Divergent effects of intracerebroventricular and peripheral leptin administration on feeding and hypothalamic neuropeptide Y in lean and obese (fa/fa) Zucker rats

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ABSTRACT

Leptin inhibits feeding and decreases body weight. It may act partly by inhibiting hypothalamic neurons that express neuropeptide Y, a powerful inducer of feeding and obesity. These neuropeptide Y neurons express the Ob-Rb leptin receptor and are overactive in the fatty (fa/fa) Zucker rat. The fa mutation affects the extracellular domain of the leptin receptor, but its impact on leptin action and neuropeptide Y neuronal activity is not fully known. We compared the effects of three doses of leptin given intracerebroventricularly and three doses of leptin injected intraperitoneally on food intake and hypothalamic neuropeptide Y mRNA, in lean and fatty Zucker rats. In lean rats, 4-h food intake was reduced in a dose-related fashion (P < 0.01) by all intracerebroventricular leptin doses and by intraperitoneal doses of 300 and 600 μg/kg. Neuropeptide Y mRNA levels were reduced by 28% and 21% after the highest intracerebroventricular and intraperitoneal doses respectively (P < 0.01 for both). In fatty rats, only the highest intracerebroventricular leptin dose reduced food intake (by 22%; P < 0.01). Neuropeptide Y mRNA levels were 100% higher in fatty rats than in lean animals, and were reduced by 18% (P < 0.01) after the highest intracerebroventricular leptin dose. Intraperitoneal injection had no effect on food intake and neuropeptide Y mRNA. The fa/fa Zucker rat is therefore less sensitive to leptin given intracerebroventricularly and particularly intraperitoneally, suggesting that the fa mutation interferes both with leptin’s direct effects on neurons and its transport into the central nervous system. Obesity in the fa/fa Zucker rat may be partly due to the inability of leptin to inhibit hypothalamic neuropeptide Y neurons.

INTRODUCTION

Leptin, the product of the obese (ob) gene, has been suggested to signal fat mass to the brain [1]. It is synthesized in the adipocytes which secrete the mature product into the circulation, from where it can enter the mediobasal hypothalamus directly and may be transported across the choroid plexus into the cerebrospinal fluid (CSF). Administration of leptin to rats and mice reduces feeding and body weight and increases energy expenditure [2–5]. Mutations of the ob gene, which prevent the production of biologically active leptin, are responsible for obesity in the ob/ob mouse [1,2]. Leptin appears to act through various isoforms of its

Key words: feeding, leptin, neuropeptide Y, Zucker rats
Abbreviations: ARC, arcuate nucleus; CSF, cerebrospinal fluid; ICV, intracerebroventricular; IP, intraperitoneal; NPY, neuropeptide Y; PVN, paraventricular nucleus.
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Neuropeptide Y (NPY) is a potent appetite-stimulating peptide [12], synthesized in neurons of the hypothalamic arcuate nucleus (ARC), which project mainly to the paraventricular nucleus (PVN) [13,14]. The ARC–PVN neurons apparently act to maintain energy balance, as they are activated in conditions of negative energy balance [13]. ARC neurons, including NPY-immunoreactive ones, express the Ob-Rb receptor [15], and leptin administration has been shown to reduce NPY and NPY mRNA levels in both rats and mice [4,5,16,17] and prevents the fasting-induced rise in NPY mRNA levels in mice [18]. Activity of the NPY-immunoreactive ARC neurons is also increased in the fatty Zucker rat, possibly because the fa mutation prevents leptin’s normal inhibitory action [19–21].

Our aims were to determine whether fatty Zucker rats are insensitive to the direct neuronal effects of leptin; whether such insensitivity would be exacerbated by intraperitoneal (IP) administration, implying an additional defect in leptin transport into the central nervous system; and whether leptin administration fails to downregulate the ARC NPY neurons in the fatty Zucker rat. In these studies, we investigated the effects on feeding of leptin injected into the lateral ventricle and given intraperitoneally, and we compared dose–response curves of leptin’s inhibition of feeding between lean and fatty Zucker rats. We also examined the effects of ICV and IP leptin on hypothalamic NPY mRNA.

METHOD

Adult male obese (fa/fa) and lean (Fa/?) Zucker rats (IFFA Credo, France) were kept on a 12-h light–dark cycle (lights on 23.00–11.00 hours), with free access to laboratory chow and water.

Fatty and lean rats (n = 8 per group) received an IP injection of either murine leptin [5], at doses of 150, 300 or 600 μg/kg (in 0.3 ml of saline) or saline, in random order, with 2 days between each dose, at the start of the dark cycle and food intake was measured for 4 h. For ICV administration of leptin into the lateral ventricle, other fatty and lean rats (n = 8 per group) had a 3.9 mm guide cannula implanted stereotactically (0.8 mm caudal to bregma, 1.4 mm lateral to the midline) under diazepam/Hypnorm™ anaesthesia, and a stylet was inserted into the cannula to prevent blockage. After 3 days recovery the rats received leptin (6, 30 or 60 μg/kg; in 3 μl of artificial CSF) or 3 μl of CSF by injection, in random order as described above, and food intake was measured up to 4 h later.

Two days later, fatty and lean rats from each study were given either leptin (60 μg/kg) or CSF ICV, or leptin (600 μg/kg) or saline IP, and killed after 4 h and plasma separated and stored at −40 °C. To measure NPY mRNA, a block of mediobasal hypothalamus was dissected, snap-frozen in liquid nitrogen and stored at −70 °C, as described previously [5].

RNA was extracted from each hypothalamic tissue block using the guanidinium thiocyanate–phenol–chloroform method, separated by electrophoresis and transferred to a charged nylon membrane (Boehringer Mannheim, Lewes, U.K.) by capillary blotting, and fixed with UV light [5].

NPY mRNA was detected using a 42-mer antisense oligonucleotide probe (25 ng/ml) end-labelled (5’) with digoxigenin (Boehringer), an antibody against digoxigenin (Fab fragment, Boehringer) conjugated to alkaline phosphatase and CDP star chemiluminescence substrate (Cambridge Biosciences, Cambridge, U.K.) as described previously [22,23]. Signals were visualized on film and quantified by densitometry, and the NPY mRNA signal was normalized against that for 18 S rRNA with a 31-mer phosphatase and CDP star chemiluminescence substrate (Cambridge Biosciences, Cambridge, U.K.) as described previously [22,23]. Signals were visualized on film and quantified by densitometry, and the NPY mRNA signal was normalized against that for 18 S rRNA with a 31-mer digoxigenin-labelled antisense oligonucleotide probe.

Plasma insulin and leptin levels were measured using radioimmunoassay kits (Pharmacia Upjohn, St Albans, U.K., and Biogenesis, Poole, U.K.) with intra-assay coefficients of variation of 5% and 4% respectively. Plasma glucose was determined by an enzymic kit (Boehringer Mannheim).

Food intake data were analysed using repeated measure two-way ANOVA followed by multiple Bonferroni t-tests. A significance level of P ≤ 0.05 was chosen for all data.

RESULTS

Lean rats
Two-way ANOVA showed a significant effect of ICV leptin injection on food intake over the 4 h (F9,118 = 105; P < 0.001). Food intake was significantly reduced in a dose-dependent fashion by all three leptin doses (by 26%, 39% and 43% respectively) compared with the CSF-injected rats (all P < 0.01) (Figure 1). With IP leptin, two-way ANOVA showed a significant treatment effect
Neuropeptide Y and leptin in Zucker rats

Figure 1 Effect of ICV administration of leptin on food intake in fatty and lean Zucker rats
(a) Food intake up to 4 h after ICV leptin (6, 30, 60 μg/kg) or CSF injection. (b) Percentage fall in 4 h food intake after ICV leptin injection. n = 8 per group. Error bars are S.E.M. *P < 0.01 compared with respective controls. (F$_{3,112}$ = 103; P < 0.01), with food intake decreased after 1 h with 300 and 600 μg/kg leptin (Figure 2a). The 4-h food intake was significantly reduced at IP leptin doses of 300 and 600 μg/kg (by 28% and 40% respectively) compared with controls (all P < 0.01) (Figure 2b). There were no changes in plasma glucose or insulin levels after either ICV or IP injections of leptin, at any dose, compared with the control groups (Table 1).

Both ICV (60 μg/kg) and IP (600 μg/kg) injection of leptin significantly reduced NPY mRNA levels compared with controls (by 28% and 21% respectively; both P < 0.01) (Figure 3).

Fatty rats
With ICV injection of leptin, two-way ANOVA showed an overall treatment effect (F$_{3,112}$ = 43.3; P < 0.001); 60 μg/kg significantly decreased food intake, but the lower doses failed to reduce food intake over the treatment period (Figure 1a). The 4-h food intake after 600 μg/kg ICV leptin was significantly decreased (by 22%) compared with the controls (P < 0.01) (Figure 1b).

All three doses of IP leptin had no effect on feeding in the fatty rats over the treatment period (Figure 2a) and 4-h food intake was unchanged (Figure 2b). Insulin levels in the fatty rats were significantly higher than those in the lean rats (P < 0.01) (Table 1), and plasma leptin levels were also increased in the control IP fatty rats compared with their lean counterparts (P < 0.01) (Table 1). There were no changes in plasma glucose or insulin concentrations in the fatty rats after either ICV or IP injection of leptin (Table 1). NPY mRNA levels were significantly increased (by 100%) in the fatty rats compared with their lean littermates (P < 0.01).

NPY mRNA levels were significantly reduced (by 18%) after ICV leptin (60 μg/kg) compared with the controls (P < 0.01) (Figure 3a), but were unchanged after IP injection of 600 μg/kg leptin (Figure 3b).
Table 1  Body weight and plasma hormone levels in lean and fatty Zucker rats 4 h after ICV (60 μg/kg) or IP (600 μg/kg) leptin injection (n = 8 per group)

<table>
<thead>
<tr>
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<th>ICV</th>
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<tr>
<td></td>
<td>Control</td>
<td>Leptin</td>
<td>Control</td>
<td>Leptin</td>
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<tr>
<td>Lean rats</td>
<td></td>
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<td>Initial body weight (g)</td>
<td>268 ± 6</td>
<td>266 ± 2</td>
<td>273 ± 5</td>
<td>270 ± 4</td>
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<td>Insulin (m-units/l)</td>
<td>31.0 ± 1.9</td>
<td>29.7 ± 1.5</td>
<td>30.5 ± 1.5</td>
<td>32.2 ± 1.8</td>
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<td>Glucose (mmol/l)</td>
<td>8.0 ± 0.2</td>
<td>7.7 ± 0.2</td>
<td>7.9 ± 0.4</td>
<td>8.0 ± 0.3</td>
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<tr>
<td>Leptin (ng/ml)</td>
<td>5.80 ± 0.5</td>
<td>–</td>
<td>5.74 ± 0.4</td>
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<tr>
<td>Fatty rats</td>
<td></td>
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<tr>
<td>Initial body weight (g)</td>
<td>386 ± 8</td>
<td>391 ± 9</td>
<td>393 ± 7</td>
<td>388 ± 9</td>
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<tr>
<td>Insulin (m-units/l)</td>
<td>102 ± 27 ++</td>
<td>112 ± 19</td>
<td>100 ± 17 ++</td>
<td>107 ± 22</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>8.1 ± 0.3</td>
<td>7.9 ± 0.2</td>
<td>8.0 ± 0.4</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>12.8 ± 0.5 ++</td>
<td>–</td>
<td>12.7 ± 0.6 ++</td>
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DISCUSSION

The point mutation (Gln269Pro) in the fatty rat (fa) occurs in the extracellular domain of the Ob leptin receptor [2,10], and will therefore affect all the receptor isoforms, including the neuronal Ob-Rb receptors that occur on NPY neurons, and the short Ob-Ra form expressed in the choroid plexus which could be involved in transport into the CSF [2,7]. Consistent with a direct effect on neuronal activity, there is a decrease in the effect of leptin on synaptic transmission in the ARC in the fa/−fa rat [24], and other studies have suggested that the mutant has reduced sensitivity to the hypophagic action of ICV leptin at low doses [4,11]. Additionally, the obesity in the fatty (f/f) Koletsky rat is also due to a mutation in the leptin receptor gene, and both fatty Koletsky and Zucker rats appear to have decreased leptin transport into the CSF [25].

We examined systematically the effects of both IP and ICV administration of leptin in lean and fatty rats, and constructed dose–response curves of leptin’s hypophagic effect for these routes. The range of doses used were comparable with other studies, although we did include some higher doses in order to produce complete dose–response curves, which allowed us to investigate the precise level of insensitivity to leptin previously reported with ICV administration in fatty rats [11], and to predict the effects with IP injection. In fatty rats, we found reduced sensitivity to ICV leptin [11], presumably due to a direct effect of the mutation on neuronal action, although the fact that ICV leptin must cross the ependyma to reach the hypothalamus may also indicate reduced transport. In agreement with another report [11], we also found a reduced sensitivity to IP leptin in the fatty rats when compared with ICV administration, which suggests an additional obstacle to leptin action with this route, presumably in leptin transport into the
central nervous system. Transport could be reduced due to
the intrinsic fa defect in the leptin receptor and/or the
high circulating leptin levels, causing down-regulation of
leptin receptors. The latter possibility is strengthened by
the finding that diet-induced obese mice develop develop
peripheral but not central resistance to leptin [26].

Leptin has been shown to inhibit aspects of the ARC
NPY neurons [4,15,11,17], which express the Ob-Rb receptor [15]. Defects in all the leptin receptor isoforms
would remove the inhibitory action of leptin, leading to
overactivity of the ARC NPY neurons, as has been
shown in the fatty Zucker rat [19–21] and suggested here
by the raised NPY mRNA levels. This in turn would be
expected to increase feeding, stimulate insulin secretion
and reduce thermogenesis, leading to obesity.

ICV leptin (60 μg/kg) significantly reduced NPY
mRNA levels in lean rats, as reported previously [4,5],
and interestingly the same doses also reduced the elevated
NPY mRNA levels in fa/fa rats. This may suggest that
NPY synthesis by these neurons remains relatively
sensitive to leptin if it can gain access to them, which
would in turn suggest that the crucial defect in fa/fa rats
is the impairment of transport into the central nervous
system [25]. The fact that food intake was not inhibited
despite the reduction in NPY mRNA might point to
other hypothalamic targets for leptin, such as neurons
expressing glucagon-like peptide-1 or the glucose-sensitiveneurons of the lateral hypothalamus [27,28]. Also,
leptin may also act as a result of its effect on other
receptors; there is cross-reactivity between leptin and
cytokine receptors [29]. Indeed, some cytokines have
been found to increase leptin levels in mice [30], which
may influence hypothalamic NPY, as others reduce NPY
levels in rats [31]. On the other hand, leptin may not be
inhibiting the increased NPY release from the ARC
neurons of fa/fa rats [19], even though NPY mRNA
levels fall. After IP injection (600 μg/kg) a similar fall in
NPY mRNA levels was seen in lean rats, but the decrease
in the fa/fa rats was small and failed to reach significance.
This may again reflect the inability of circulating leptin
to enter the hypothalamus.

These findings therefore support the hypothesis that
overactivity of the ARC NPY neurons is involved in the
obesity of the fatty Zucker rat, and that this is due to the
fa mutation interfering with leptin inhibition. However,
there are other leptin-sensitive neuronal targets involved,
because leptin still inhibits feeding in the NPY-deficient
mouse which has a normal food intake [32].

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