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Effects of adrenal hormones on the expression of adiponectin and adiponectin receptors in adipose tissue, muscle and liver

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ABSTRACT

Background: Adiponectin, an insulin-sensitive hormone that is primarily synthesized in adipose tissue, exerts its effects by binding to two receptors, adipoR1 and adipoR2. Little is known regarding the effects of glucocorticoids on the expression of adiponectin receptors.
Methods: Male Wistar rats were bilaterally adrenalectomized and treated with dexamethasone (0.2 mg/100 g) twice daily for 3 days. To analyze the potential effects of glucocorticoids, rats received two daily injections of the glucocorticoid receptor antagonist (RU-486, 5.0 mg) over the course of 3 days. Additionally, 3T3-L1 adipocytes and C2C12 myotubes were treated with dexamethasone, adrenaline or RU-486. The gene expression of adiponectin, adipoR1 and adipoR2 was determined by real-time PCR, and protein secretion was examined by Western blotting using lysates from retroperitoneal, epididymal and subcutaneous adipose tissue depots, liver and muscle.
Results: In rats, excess glucocorticoids increased the levels of insulin in serum and decreased serum adiponectin concentrations, whereas adrenalectomy decreased the mRNA expression of adiponectin (3-fold) and adipoR2 (7-fold) in epididymal adipose tissue and increased adipoR2 gene expression in muscle (3-fold) compared to control group sham-operated. Dexamethasone treatment did not reverse the effects of adrenalectomy, and glucocorticoid receptor blockade did not reproduce the effects of adrenalectomy. In 3T3-L1 adipocytes, dexamethasone and adrenaline both increased adipoR2 mRNA levels, but RU-486 reduced adipoR2 gene expression *in vitro*.
Conclusion: Dexamethasone treatment induces a state of insulin resistance but does not affect adiponectin receptor expression in adipose tissue. However, the effects of catecholamines on insulin resistance may be due to their effects on adipoR2.

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1. Introduction

Adiponectin, which is also known as ACRP30 (adipose complement-related protein of 30 kDa), GBP28 (gelatin-binding protein of 28 kDa), adipoQ and apM1 (adipose most abundant gene transcript 1), is the most abundant secreted plasma protein. Although it is primarily synthesized by adipocytes, it has also been detected in skeletal muscle [1–3], cardiomyocytes [4,5], osteoblasts [6], lymphocytes [7], the adrenal gland [8] and liver tissue [9,10]. Unlike other adipokines produced by adipose tissue, plasma levels of adiponectin have been shown to be lower in obese humans and animals than in lean controls [11–13]. Thus, plasma levels of adiponectin are inversely correlated with body mass index (BMI), visceral adiposity and plasma levels of triglycerides and low-density lipoprotein (LDL) [14–17] and positively correlated with plasma levels of high-density lipoprotein (HDL) [18].

Adiponectin is an insulin-sensitive hormone that has been shown to be reduced in clinical conditions associated with insulin resistance, such as obesity, type 2 diabetes, dyslipidemia and hypertension [19–21]. This adipokine has been reported to exhibit anti-inflammatory, anti-diabetic and anti-atherogenic properties [22–25] and is known to play a central role in lipid and glucose metabolism in muscle and liver [26,27].

The effects of adiponectin are mediated through two receptors. AdipoR1 has a strong affinity for adiponectin and is abundantly expressed in skeletal muscle. AdipoR2, exhibits an intermediate

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affinity for adiponectin and is mainly expressed in the liver [28–30]. Adiponectin receptors have also been detected in pancreatic beta cells [31], macrophages [32], osteoblasts [6], the adrenal gland [8], the human endometrium [33] and in adipose tissue, both in isolated adipocytes and in other cell types from the stroma-vascular fraction [30,34,35]. Although plasma adiponectin levels are inversely correlated with obesity and insulin resistance, Blüher et al. [36] have shown that the mRNA expression levels of the adiponectin receptors, adipoR1 and adipoR2, are positively associated with obesity, glucose intolerance and insulin resistance in human muscle, findings that have been replicated by other studies [37,38].

Several hormones have been shown to regulate adiponectin levels, including catecholamines [39,40], testosterone [41,42], prolactin [43], growth hormones [44,45] insulin [37,34,46,47] and glucocorticoids [44,48]. The production of glucocorticoids, which are adrenal steroid hormones, is primarily controlled by the hypothalamic–pituitary–adrenal axis, but glucocorticoids can also be generated locally from their metabolites. For example, this type of glucocorticoid recycling occurs in adipose tissue, where cortisone is converted into cortisol, wich has higher glucocorticoid activity than cortisone, by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) [49]. This hormone acts in a variety of physiological processes, including the immune response, the regulation of cardiovascular activity, brain function, glucose and lipid metabolism, insulin secretion and contribute to the development of obesity and insulin resistance [50–53].

Despite the similarity of the symptoms observed in metabolic syndrome and Cushing's syndrome, which is characterized by an excess of cortisol and is often observed in abdominal obesity, insulin resistance, diabetes and dyslipidemia, obese individuals frequently have normal or low plasma cortisol concentrations, despite elevated cortisol secretion and an elevated peripheral turnover rate [54].

The effects of glucocorticoids on the expression and secretion of adiponectin remain controversial. Both *in vitro* and *in vivo* studies have shown that glucocorticoids reduce plasma levels of adiponectin and inhibit adiponectin expression [48,55–59]. However, Shi et al. [60] observed decreased serum levels of adiponectin and reduced adiponectin mRNA expression in the adipose tissue of both obese and non-obese rats treated with glucocorticoids. Adrenalectomy has been shown to increase adiponectin levels in patients with Cushing's syndrome [61], to stimulate adiponectin expression in the white adipose tissue of ob/ob mice [57] and to reverse hyperglycemia in several models of obesity [62,63]. Other studies in humans have shown that dexamethasone injections (over the course of 2 or 5 days) do not modify adiponectin levels [64,65]. However, Jang et al. [59] observed a significant increase in serum adiponectin levels after dexamethasone treatment in humans, an effect that was suggested to be an adaptative mechanism compensating for the glucocorticoid-induced reduction in insulin sensitivity.

Relatively few studies have analyzed the effects of glucocorticoids on the expression of adiponectin receptors, and the few studies that do exist reported somewhat contradictory results. In hepatocytes, dexamethasone has been shown to stimulate the adipoR2 promoter, an effect that can be abolished by treatment with the glucocorticoid receptor antagonist, RU486 [66]. On the other hand, dexamethasone has been shown to decrease adipoR2 mRNA expression in human skeletal muscle [59].

In this study, we investigated the effects of dexamethasone treatment on the expression of adiponectin and its receptors in insulin- and glucocorticoid-responsive tissues, including adipose, skeletal muscle and liver, to determine a potential role for adiponectin and its receptors in the actions of glucocorticoids.

2. Materials and methods

2.1. Animals

Twelve-week-old male Wistar rats were obtained from the Federal University of São Paulo, Centro de Desenvolvimento de Modelos Experimentais (CEDEME). Rats were group housed in an animal facility under controlled temperature. Rats were kept on a 12-h light/dark cycle at 22 ± 1 °C with free access to food and water. All animal procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Committee on Animal Research Ethics of the Federal University of São Paulo (Process No. 01201-6).

2.2. Surgery and dexamethasone treatment

After 1 week of acclimatization, rats were anesthetized using a mixture of ketamine and xylazine (66.6 and 13.3 mg/kg, respectively, via intraperitoneal injection) and divided into three different experimental groups: adrenalectomized animals (ADREC) that underwent a bilateral adrenal gland removal via a dorsal incision; adrenalectomized animals that were injected subcutaneously (s.c.) with a supra-physiological (pharmacological) dose of dexamethasone (A-DEX; Aché; 2 mg/Kg body weight, 2×/day); and sham-operated animals (S-ADREC) that underwent a similar surgery without removal of the adrenal glands. We used a pharmacological dose of dexamethasone to ensure an excess of this hormone. Both the ADREC and the S-ADREC groups received two daily s.c. injections of saline solution (NaCl 0.9%). Saline solution was also provided to adrenalectomized rats. Animals were sacrificed by decapitation without sedation 72 h after surgery. Retroperitoneal (RET), epididymal (EPI) and subcutaneous (SUB) adipose tissues, gastrocnemius muscle (MUSC) and liver were collected, frozen in liquid nitrogen and stored at −80 °C until the extraction of total RNA or protein.

2.3. The effects of blocking type II glucocorticoid receptors

A group of rats (12-weeks old) received s.c. injections of either the type II glucocorticoid receptor antagonist, RU-486 (Mifepristone, Sigma–Aldrich; 5.0 mg per animal, 2×/day), or vehicle for 72 h (control group). Fourteen hours after the last injection, rats were sacrificed by decapitation without sedation. RET, EPI and SUB adipose tissues, gastrocnemius muscle and liver were collected, frozen in liquid nitrogen and stored at −80 °C until the extraction of total RNA or protein.

2.4. Hormone analysis

Trunk blood was collected and immediately centrifuged, and the resulting serum was stored at −80 °C until the measurement of serum hormones. The levels of insulin and adiponectin in rat serum were determined using commercial ELISA kits (LINCO, USA and AdipoGen, San Diego, USA, respectively). Glucose levels were assessed using the Glucose PAP Liquiform kit (Labtest, Brazil), and serum corticosterone was assayed using an Enzyme Immunoassay (EIA) (Cayman Chemical Co., USA).

2.5. 3T3-L1 adipocyte and C2C12 myocyte cell cultures

3T3-L1 cells obtained from the American Type Culture Collection (ATCC, USA) and C2C12 cells obtained from the Rio de Janeiro Cell Bank (UFRJ, Brazil) were cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air. 3T3-L1cells were grown in culture medium (DMEM culture medium supplemented with 25 mM

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