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Resistin, but not adiponectin, inhibits dopamine and norepinephrine release in the hypothalamus

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Abstract

Adiponectin (Adipocyte Complement-Related Protein of 30 kDa, ACRP30) and resistin are adipocyte-derived polypeptide hormones playing a role in metabolic homeostasis. Their plasma levels are inversely (adiponectin) or directly (resistin) correlated to obesity and they have opposite effects on insulin sensitivity. Adipose tissue hormones such as leptin have been shown to modulate neurotransmitters which control feeding in the hypothalamus. We have studied the effects of adiponectin and resistin on dopamine, norepinephrine and serotonin release from hypothalamic neuronal endings (synaptosomes) in vitro. We have found that adiponectin does not modify either basal or depolarization-induced amine release, while resistin inhibits the stimulated release of dopamine and norepinephrine, leaving unaffected serotonin release. We can conclude that, similarly to leptin, but differently from adiponectin, the adipose tissue hormone resistin could affect the central mechanisms of feeding by inhibiting catecholamine release in the hypothalamus.

Keywords: Adiponectin; Resistin; Hypothalamus; Dopamine; Norepinephrine; Serotonin

1. Introduction

Feeding and energy expenditures are modulated by the interplay of hormones and neurotransmitters in the central nervous system, where the hypothalamus plays a pivotal role in the transduction of peripheral afferents into satiety and feeding signals (Kalra et al., 1999). The adipose tissue, besides its role of energy storage tissue, has increasingly been regarded as an endocrine-like tissue, since the discovery of adipocyte-derived peptides, or adipocytokines, such as leptin, which are able to modulate a wide range of biological functions, among which energy homeostasis, both peripherally, and at the hypothalamic level (Ahima and Flier, 2000). Adiponectin (Adipocyte Complement-Related Protein of 30 kDa, ACRP30) is expressed exclusively in the adipocytes (Scherer et al., 1995), and has been shown to be inversely correlated to obesity and insulin resistance (Weyer et al., 2001). Available evidence suggests that adiponectin controls energy homeostasis at the peripheral level, decreasing body weight independently from food intake (Fruebis et al., 2001; Tsao et al., 2002). Resistin is another adipocyte-derived polypeptide hormone playing a role in metabolic homeostasis seemingly opposite to adiponectin. Plasma resistin levels are increased in diet-induced and genetic forms of obesity and are causally related to insulin resistance in the mouse (Steppan et al., 2001), although other studies have shown decreased resistin levels in different mouse models of obesity (Way et al., 2001). In humans, plasma resistin is positively correlated to body fat mass (Yannakoulia et al., 2003).

It has been shown that circulating peptides can gain access to the central nervous system (CNS) either by simple lipophilic diffusion or by saturable transport systems, such as the case of leptin (Banks et al., 1996; Kastin et al., 1999). In the hypothalamus, energy homeostasis regulatory peptides delivered from the periphery interact with CNS neuropeptides and neurotransmitters, among which catecholamines and serotonin play a major role in

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the regulation of feeding and energy expenditures (Kalra et al., 1999). We have previously reported that peripheral hormones such as leptin, amylin and ghrelin modulate dopamine, norepinephrine and serotonin release in the hypothalamus, which could partially mediate their appetite regulatory effects (Brunetti et al., 1999, 2002).

In order to evaluate whether adiponectin and resistin could also play a role at the CNS level, in the present study we have evaluated their effects on dopamine, norepinephrine and serotonin release from hypothalamic neuronal endings (synaptosomes) in vitro.

2. Materials and methods

Hypothalamic synaptosomes were obtained from male Wistar rats (200–250 g), as previously described (Brunetti et al., 1999). They were loaded with either [^{3}H]dopamine, [^{3}H]norepinephrine, or [^{3}H]serotonin, perfused in water-jacketed superfusion chambers with Krebs–Ringer buffer (0.6 ml/min), and perfusate was collected (1 min fractions for serotonin, and 2 min fractions for dopamine and norepinephrine release) to detect released [^{3}H] by liquid scintillation scanning. The European Community guidelines for the use of experimental animals have been adhered to and the protocol was approved by the institutional ethics committee. In a first set of experiments, either adiponectin or resistin were added to the perfusion buffer, in graded concentrations, for 5 min in the serotonin release experiments, and for 10 min in the dopamine and norepinephrine release experiments, followed by 8 min with Krebs buffer alone. Amine release was calculated as the means ± S.E.M of the percentage of [^{3}H] recovered in the stimulus and return to basal fractions (a total of 11 fractions for serotonin, and 10 fractions for dopamine and norepinephrine), compared to total loaded [^{3}H]. A second set of experiments was run to evaluate the effects of the peptides on neurotransmitter release induced by a mild depolarizing stimulus. After a 30-min equilibration perfusion with buffer alone, a 23-min perfusion with the peptides (0.1–10 nM) was started, where in the final 3 min, K^+ concentration in the perfusion buffer was elevated to 15 mM (after removal of equimolar concentrations of Na^+). A time–response curve relative to the percentage of [^{3}H] recovered in each perfusate fraction compared to total loaded [^{3}H] was plotted, and amine release was calculated as the area under the time–response curve (AUC) corresponding to 3-min depolarization + return to basal period in Krebs–Ringer buffer (a total of eight fractions). Preliminary experiments showed that monoamine reuptake is negligible due to the rapid removal of released amines by perfusion flow, intrasynaptosomal metabolism is negligible for dopamine and norepinephrine, while in the experiments evaluating serotonin release, a column chromatography of the perfusate proved necessary to separate serotonin from its metabolites, as previously described (Orlando et al., 2001).

Data represent the group means ± S.E.M. of three to five experiments performed in triplicate. Treatment and control group means were compared by the analysis of variance (ANOVA) followed by Student–Newman–Keul's multiple comparison test (GraphPad Prism 2.00 software).

Human adiponectin, 0.1 mg, and human resistin, 0.1 mg, were purchased from Phoenix Pharmaceuticals, USA. Considering that adiponectin and resistin circulate in plasma in concentrations around 1 μM and 10 nM, respectively (Yannakoulia et al., 2003), they were tested in the concentration ranges 0.01–1 μM for adiponectin, and 0.1–10 nM for resistin. [^{3}H]dopamine (40–60 Ci/mmol, 250 μCi pack size), [^{3}H]norepinephrine (30–50 Ci/mmol, 250 μCi pack size), and [^{3}H]serotonin (10–20 Ci/mmol, 1 mCi pack size), were purchased from Amersham Pharmacia Biotech, Italy.

3. Results

Basal amine release was not modified by either adiponectin, or resistin.

3.1. Adiponectin

Means ± S.E.M. of the percentage of [^{3}H]amine recovered in the stimulus and return to basal fractions respect to total loaded [^{3}H]. [^{3}H]dopamine: control, 1.44 ± 0.06; 0.01 μM, 1.46 ± 0.01; 0.1 μM, 1.46 ± 0.02; 1 μM, 1.47 ± 0.02; [^{3}H]norepinephrine: control, 1.47 ± 0.02; 0.01 μM, 1.48 ± 0.04; 0.1 μM, 1.49 ± 0.01; 1 μM, 1.46 ± 0.03; [^{3}H]serotonin: control, 0.97 ± 0.06; 0.01 μM, 0.98 ± 0.05; 0.1 μM, 0.99 ± 0.04; 1 μM, 0.96 ± 0.03.

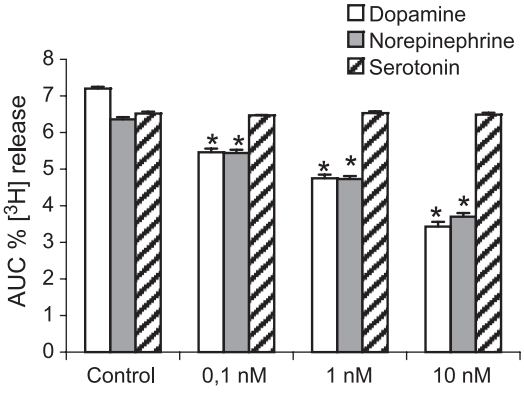

Fig. 1. Effects of resistin (0.1–10 nM) on depolarization-induced dopamine, norepinephrine and serotonin release. The synaptosomes were perfused with K^+ (15 mM) in Krebs–Ringer buffer for 3 min (control) or with graded concentrations of resistin in K^+ (15 mM) Krebs–Ringer buffer for 3 min, after a 20-min pre-incubation with the peptide in Krebs–Ringer buffer. The columns represent the area under the time–response curve (AUC) of the percentage of [^{3}H]amine recovered, respect to total (fractions + filters); each column represents the mean ± S.E.M. of three to five experiments performed in triplicate; ANOVA, $P < 0.0001$; *$P < 0.001$ vs. the respective control.

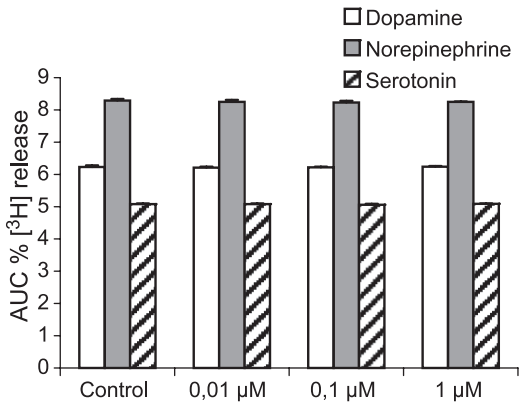

Fig. 2. Effects of adiponectin (0.01–1 μM) on depolarization-induced dopamine, norepinephrine and serotonin release. The synaptosomes were perfused with K^+ (15 mM) in Krebs–Ringer buffer for 3 min (control) or with graded concentrations of adiponectin in K^+(15 mM) Krebs–Ringer buffer for 3 min, after a 20-min pre-incubation with the peptide in Krebs–Ringer buffer. The columns represent the area under the time–response curve (AUC) of the percentage of [^{3}H]amine recovered, respect to total (fractions + filters); each column represents the mean ± S.E.M. of three to five experiments performed in triplicate.

3.2. Resistin

Means ± S.E.M. of the percentage of [^{3}H]amine recovered in the stimulus and return to basal fractions respect to total loaded [^{3}H]. [^{3}H]dopamine: control, 1.66 ± 0.05; 0.1 nM, 1.74 ± 0.03; 1 nM, 1.71 ± 0.10; 10 nM, 1.70 ± 0.03; [^{3}H]norepinephrine: control, 0.89 ± 0.02; 0.1 nM, 0.85 ± 0.02; 1 nM, 0.86 ± 0.01; 10 nM, 0.88 ± 0.03; [^{3}H]serotonin: control, 1.43 ± 0.05; 0.1 nM, 1.38 ± 0.03; 1 nM, 1.40 ± 0.05; 10 nM, 1.42 ± 0.03.

After preincubating the synaptosomes with graded concentrations of adiponectin and resistin, and then perfusing with depolarizing buffer (K^+ 15 mM), we observed that resistin inhibited the stimulated release of dopamine and norepinephrine, leaving unaffected serotonin release (Fig. 1), while adiponectin did not modify either amine release (Fig. 2).

4. Discussion

A wide body of evidence suggests that the adipose tissue hormones regulate fuel homeostasis both peripherally and at the hypothalamic level, where catecholamines and serotonin can be regarded as central transducers of peripheral signaling (Kalra et al., 1999; Ahima and Flier, 2000). The role played by dopamine in the central regulation of food intake is controversial. Some data support inhibition of feeding after dopamine administration into the hypothalamus (Gillard et al., 1993) or following dopamine re-uptake inhibition induced by anorectic drugs such as the amphetamines (Samanin and Garattini, 1993). On the contrary, other studies have shown that dopamine administration into the lateral hypothalamus stimulates food intake and obese rats have increased dopamine levels in the hypothalamus (Yang and Meguid, 1995). Dopamine is required for the hyperphagia that is associated with leptin deficiency (Szczypka et al., 2000) and we have previously shown that leptin inhibits dopamine release from hypothalamic synaptosomes (Brunetti et al., 1999), which could be related to its central anorectic effects. Moreover, the pancreatic hormone amylin, which inhibits feeding, as well as the anorectic peptides cocaine and amphetamine-regulated transcript (CART) peptide-(55–102) and thyrotropin releasing hormone (TRH), also inhibit hypothalamic dopamine release (Brunetti et al., 2000, 2002). The present findings support a central role for resistin, but not adiponectin, in inhibiting hypothalamic dopamine release (Figs. 1 and 2), which could add to the peripheral effects of both peptides in regulating fuel homeostasis.

Hypothalamic norepinephrine has also been shown to be involved in feeding regulation, with either inhibition or stimulation of food intake mediated by α_1- or α_2-adrenoceptors, respectively (Wellman et al., 1993) On the other hand, chronic norepinephrine infusion into the ventromedial hypothalamus induces obesity in rats (Shimazu et al., 1986). We have previously reported that out of several hormones and neuropeptides involved in feeding regulation, only leptin inhibits norepinephrine release from hypothalamic synaptosomes (Brunetti et al., 1999), while CART peptide-(55–102), TRH, amylin, and ghrelin do not modify norepinephrine release (Brunetti et al., 2000, 2002). Now, we have observed a dual inhibitory effect of resistin on both dopamine and norepinephrine release, while adiponectin has no activity on either catecholamines (Figs. 1 and 2). Leptin, resistin, and adiponectin are all produced in the adipocyte and their plasma concentrations are directly (leptin and resistin) or inversely (adiponectin) related to total adipose store (Frederich et al., 1995; Steppan et al., 2001; Weyer et al., 2001). All three peptides play a role in the obesity associated insulin resistance mainly acting at the peripheral level (Steppan and Lazar, 2002), but only leptin has been clearly shown to affect food intake by CNS mechanisms (Campfield et al., 1996). The present findings show that resistin effects closely mirror leptin-induced inhibition of dopamine and norepinephrine release in the hypothalamus (Brunetti et al., 1999), supporting a role for both catecholamines as central mediators of adipocyte-repleted signaling in the CNS.

Serotonin is known to inhibit feeding at the hypothalamic level (Leibowitz and Alexander, 1998), and we have previously shown that orexin-A, orexin-B, and ghrelin inhibit serotonin release from hypothalamic synaptosomes, which could be related to their physiological role of feeding stimulatory peptides. On the other hand, anorectic peptides such as leptin, CART peptide-(55–102) and amylin do not affect hypothalamic serotonin release (Orlando et al., 2001; Brunetti et al., 2002). The present data seem to exclude an

involvement of serotonin in mediating the effects of adiponectin and resistin at the hypothalamic level.

In conclusion, resistin, but not adiponectin, besides its peripheral activities in the regulation of energy metabolism, could be involved in the CNS mechanisms of feeding, by inhibiting dopamine and norepinephrine release in the hypothalamus.

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