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Aldosterone Inhibits Uncoupling Protein-1, Induces Insulin Resistance, and Stimulates Proinflammatory Adipokines in Adipocytes

Abstract

Aldosterone is a mineralocorticoid hormone that regulates blood pressure and salt/water balance. Increased aldosterone levels are found in states of disturbed energy balance such as the metabolic syndrome. Adipose tissue has been recognized to play a pivotal role in the regulation of energy homeostasis. We investigated direct aldosterone effects on brown adipocyte function. Aldosterone dose-dependently inhibited expression of uncoupling protein-1 (UCP-1) by 30% ($p < 0.01$). Furthermore, aldosterone dose-dependently impaired insulin-induced glucose uptake by about 25% ($p < 0.01$). On a transcriptional level, mRNA of the proinflammatory adipokines leptin and monocyte chemoattractant protein-1 (MCP-1) was increased by 5,000% and 40%, respectively, by aldosterone exposure ($p < 0.05$). This study demonstrates that aldosterone directly impacts on major adipose functions including stimulation of proinflammatory adipokines.

Key words
Adipose tissue · Metabolic syndrome · Monocyte-chemoattractant protein-1

Introduction

The renin-angiotensin-aldosterone system (RAAS) is involved in the regulation of blood pressure and salt/water balance, increasing secretion of aldosterone from the adrenal cortex. Aldosterone appears to be linked to several components of the insulin resistance syndrome comprising hypertension, insulin resistance or frank diabetes, dyslipidemia, and obesity [1]. Obese subjects are frequently found to have hypertension due to an activated RAAS involving elevated aldosterone levels [2–4]. Adipose tissue plays a pivotal role in the regulation of energy homeostasis [5]. First, adipocytes are insulin-sensitive, and disruption of insulin signaling in adipose tissue entails significant systemic consequences [6]. Depending on adipose tissue type and signaling level affected, these changes range from obesity resistance through diabetes to increased longevity [6]. Second, adipose tissue also responds to sympathoadrenergic activation-β-adrenergic stimulation of brown adipocytes enhances thermogenesis, and hence energy expenditure-by increasing the expression of uncoupling protein-1 (UCP-1) [7]. In humans, brown adipose tissue can be found at any age [7], accounting for approximately 2% of body fat in adults [8]. In insulin-resistant subjects, decreased expression of brown adipogenic genes has been reported, which possibly indicates a brown fat-specific basal gene expression profile involved in the regulation of insulin sensitivity [9]. Third, adipocytes secrete hormones known as adipokines, which regulate a broad range of physiological processes including glucose and energy homeostasis as well as inflammation and atherosclerosis [5].

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Accumulating evidence suggests a link between mineralocorticoid actions, adipose tissue, and the pathogenesis of features of the metabolic syndrome [10,11]. Adipose tissue may contribute to the systemic RAAS by expression and secretion of angiotensinogen and other components of a local RAAS [12]. Aldosterone itself does not appear to be synthesized in fat cells. However, expression of the mineralocorticoid receptor can be found in adipose tissue [13]. Here, we investigate molecular and functional effects of aldosterone on adipocyte metabolism and endocrine activity. Our study demonstrates a direct impact of this mineralocorticoid hormone on thermogenesis, insulin sensitivity, and regulation of anorexigenic and proinflammatory adipokines.

Materials and Methods

Materials

Aldosterone was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Merck Bioscience (Darmstadt, Germany). Phosphospecific antibodies against p44/p42 mitogen-activated protein (MAP) kinase, and Akt (S473) were from Cell Signaling Technology Inc. (Beverly, MA, USA). Glucose uptake assays were performed with 2-deoxy-[3H]glucose from NEN Life Technologies (Deirich, Germany). Primers for expression analysis were ordered from Biometra (Göttingen, Germany) and TIB Molbiol (Berlin, Germany). All other chemicals were from Sigma-Aldrich Co. (St. Louis, MO, USA) unless stated otherwise.

Cell Culture

We used SV40 T-immortalized murine brown preadipocytes in all experiments [14,15]. Cells were cultured in differentiation medium consisting of Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Paisley, Scotland) with 4.5 g/l glucose and supplemented with 20 nmol/l insulin, 1 nmol/l triiodothyronine, 20% fetal bovine serum, and penicillin/streptomycin (Bio Whittaker, Vervier, Belgium). When confluence was reached, 500 μmol/l isobutylmethylxanthine, 250 μmol/l indomethacine, and 2 μg/ml dexamethasone were added to the differentiation medium for a 24 h induction period. Following induction, cell culture was continued with differentiation medium for four to seven days. Cells were starved overnight in serum-free DMEM prior to experimentation.

Analysis of gene expression

UCP-1, leptin, and MCP-1 mRNA expression was determined by reverse transcription (RT) and real-time polymerase chain reaction (PCR). Total RNA was isolated using RNAwitz (Ambion, Austin, TX, USA) or TRIzol reagents (Invitrogen, Karlsruhe, Germany), followed by RNA clean-up using the RNeasy kit (Qiagen, Hilden, Germany). RNA quality was tested by photometric analysis and RNA visualization on agarose gel. Up to 2 μl of RNA isolate were reverse-transcribed using Superscript II (Invitrogen, Karlsruhe, Germany) and oligo p(DT)15 primer (Roche Molecular Biochemicals, Mannheim, Germany) in the presence of RNase inhibitor (Roche Molecular Biochemicals). 1 μl of each RT reaction was amplified in a PCR reaction containing 250 nM of each primer and 1 × QuantiTect SYBR Green RT-PCR-Mix (Qiagen, Hilden, Germany) in GeneAmp 5700 and Prism 7000 sequence detection systems (Applied Biosystems, Foster City, CA, USA). For quantitative RNA analysis, the following primers were used: UCP-1 (acc. nos. M21222 and M21244) 5'-ATG GTG AAC CCG ACA ACT TCC GAA GTG-3' (sense) and 5'-GTA CGT GAA GCC TGG CCT TCA CCT TGG-3' (antisense); leptin (acc. no. NM008493) 5'-GTG CCT ATC CAG AAA GTC CAG GAT G-3' (sense) and 5'-CTG GTG AGG ACC TGT TGA TAG AC-3' (antisense); MCP-1 (acc. no. NM011333) 5'-GCC CCA CTC ACC TGC TGC TAC T-3' (sense) and 5'-CCT GCT GCT GGT GAT CCT CTT GT-3' (antisense); 36B4 (acc. no. NM007475) 5'-AAG CGC GTC CTG GCA TTG TCT-3' (sense) and 5'-CCG CAG GGG CAG CAG TGG T-3' (antisense). PCR was performed as follows: initial denaturation at 95 °C for 90 s, 40 cycles with 95 °C for 30 s, 60 °C for 60 s, 72 °C for 60 s (MCP-1) or 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s (UCP-1, leptin). Specific amplification was confirmed using melting curve profiles. Optimized relative quantification was done using the Relative Expression Software Tool (REST©) [16] normalized to 36B4 as housekeeping gene [17].

Glucose uptake assay

Glucose uptake assays were essentially carried out as described elsewhere [14]. In brief, fully differentiated cells were starved overnight in serum-free medium and then washed twice in Krebs-Ringer HEPES. Cells were then stimulated with or without insulin (100 nM) for 30 min. At the end of the stimulation period, the cells were incubated with 2-deoxy-[3H]glucose for 4 min, washed in phosphate-buffered saline, and lysed with 0.1% sodium dodecyl sulfate (SDS). The incorporated radioactivity was determined by liquid scintillation counting.

Western blotting

Fully differentiated cells were starved for up to 24 h in serum free-medium prior to stimulation. Cells were washed with ice-cold phosphate-buffered saline and proteins were isolated using whole-cell lysis buffer containing 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 mmol/l vanadate and 26 mmol/l phenylmethylsuphonyl fluoride (Fluka Chemie, Neu-Ulm, Germany). Protein content was measured by the Bradford method using the dye from Bio-Rad (Hercules, CA, USA). Whole-cell protein lysates were subjected to SDS polyacrylamide gel electrophoresis. Proteins were then transferred to nitrocellulose membranes. Membranes were blocked overnight either with rinsing buffer (10 mmol/l Tris, 150 mmol/l NaCl, 0.05% Tween 20, pH 7.2) supplemented with 3% bovine serum albumin ('blocking solution'). Membranes were then incubated with the antibodies for 1 – 2 h. Protein bands were visualized using chemiluminescence (Roche Molecular Biochemicals, Mannheim, Germany) with enhanced chemiluminescence films (Amersham Pharmacia Biotech, Freiburg, Germany).

Statistical analysis

Statistical analysis was performed using Sigma Stat (SPSS Science, Chicago, IL, USA). Data are presented as means ± SEM. Statistical significance was determined using the unpaired Student *t*-test or Mann-Whitney U-test as appropriate. Values of $p < 0.05$ were considered significant, and $p < 0.01$ highly significant.

Results

Aldosterone inhibits expression of thermogenic UCP-1

Given a potential role for aldosterone in energy homeostasis [4], we studied the direct effect of aldosterone treatment on the ex-

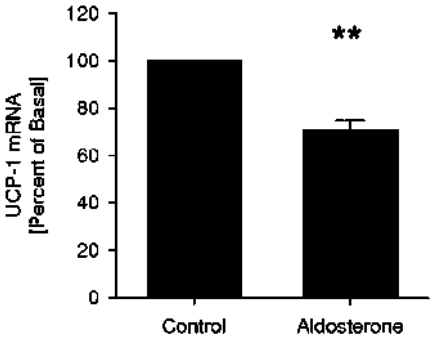

Fig. **1** Aldosterone inhibits thermogenesis. Differentiated adipocytes were treated with 100 nmol/l aldosterone for 8 h or left untreated.

Quantitative analysis of UCP-1 mRNA was performed. The bar graph shows the mean of three independent experiments. **p < 0.01 comparing treated to untreated cells.

pression of thermogenic UCP-1 protein. Stimulation of fully differentiated brown adipocytes with 100 nmol/l aldosterone for eight hours diminished basal UCP-1 mRNA by 30% (Fig. **1**). This effect was dose-dependent, and independent of any effect of aldosterone on adipocyte differentiation as measured by Oil Red-O staining (data not shown).

Aldosterone induces insulin resistance

UCP-dependent thermogenesis is coupled to glucose uptake. We found that insulin, but not aldosterone, induced basal glucose uptake about ten-fold (Fig. **2a**). However, when pre-treating cells with aldosterone for twenty-four hours prior to the stimulation with insulin, glucose uptake was dose-dependently reduced by up to 25% (Fig. **2a**). Concomitantly, the activation of the major insulin signaling elements protein kinase B and mitogen-activated protein (MAP) kinase as measured by phosphospecific Western blotting was diminished by aldosterone pre-treatment (Fig. **2b**).

Aldosterone stimulates leptin and MCP-1 expression

Leptin is the prototypic anorexigenic adipokine [18], and MCP-1 is a proinflammatory chemokine that has been implicated in the pathogenesis of insulin resistance [19,20]. MCP-1 mRNA expression transiently increased to 140% of basal levels after stimulation with 100 nmol/l aldosterone for 0.5 to 1 h (Fig. **3a**). This effect was non-significantly sustained for up to eight hours (data not shown). Moreover, thirty minutes of aldosterone treatment strongly augmented leptin mRNA expression to 5,000% (Fig. **3b**). Again, this effect appeared to be sustained for up to eight hours (data not shown).

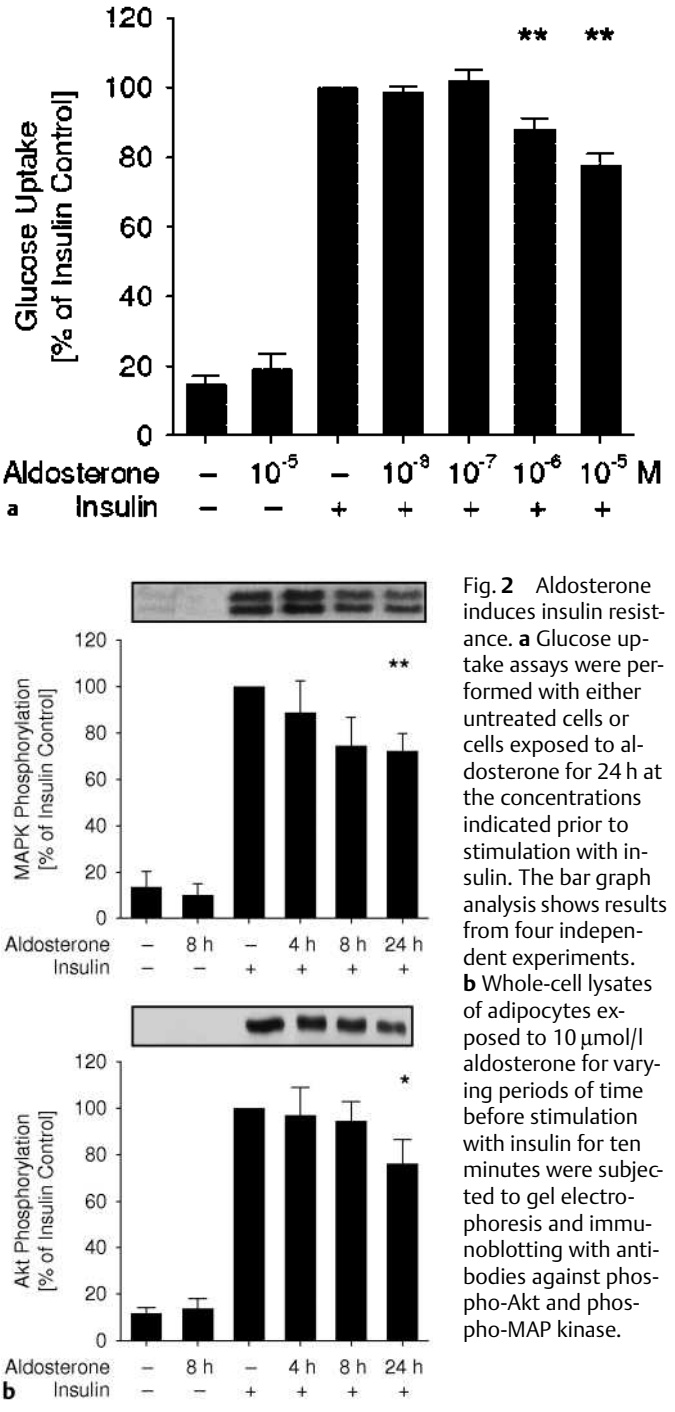

Fig. **2** Aldosterone induces insulin resistance. **a** Glucose uptake assays were performed with either untreated cells or cells exposed to aldosterone for 24 h at the concentrations indicated prior to stimulation with insulin. The bar graph analysis shows results from four independent experiments. **b** Whole-cell lysates of adipocytes exposed to 10 µmol/l aldosterone for varying periods of time before stimulation with insulin for ten minutes were subjected to gel electrophoresis and immunoblotting with antibodies against phospho-Akt and phospho-MAP kinase.

The bar graph shows analyses of at least three independent experiments and representative immunoblots. *p < 0.05 and **p < 0.01 as compared to cells treated with insulin alone.

Discussion

In the present study, we demonstrate direct aldosterone action in adipocytes resulting in reduced thermogenesis, impaired insulin sensitivity, and altered proinflammatory hormone expression.

Individuals with the metabolic syndrome are prone to have an activated RAAS with elevated aldosterone serum levels [4]. Aldosterone interferes with insulin secretion and/or signaling in these patients [4]. Adipocytes importantly contribute to the control of energy homeostasis. In this study, aldosterone depressed basal UCP-1 mRNA levels. Consistent with our findings, aldosterone has been reported to impair UCP-1 mRNA expression induced by isoproterenol and retinoic acid, with doses and kinetics comparable to our results in a brown adipose tumor cell line [21]. However, this is the first study to demonstrate direct effects of aldosterone on basal UCP-1 levels. Although UCP-1 is a marker of adipocyte differentiation, the effect shown here is not caused by impaired differentiation since adipocyte differentiation was unaltered under chronic direct aldosterone exposure (data not shown). Indeed, aldosterone has been reported to facilitate rather than hinder adipocyte differentiation [22].

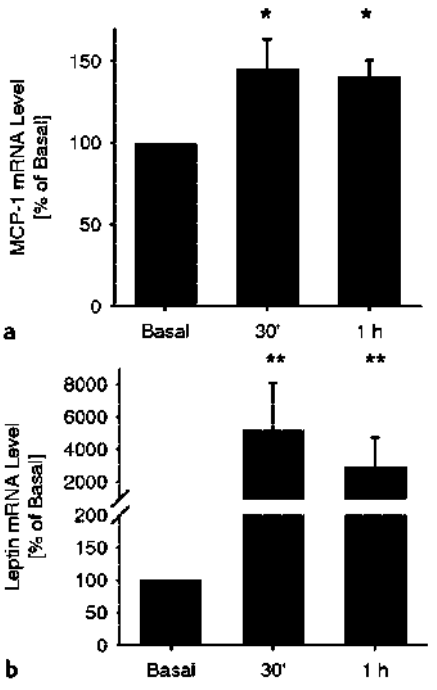

Fig. **3** Aldosterone modulates proinflammatory hormone expression. Mature adipocytes were starved overnight in serum-free medium and stimulated with 100 nmol/l aldosterone for the time periods indicated. **a** Quantitative MCP-1 mRNA expression was determined. **b** Quantitative leptin mRNA was determined. Bar graph analyses of at least four independent experiments are shown. $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared to untreated cells.

Coupled to this impairment of UCP-1 expression, we found a direct inhibition of insulin-induced intracellular glucose uptake. Accordingly, insulin signaling elements such as protein kinase B and mitogen-activated kinase were inhibited.

Finally, we have characterized the modulation of adipose endocrine function by aldosterone. As we [23] and others [24] have demonstrated, brown adipocytes produce and secrete leptin, a peptide hormone with central and peripheral effects on multiple physiologic systems including energy homeostasis and the immune system [25]. In this study, we have described a strong stimulation of leptin mRNA expression in cultured adipocytes. In contrast to our findings, patients suffering from primary hyperaldosteronism appear to have decreased leptin levels [26,27]. This discrepancy may highlight differences between acute and chronic effects of this mineralocorticoid hormone. Furthermore, aldosterone induces the expression of MCP-1, a proinflammatory adipokine that has recently been implicated in insulin resistance and the metabolic syndrome [20]. Thus, aldosterone action may favor a proinflammatory profile of adipokine secretion that mediates cardiovascular complications associated with the syndrome [28].

In summary, this study describes direct actions of aldosterone on adipocyte metabolism and endocrine function. The mineralocorticoid hormone impairs thermogenesis, induces insulin resistance, and modulates the expression of proinflammatory adipokines. These functional changes may play a pathogenic role in states characterized by an activated RAAS such as the metabolic syndrome.

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