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Effects of leptin and leptin fragments on steroid secretion of freshly dispersed rat adrenocortical cells

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Abstract

The biological actions of leptin on target tissues are mediated via several isoforms of receptors (Ob-Rs), which may differently interact with native leptin and its fragments. Based on the presence in the rat adrenals of at least two Ob-R isoforms and the conflicting findings on the effect of leptin on adrenocortical secretion, we investigated the effects of the native leptin and several leptin fragments (10^{-8} and 10^{-6} M) on aldosterone and corticosterone secretion from freshly dispersed rat zona glomerulosa (ZG) and zona fasciculata-reticularis (ZF/R) cells. Reverse transcription (RT)-polymerase chain reaction (PCR) showed the expression of Ob-Ra and Ob-Rb mRNAs in both ZG and ZF/R cells. Native murine leptin (1–147) enhanced aldosterone and corticosterone secretion from dispersed ZG and ZF/R cells, and similar effects were elicited by murine leptin fragment 116–130, and human leptin fragments 138–167, 150–167 and [Tyr] 26–39. Human leptin fragment 93–105 was ineffective, while fragment 22–56 decreased corticosterone output without affecting aldosterone secretion. Taken together, our findings indicate that in rat adrenocortical cells leptin and leptin fragments may differently interact with Ob-Rs or interact with different Ob-R isoforms. Moreover, they suggest that (1) the direct adrenocortical secretagogue effect of leptin mainly depends on the C-terminal sequence 116–166; and (2) the N-terminal sequence is not needed for leptin to activate Ob-Rs positively coupled to steroidogenesis, but is possibly responsible for a direct inhibitory effect on glucocorticoid secretion.

Keywords: Leptin; Leptin fragments; Adrenocortical cells; Aldosterone; Corticosterone

1. Introduction

Leptin, the protein product of the *ob* gene transcription, is an adipose tissue-secreted hormone, which regulates satiety, metabolic rate and thermogenesis (for review, see [1]). Leptin acts on target tissues via specific receptors, named Ob-Rs, of which several variants have been described (from Ob-Ra to Ob-Rf). Ob-R isoforms may be responsible for the different responses of cells to native leptin and its fragments [2].

Several studies demonstrated the expression of Ob-Rs in the rat and human adrenal cortex [3–6], and the predominance of Ob-Ra and Ob-Rb isoforms in the rat [7]. However, available data on the direct effect of leptin on adrenocortical-cell secretory activity are controversial. Although earlier investigations demonstrated a stimulating effect of leptin on basal aldosterone and corticosterone secretion from dispersed rat adrenocortical cells [8], subsequent studies reported an inhibitory effect of leptin on both basal and ACTH-stimulated corticosterone output [7,9]. Moreover, an inhibitory effect of leptin on cortisol release has been described in cultured human adrenocortical and NCI-H295 adrenal carcinoma cells [4,5,9,10].

In light of the expression of at least two Ob-R isoforms in the rat adrenals, and their possible different interactions with leptin fragments, we decided to investigate the direct effects of several leptin fragments on steroid secretion of freshly dispersed rat adrenocortical cells.

2. Materials and methods

Recombinant murine leptin (1–147) was purchased from Prepro. Tech. EC (London, UK). Leptin (116–130) amide (mouse), and human leptin fragments 150–167, 138–167, 93–105, 22–56 and [Tyr] 26–39 were obtained from Bachem

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AG (Bubendorf, Switzerland). All other laboratory reagents were provided from Sigma (St. Louis, MO). Adult female Wistar rats (160–180 g body weight), bred in our laboratory facilities, were used, and the experimental protocol was approved by the local Ethical Committee for Animal Studies.

Rats were decapitated, and their adrenal glands were promply removed and gently decapsulated to separate zona glomerulosa (ZG) from the zona fasciculata-reticularis (ZF/R). Dispersed capsular (ZG) and inner (ZF/R) cells were obtained by collagenase digestion and mechanical disaggregation [11]. Inner-cell contamination of capsular-cell preparation, as checked by phase microscopy, was less than 8%. The viability of isolated cells, as assayed by the trypan-blue exclusion test, was higher than 92%. Dispersed cells obtained from six to eight rats were pooled to obtain a single cell suspension, and six cell suspensions for each incubation experiment were employed. Aliquots of each cell suspension was frozen at −80 °C and used for gene expression studies.

Total RNA was extracted from frozen dispersed cells, and its reverse transcription (RT) to cDNA was carried out as previously detailed [12]. Polymerase chain reaction (PCR) was performed following the procedures described earlier [13], using the primers for Ob-Ra, Ob-Rb and Ob-Rf published by Tena-Sempere et al. [14]. In a thermal cycler (Delfi 100; MJ Research Inc., Waterston, MA), after a predenaturation step at 97 °C for 5 min, we used a denaturation step at 96 °C for 90 s, an annealing step at 55 °C for 90 s, and an extension step at 72 °C for 3 min for a total of 33 cycles. An additional elongation step at 72 °C for 10 min was then carried out. As positive control the expression of the househeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was detected. To rule out the possibility of amplifying genomic

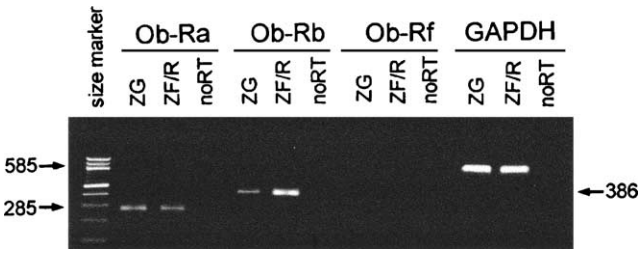

Fig. 1. Ethidium bromide-stained 2% agarose gel showing cDNA amplified with rat Ob-R and GAPDH specific primers from RNA of dispersed rat ZG and ZF/R cells. The following primers pairs were used: Ob-Ra sense, 5′-CCT ATC GAG AAA TAT CAG TTT A-3′ and ob-Ra antisense, 5′-TCA AAG AGT GTC CGC TCT CT-3′ (amplicon, 285 bp); Ob-Rb sense, 5′-TGG CCC ATG AGT AAA GTG AAT-3′ and Ob-Rb antisense, 5′-CCA GAA GAA GAG GAC CAA ATA-3′ (amplicon, 386 bp); Ob-Rf sense, 5′-AGA GGA TAT ATA GTG GAT GCC G-3′ and Ob-Rf antisense, 5′-CAC AAA TGA GCC ATC TTC AAA CC-3′ (amplicon, 411 bp); and GAPDH sense, 5′-CCC TCC ATT GAC CTC AAC TA-3′ and GAPDH antisense, 5′-GCC AGT GAG CTT CCC GTT CA-3′ (amplicon, 585 bp). Lane 1 was loaded with 200 ng of a size marker (Marker VIII; Roche Molecular Biochemicals, Indianapolis, IN). No amplification of PCR mixture without prior RT is shown as negative control.

DNA, one PCR was performed without prior RT of the RNA. Detection of the PCR amplification products was first carried out by size fractionation on 2% agarose-gel electrophoresis. Then, after purification using the QIAQuick PCR purification kit (Qiagen, Hilden, Germany), PCR products were identified by sequencing on an Alf sequencer (Pharmacia Biothech, Freiburg, Germany). Aliquots of each fresh-cell suspension (10^4 cells/ml, in Krebs-Ringer bicarbonate buffer with 0.3% glucose and 0.2% bovine serum albumin) were incubated with leptin or leptin fragments at the concentration of 10^{-8} or 10^{-6} M. Earlier studies showed that in our

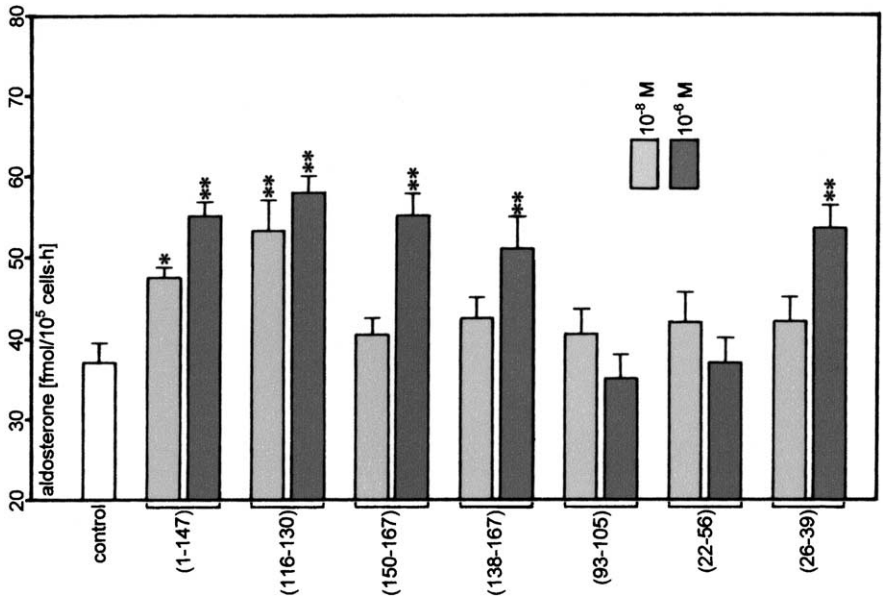

Fig. 2. Effects of leptin (1–147) and leptin fragments on basal aldosterone secretion from dispersed rat ZG cells. Bars are mean ± S.E.M. of six separate experiments: $^{*}P < 0.05$ and $^{**}P < 0.01$ from control.

female rats the plasma concentration of leptin was about 0.2×10^{-9} M [15]. However, we used these higher concentrations of leptin, because 10^{-8} M was previously found to be the minimal effective one in stimulating in vitro secretion from rat adrenocortical cells [8]. The incubation was carried out in a shaking bath at 37 °C for 60 min, in an atmosphere of 95% air–5% CO_2. At the end of the experiment, the incubation tubes were centrifuged at 4 °C and supernatants stored at −30 °C.

Aldosterone and corticosterone were extracted from incubation media and purified by HPLC [16], and their concentrations were estimated by specific RIA, as previously detailed [17]. Intra- and interassay variation coefficients were: aldosterone, 5.2 and 7.1%; and corticosterone, 7.5 and 9.4%, respectively. Data were expressed as mean ± S.E.M., and their statistical comparison was done by ANOVA, followed by the multiple range test of Duncan.

3. Results

Using the primers published by Tena-Sempere et al. [14], the expression of Ob-Ra and Ob-Rb mRNA was demonstrated in both ZG and ZF/R cells. Ob-Rf expression was not detected (Fig. 1). The expression of Ob-Ra and Ob-Rb was confirmed using the primers published by Jin et al. [18] (data not shown).

The incubation with the entire murine leptin molecule enhanced aldosterone and corticosterone secretion from dispersed ZG and ZF/R cells, respectively (Figs. 2 and 3). Similar effects were obtained by incubating cells with leptin (116–130), leptin (150–167), leptin (138–167) and Tyr-leptin (26–39), although fragments 150–167 and 26–39 elicited a significant aldosterone response only at a concentration of 10^{-6} M (Fig. 2). Leptin (22–56) did not affect aldosterone secretion, but decreased corticosterone output, while leptin (93–105) was ineffective (Figs. 2 and 3).

4. Discussion

Our present findings show that both ZG and ZF/R cells express Ob-Ra and Ob-Rb mRNAs, thereby confirming previous findings obtained in rat adrenal homogenates [7] and ruling out the possibility that Ob-R expression may be ascribed to connective tissue or blood vessels. Our results also confirm our earlier data indicating that native murine leptin has a stimulating effect on basal corticosteroid secretion from freshly dispersed rat adrenocortical cells [8]. As mentioned in the Introduction, this finding conflicts with the reported inhibitory effect of leptin on corticosterone output from isolated rat adrenocortical cells or adrenal slices [7,9], and, at present, we are not able to explain this discrepancy.

Earlier investigations revealed that under various experimental conditions leptin fragments exert biological effects both similar to and distinct from those of the native leptin molecule [7,19–25]. Structure–activity studies revealed that two segments of the leptin molecule affect its in vivo and in vitro activities: (1) the N-terminal amino-acid sequence 22–115, which is essential for the biological and receptor-binding activities; and (2) the C-terminal sequence 116–166 possessing a loop structure, that enhances the activity of the N-terminal region. The C-terminal disulfide bond is not needed for leptin activity [20,26]. Our present findings

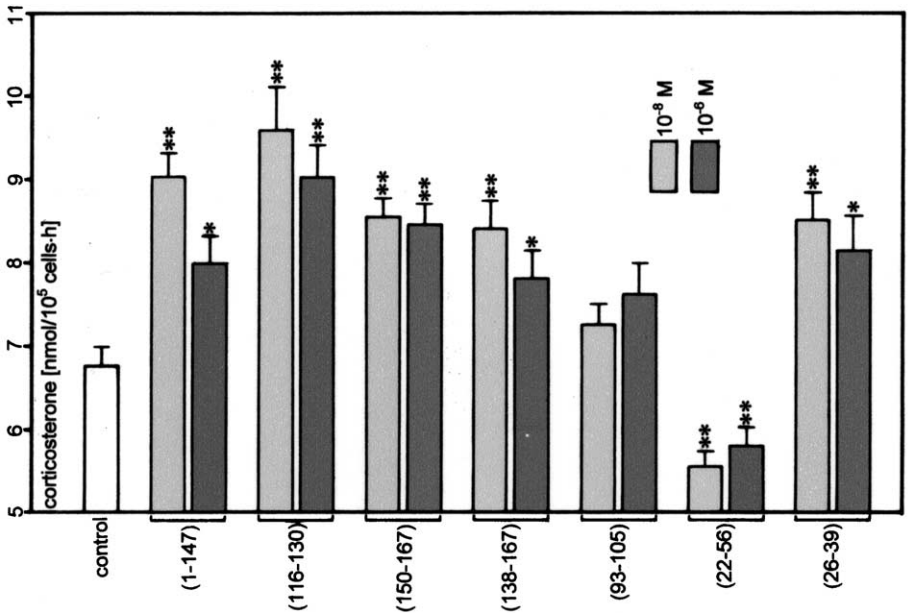

Fig. 3. Effects of leptin (1–147) and leptin fragments on basal corticosterone secretion from dispersed rat ZF/R cells. Bars are mean ± S.E.M. of six separate experiments: $^{*}P < 0.05$ and $^{**}P < 0.01$ from control.

do not accord with this view, as far as leptin effects on rat adrenocortical-cell secretion are concerned.

Murine leptin fragments 116–130, in disagreement with the reported inhibitory effect on basal and ACTH-stimulated corticosterone secretion from rat adrenal slices [7], was found to enhance basal aldosterone and corticosterone production from freshly dispersed ZG and ZF/R cells, respectively. Moreover, other human leptin fragments, lacking N-terminal 22–115 sequence, i.e. leptin (150–167) and leptin (138–167), displayed secretagogue effects similar to those of the native molecule. Since leptin (93–105) was ineffective, we suggest that (1) the stimulating effect of leptin on adrenocortical steroidogenesis depends on the C-terminal sequence 116–166; and (2) the N-terminal seuence 22–115 is not needed for leptin to bind its receptors positively coupled to steroidogenesis. In contrast, leptin (22–56) was found to markedly inhibits corticosterone secretion from ZF/R cells, without affecting aldosterone production by ZG cells, thereby suggesting that this segment of leptin molecule may be responsible of a direct inhibition of glucocorticoid secretion. However, we wish to stress that the presently observed secretagogue effect of Tyr-leptin (26–39) appears to conflict with this contention.

Rat adrenocortical cells possess at least two subtypes of Ob-Rs, and collectively our findings suggest that leptin and leptin fragments can differently interact with Ob-R isoforms, which could be either positively or negatively coupled to steroid-hormone secretion.

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