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Short communication

Leptin inhibits norepinephrine and dopamine release from rat hypothalamic neuronal endings

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Abstract

Noradrenergic and dopaminergic afferents arising from the brain stem to the hypothalamus still play a poorly characterised role in food intake control. We have studied the effect of leptin, an adipocyte-derived hormone which has been implicated in the regulation of feeding behaviour, on [^{3}H]norepinephrine and [^{3}H]dopamine release from perfused hypothalamic neuronal endings (synaptosomes) in vitro. We have found that leptin (0.01–10 nM) does not modify basal, while it inhibits depolarization-induced norepinephrine and dopamine release. We can hypothesize that at least part of the anorectic activity of leptin in the hypothalamus is effected through an inhibition of noradrenergic and dopaminergic firing.

Keywords: Leptin; [^{3}H]Norepinephrine release; [^{3}H]Dopamine release; Hypothalamus

1. Introduction

Leptin, a 16-kDa polypeptide hormone coded by the *ob* gene (Zhang et al., 1994) and released into the blood by adipocytes, plays a pivotal role in signalling adipose store repletion to the central nervous system. In *ob/ob* leptin-deficient mice, obesity spontaneously develops (Zhang et al., 1994) and leptin administration restores normal weight (Halaas et al., 1995; Pelleymounter et al., 1995); in contrast, human obesity is characterized by elevated leptin levels and it has been hypothesized that there is a resistance to its effects in the central nervous system (Considine et al., 1996). The physiological actions of leptin are mainly effected by the interaction with neuropeptides which inhibit food intake and stimulate energy expenditure (Campfield et al., 1996).

In the ventromedial hypothalamus, norepinephrine and dopamine are known to modulate food intake (Shiraishi, 1991;Wellman et al., 1993; Yang and Meguid, 1995) and receptors for leptin have been localized in the hypothalamus, including cathecolamine-stimulating neurons (Håkansson et al., 1998).

In order to further investigate the hypothalamic mediators of the anorectic effect of leptin, we have studied the role of leptin in [^{3}H]norepinephrine and [^{3}H]dopamine release from perfused hypothalamic neuronal endings (synaptosomes) in vitro, both in basal conditions, and after K^+(15 mM)-induced depolarization.

2. Materials and methods

2.1. Animals

Male adult Wistar rats (200–220 g) were housed in a thermoregulated environment (23 ± 1°C), with automatic control of light/darkness cycle. Food and water were available ad libitum.

2.2. Drugs

Recombinant human leptin was kindly provided by IRBM P. Angeletti (Pomezia, Italy).

2.3. Hypothalamic synaptosomes

Hypothalamic synaptosomes were prepared according to Gray and Whittaker (1962). Briefly, male Wistar rats (200–250 g) were sacrificed by decapitation, the hypothalami quickly dissected, homogenized in 0.32 M saccharose and centrifuged, first at $1000 \times g$ for 5 min, and then at

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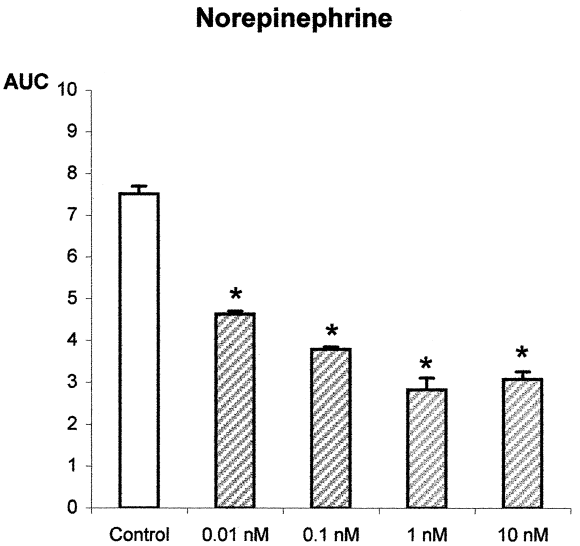

Fig. 1. Effect of leptin (0.01–10 nM) on depolarization-induced norepinephrine release. The control group (white column) was perfused with K^+ (15 mM) in Krebs–Ringer buffer for 3 min; the leptin groups (hatched columns) were perfused with graded concentrations of leptin in K^+ (15 mM) Krebs–Ringer buffer for 3 min, after a 20-min pre-incubation with leptin in Krebs–Ringer buffer. The columns represent the area under the time–response curve (AUC) of the percentage of [^{3}H]norepinephrine recovered, with respect to total (fractions + filters); each column represents the mean ± S.E.M. of three to five experiments performed in triplicate; $^*P < 0.001$.

12,000 × *g* for 20 min, to isolate neuronal endings from cell nuclei and glia. Then, the synaptosome suspension was incubated, at 37°C, under O_2/CO_2 95/5%, pH 7.2–7.4, in Krebs–Ringer buffer (mM/l: NaCl 125, KCl 3, $MgSO_4$ 1.2, $CaCl_2$ 1.2, NaH_2PO_4 5, Tris–HCl 10, glucose 10, ascorbic acid 1), with either 0.05 μM norepinephrine, [^{3}H]norepinephrine, or 0.05 μM dopamine, [^{3}H]dopamine, for 15 min, to make synaptosomes uptake [^{3}H]norepinephrine or [^{3}H]dopamine, substituting for the endogenous norepinephrine or dopamine pool. Then, synaptosomes were layered onto 0.8 μM Millipore filters, placed into 37°C water-jacketed superfusion chambers (18 different chambers for each experiment), and perfused with the above buffer (0.6 ml/min). After 30 min, to allow stable release (equilibration period), perfusate was collected in 2 min fractions, and after the first three to four fractions (basal release) testing drugs were added to the perfusion buffer for 10 min (stimulus), followed by 10 min with Krebs buffer alone (return to basal). To evaluate the effect of leptin on neurotransmitter release during depolarization [3 min perfusion with K^+(15 mM), after removal of equimolar concentrations of Na^+ in perfusion buffer], leptin was added, after the equilibration period, both 20 min prior (pre-stimulus) and 3 min during K^+(15 mM) perfusion (stimulus). Finally, beta-emission from perfusate fractions, corresponding to [^{3}H]norepinephrine or [^{3}H]dopamine release, was detected by liquid scintillation scanning.

2.4. Analysis of data

Catecholamine release has been calculated as either the means ± S.E.M. of the percentage of [^{3}H]norepinephrine or [^{3}H]dopamine recovered in each fraction respect to total (fractions + filter) or the means ± S.E.M. of the area under the time–response curve (AUC); each group represents the mean ± S.E.M. of three to five experiments performed in triplicate. Treatment and control group means are compared by the Student–Newman–Keul test and group differences considered significant for $P < 0.05$.

3. Results

We have found leptin, in the dose range 0.01–10 nM, does not modify basal norepinephrine and dopamine release (means ± S.E.M. of the percentage of [^{3}H]norepinephrine or [^{3}H]dopamine recovered in each fraction with respect to total. Norepinephrine: Krebs–Ringer buffer, 1.12 ± 0.02; leptin 0.01 nM, 1.13 ± 0.01; 0.1 nM, 1.14 ± 0.01; 1 nM, 1.12 ± 0.01; 10 nM, 1.12 ± 0.01. Dopamine: Krebs–Ringer buffer, 1.67 ± 0.04; leptin 0.01 nM, 1.55 ± 0.02; 0.1 nM, 1.54 ± 0.03; 1 nM, 1.70 ± 0.01; 10 nM, 1.69 ± 0.15). In order to investigate a possible modulatory role of leptin in neurotransmitter release, such as potentiation or inhibition of stimulated release, we have incubated the synaptosomes with leptin (0.01–10 nM) 20 min before,

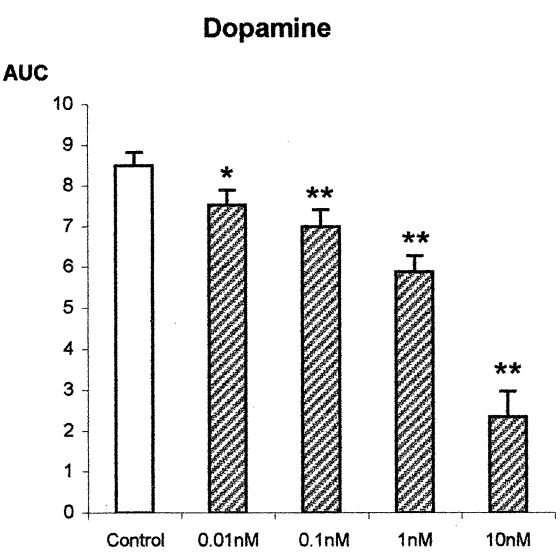

Fig. 2. Effect of leptin (0.01–10 nM) on depolarization-induced dopamine release. The control group (white column) was perfused with K^+ (15 mM) in Krebs–Ringer buffer for 3 min; the leptin groups (hatched columns) were perfused with graded concentrations of leptin in K^+ (15 mM) Krebs–Ringer buffer for 3 min, after a 20-min pre-incubation with leptin in Krebs–Ringer buffer. The columns represent the area under the time–response curve (AUC) of the percentage [^{3}H]dopamine recovered, with respect to total (fractions + filters); each column represents the mean ± S.E.M. of three to five experiments performed in triplicate; $^*P < 0.05$, $^{**}P < 0.001$.

and during a 3-min depolarizing stimulus, which was induced by raising the K^+ concentration to 15 mM in the Krebs–Ringer perfusion buffer. Leptin alone did not modify basal neurotransmitter release, while it caused a dose-dependent inhibition of depolarization-induced norepinephrine (Fig. 1) and dopamine (Fig. 2) release. The maximal inhibitory effect was similar for both neurotransmitters, but the $EC_{50} \pm$ S.E.M. was lower for norepinephrine (0.25 ± 0.02 nM) respect to dopamine (6.30 ± 0.15 nM).

4. Discussion

The hypothalamus has long been considered to be the site of the feeding regulatory center, since it was shown that lesions of the medial hypothalamus lead to increased food intake and obesity (Wellman et al., 1993). Leptin receptors have been localized throughout the hypothalamus (Håkansson et al., 1998), and it has been suggested that leptin can regulate adipose store repletion by reciprocally modulating stimulating and inhibiting transmitters in the feeding regulatory areas of the hypothalamus, resulting in a decrease in food intake and an increase in energy expenditure. Leptin has been shown to stimulate corticotrophin releasing hormone (CRH) (Costa et al., 1997; Raber et al., 1997; Brunetti et al., 1999), pro-opiomelanocortin (Schwartz et al., 1997; Thornton et al., 1997), and prostaglandin E_2 and $F_{2\alpha}$ (Brunetti et al., 1999) in the hypothalamus, which all mediate anorectic behaviour. In contrast, leptin inhibits neuropeptide Y, galanin and melanin-concentrating hormone gene expression in the hypothalamus, where these peptides display stimulatory effects on feeding (Sahu, 1998).

The present findings, which show that leptin inhibits depolarization-induced norepinephrine (Fig. 1) and dopamine (Fig. 2) release from isolated neuronal endings in vitro, further extend previous reports showing leptin is able to reduce excitatory postsynaptic currents in the hypothalamus (Glaum et al., 1996), possibly by activation of ATP-sensitive K^+ channels (Spanswick et al., 1997), and point to a possible role for catecholamines in leptin signalling in the hypothalamus. It is well-established that neurons arising from the paraventricular nucleus of the hypothalamus play a key role in food intake control, by inhibiting the nucleus of the solitary tract and the dorsal nucleus of the vagus in the brain stem (Kirchgessner and Sclafani, 1988) and leptin receptors have been demonstrated in the paraventricular nucleus (Håkansson et al., 1998). The paraventricular nucleus, in turn, is largely innervated by noradrenergic and dopaminergic fibers arising from the brain stem (Swanson and Sawchenko, 1983). Norepinephrine release from presynaptic terminals in the paraventricular nucleus stimulates food intake (Leibowitz and Brown, 1980), an effect which seems to be mediated by α_2-adrenoceptors (Wellman et al., 1993). Moreover, chronic infusion of norepinephrine into the ventromedial hypothalamus induces obesity in rats (Shimazu et al., 1986). Interestingly, *ob/ob* genetically obese mice, which are characterized by loss of the leptin gene, and *db/db* genetically obese mice, which are lacking in the leptin receptor gene, have been found to display increased norepinephrine levels in the hypothalamus (Lorden et al., 1975; Currie and Wilson, 1992): this has been hypothesized to underlie their obesity, even if increased brain norepinephrine levels in leptin-deficient rodents do not imply increased noradrenergic function, but could result from decreased norepinephrine release. On the other hand, the role of hypothalamic dopamine in feeding control is still unsettled: on one side, both amphetamine, a dopamine reuptake inhibitor, and dopamine itself microinjected into the perifornical hypothalamus have been shown to inhibit food intake (Gillard et al., 1993); on the other hand, dopamine dose-dependently increases food intake when injected into the lateral hypothalamus (Shiraishi, 1991) and hypothalamic dopamine levels are significantly higher in obese than in lean rats (Yang and Meguid, 1995).

In conclusion, our findings, which show that leptin is capable of inhibiting depolarization-induced norepinephrine and dopamine release (Figs. 1 and 2), support an enhancing action on feeding by norepinephrine and dopamine in the hypothalamus. This could explain part of the anorectic activity of leptin by inhibition of dopaminergic and noradrenergic fibers in the hypothalamus.

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