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ORIGINAL ARTICLE

The role of leptin in the regulation of pituitary hormones release

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Abstract

OBJECTIVE: It has been reported that leptin plays an important role in the regulation of food intake and energy expenditure. There are controversial opinions about effects of leptin on the hormonal system. The aim of this study was to estimate the of leptin on pituitary hormones release after central and peripheral administration.
METHODS: Leptin was injected intracerebroventricularly (icv) in a dose of 0.5 μg/5μl aCSF (artificial cerebrospinal fluid) for 5 min in Wistar Kyoto rats. At 60 and 120 min after injections the animals were decapitated.
Leptin in a dose of 10 μg in 300 μl of saline was administered intravenously (i.v). At 60 mins the animals were decapitated.
Serum rLH, rFSH, rPRL, rTSH, rGH concentrations were measured with RIA methods.
RESULTS: After central (icv) injection of leptin we observed an increase of rGH, rTSH and a decrease of rPRL. However, after peripheral (iv) injection of leptin we found a decrease of rGH and rTSH and an increase of rPRL. We did not fnd any significant changes in LH and FSH after icv and iv injection of leptin.
CONCLUSIONS: The opposite effects of leptin on pituitary hormones release were observed due to the method of leptin administration. Leptin may play a modulating role in the mechanism of pituitary hormones release.

Introduction

Leptin – the product of the OB gene, an adipocyt secreted hormone plays an important role in the regulation of food intake and energy expenditure [1, 2]. It has been reported that leptin is synthesized mainly by adipocytes and its plasma levels in humans are strongly correlated with body mass index (BMI) and fat mass [2, 3, 4, 6]. Moreover, leptin is involved in the regulation of thermogenesis, modulation of immune system and many neuroendocrine functions. Leptin could exert a central and peripheral action through hypothalamic pathways as well as through direct effects on hormones release. There are controversial opinions about effects of leptin on the hormonal system.

The aim of this study was to estimate the effect of leptin on pituitary hormones release after central and peripheral administration.

Material and methods

Female Wistar-Kyoto rats (240–260g) were kept under controlled light schedule of 12-h light, 12-h dark (lights on at 0600h) in a temperature-controlled environment (22–24 ºC) with free access to food and water.

All experimental procedures were approved by the First Warsaw Ethic Committee for Experiments on Animals (the M. Nencki Institute of Experimental Biology, the Polish Academy of Sciences).

Experiment I.

Intracerebroventricular administration of leptin.

Surgical procedure

Three weeks after ovariectomy (OVX) rats were anesthetized with ketamine and implanted with permanent stainless steel cannulas into the third ventricle of the brain according to the rat stereotaxic atlas. The guide cannula location was confirmed with the flow of cerebrospinal fluid and a removable stylet was inserted so that its tip was flush with the tip of guide cannula. During a 7-day period of recovery, rats were transferred to individual cages and handled daily to minimize stress-related effects of the injection procedures. On the day of experiment, freely moving rats were connected to an automatic pump (CMA/100, Sweden) and received an intracerebroventricular (icv) microinjections for 5 min. of 5 µl artificial cerebrospinal fluid (aCSF), or 0,5 µg leptin dissolved in 5 µl aCSF.

Intracereboventricular injections of leptin or vehicle were performed in the morning (between 09.00 – 11.00). At 60, 120 min. after the microinjection of leptin or vehicle, animals were decapitated and trunk blood was collected in plastic tubes containing 1000 I.U. Trascolane (inhibitor of proteases) per each ml of blood. Blood samples were centrifuged (3000rpm for 20 min.) and serum was frozen until hormonal analyses were performed.

Experiment II.

Intravenous injection of leptin

Leptin in a dose of 10 µg in 300 µl of saline was injected intravenously (i.v) by tail vein in rats. At 60 mins the animals were decapitated and the trunk blood was collected in plastic tubes containing inhibitor of proteases (Trascolan). The serum samples were stored at – 20 C until assayed by RIA methods for rLH, rFSH, rPRL, rTSH, rGH.

Results

Effects of leptin administered intracerebroventricularly (icv) on pituitary hormones release were presented in *Table 1*.

Effects of leptin injected intravenously (i.v) were shown in *Table 2*.

A marked increase in rGH ($p<0.001$) was observed after icv injection of leptin. However, rGH concentration was decreased in response to i.v leptin administration.

We found a significant increase in rTSH ($p<0.05$) after leptin administered centrally.

Serum TSH concentration was decreased after peripheral administration of leptin but those changes were not significant.

Serum rPRL concentration after icv leptin injection was significantly lower ($p<0.01$) than in the control group. The rPRL level significantly increased after i.v leptin injection ($p<0.05$).

We did not find any significant changes in LH and FSH release after icv and i.v injection of leptin.

Discussion

Leptin is a very important factor in the regulation of food intake and energy expenditure and it is also involved in the mechanism of sexual maturation and reproduction, in the regulation of the hypothalamo-pituitary-adrenal, thyroid and growth hormone (GH) axes.

Leptin activates gonadal hormones release through central stimulation of GnRH, whereas peripheral effect of leptin on steroid synthesis is inhibitory [7].

Table 1: effects of leptin injected intracerebroventricularly (icv) on pituitary hormones release in ovariectomized rats (ovx)

HORMONES	CONTROL(CSF icv)	**LEPTIN icv**
	$\bar{x} \pm$ SEM	$\bar{x} \pm$ SEM
rLH ng/ml	5.4 ± 0.3	5.6 ± 0.8n.s
rFSH ng/ml	7.9 ± 1.0	8.0 ± 0.9n.s
rPRL ng/ml	1.7 ± 0.2	**0.9** ± 0.01 ($p<0.01$)
rTSH ng/ml	3.2 ± 0.2	**4.5** ± 0.2 ($p<0.05$)
rGH ng/ml	27.3 ± 4.0	**44.5** ± 5.0 ($p<0.001$)

Table 2: Effects of leptin injected intravenously (i.V) on pituitary hormones release in ovariectomized rats (ovx)

HORMONES	CONTROL(0.9 % NaC) i.v	**LEPTIN i.v**
	$\bar{x} \pm$ SEM	$\bar{x} \pm$ SEM
rLH ng/ml	4.3 ± 0.4	4.0 ± 0.6n.s
rFSH ng/ml	23.2 ± 0.9	24.7 ± 1.8n.s
rPRL ng/ml	0.8 ± 0.07	**1.1** ± 0.05 ($p<0.05$)
rTSH ng/ml	3.5 ± 0.2	3.4 ± 0.4n.s
rGH ng/ml	35.2 ± 5.0	**23.6** ± 6.0 ($p<0.01$)

"*In vitro*" studies showed that leptin can suppress ovarial production of oestradiol and progesterone [8, 9]. Leptin plays a role in the acceleration of the onset of puberty [10] through triggering the pulsatile release of GnRH.

Leptin could act by stimulation of the secretion of GnRH by hypothalamic neurons or through direct effect on pituitary and stimulation of gonadotrophins [11, 12]. The NPY 1 receptor regulates leptin-mediated control of energy homeostasis and reproductive functions. On the other hand, oestrogens can [13] stimulate leptin secretion by adipocytes *in vitro* [13].

In our study we did not observe any significant changes in serum rLH and rFSH concentrations at 1h after both central and peripheral injections of leptin. In future we are going to measure serum gonadotrophins at the same short times after injection of leptin.

It has been published that leptin can regulate hypothalamo-pituitary-adrenal (HPA) axis both centrally through CRH release and peripherally at adrenal gland. Leptin could suppress HPA axis by inhibition of neuropeptide Y (NPY), which activates the HPA axis [15, 16]. On the contrary, Malendowicz et al. [17] showed that acute leptin injection augmented ACTH release. In studies in vitro leptin blunted the release of CRH induced by hypoglycaemia in isolated hypothalamic neurons but leptin did not alter the secretion of ACTH from isolated pituitary cells [18]. However, leptin can affect adrenal steroidogenesis and causes inhibition of corticosterone, aldosterone and dehydroepiandosterone [19, 20].

There are some controversial opinions about the effect of leptin on GH secretion. Some authors demonstrated that central infusion of leptin in rats strongly stimulated GH release [21, 22, 23, 24].

Leptin may regulate GH secretion by acting on GHRH and somatostatinergic activity [22, 24].

Watanobe and Habu [25] showed that leptin could increase GH release and alter in vivo the release of both GHRH and somatostatin, but not NPY release in rat hypothalamus.

However, Isozaki et al. [26] showed that leptin pretreatment of pituitary cells in culture did not change GHRH induced GH secretion.

Our results indicated a marked increase of GH release after icv injection of leptin. However, i.v administration of leptin leads to the decrease of serum GH concentration.

The opposite effects of leptin on PRL release after icv and iv injection were also observed.

It has been known that leptin and thyroid hormones have similar effects on thermogenesis and energy balance.

Leptin may influence the feedback regulation of TRH secreting neurons by thyroid hormones [7].

Some authors suggest that the central effect of leptin may be mediated through NPY and /or CRH, as neurons interacts with TRH [27, 28].

Legradi et al. [29] suggested that leptin can modulate the hypothalamic-pituitary-thyroid axis by regulation proTRH gene expression in the PVN.

However Gones et al. [30] did not find any correlation between leptin levels and pituitary- thyroid axis in healthy humans.

In our studies in vivo we have observed stimulating effects of leptin administered centrally on TSH release. Leptin has an acute stimulatory effect on TSH in vivo, but direct effect of leptin on pituitary is inhibitory [31].

Our results have confirmed studies of other authors that leptin plays an important role in the regulation of metabolic and neuroendocrine functions.

Conclusions

1. The opposite effects of leptin on pituitary hormones release were observed due to the method of leptin administration
2. Leptin may play a modulating role in the mechanism of pituitary hormones release.

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