further supporting the potential of MR antagonists as novel pharmacological agents in programs of metabolic rehabilitation for subjects with obesity and metabolic syndrome. However, the molecular mechanisms downstream MR, which mediate the mentioned effects on adipocyte function, are poorly known. Indeed, deeper understanding of the molecular pathways modulated by MR is necessary to design efficient therapies against AT dysfunctions and obesity.

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