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Leptin-derived peptides that stimulate food intake and increase body weight following peripheral administration

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ABSTRACT

We previously showed that peptides containing leptin sequences 1–33 or 61–90 are taken up by the rat brain. We now report the effects of these peptides on food intake and body weight in mature rats. Peptides were infused intravenously for 4 weeks, using Alzet minipumps. Dosages were 20 μg/kg/day in experiment I, and 60 μg/kg/day in experiment 2. In experiment 1, female rats receiving peptides 1–33 and 61–90 each underwent an approximate doubling of the weight gain of control rats. These peptides also increased food intake in female rats. Peptide 15–32, which has a lesser degree of brain uptake, gave a smaller weight gain. Peptide 83–108, which is not taken up by the brain, had no effect on weight gain or food intake. Similar results were obtained in experiment 2. In male rats, however, none of the peptides caused significant changes in food intake or body weight. This was at least partly due to the fact that all male rats underwent vigorous weight increases. We conclude that peptides 1–33 and 61–90 acted as leptin antagonists, stimulating food intake and body weight increases, at least in female rats. These peptides may lead to clinical applications in conditions such as anorexia and cachexia.

1. Introduction

Leptin is one of a small number of peptide and protein signaling molecules that are known to be taken up across the blood-brain barrier. Since leptin is produced by adipocytes, and some of its most important signaling actions are on the brain, brain uptake is essential to its normal functioning. Leptin acts on hypothalamic neurons to reduce food intake and body weight, while leptin receptors are also found in many other brain regions [1]. Leptin receptors also occur on many cells in peripheral tissues, where leptin has modulatory roles on reproductive, endocrine and immune system function [2–5]. The leptin brain uptake mechanism is saturable and has a rather low capacity [6–8]. Indeed, brain uptake may be an important limiting factor that restricts the effectiveness of leptin in regulation of body weight. The majority of human obese patients have high leptin levels [9], yet their obesity persists. When leptin levels are raised further by giving exogenous leptin, the effect on body weight has been disappointing [10]. This is the phenomenon of leptin resistance, in which inadequate brain uptake may be a key factor [7,11,12].

We previously screened leptin peptide fragments for their brain uptake ability. We found that peptide sequences 1–33 and 61–90, and some shorter peptides within these regions, displayed good brain uptake [13]. We therefore decided to investigate the *in vivo* effects of these peptides on regulation of body weight and food intake. These effects are the subject of the present report.

Peptides 1–33 and 61–90, which display brain uptake on a par with leptin itself, both contain important receptor binding sequences. Leptin itself contains 3 receptor binding sites; site II is almost solely responsible for high affinity binding [14,15], while site III, and to a lesser extent site I, are crucial for receptor activation [15]. Binding site II is formed by the adjacent, anti-parallel helices A and C, which consist of residues 2 to 26 and 71 to 94, respectively [16]. Peptides 1–33 and 61–90 correspond approximately to these helices. Indeed, virtually all of the leptin residues that have been determined to be necessary for high affinity receptor binding are found within the regions spanned by these two peptides [14]. This observation, that peptides with the highest brain uptake also contain the residues necessary for receptor binding, strengthens the evidence that the leptin receptor is involved in brain uptake [13].

Since peptides 1–33 and 61–90 each contain important receptor-binding residues, we undertook *in vivo* experiments to look for antagonistic or agonistic actions. We expected the peptides to be able to access hypothalamic receptors when administered peripherally, since they have good brain uptake. Leptin itself causes dramatic weight loss when administered to obese, leptin-deficient *ob*/*ob* mice [17–19], and also decreases food intake and body weight in normal (non-leptin-deficient) mice [20,21] and rats [22–24], albeit to a milder

Abbreviations: HPLC, High Pressure liquid Chromatography; PBS, Phosphate buffered saline; SEM, Standard Error of the Mean.

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degree. Conversely, leptin antagonists, usually made by site-specific mutagenesis of the binding site III receptor activation site, have been shown to be orexigenic agents, capable of stimulating food intake and increasing body weight in normal mice [25–28].

Our investigation was undertaken using normal Sprague–Dawley rats, approximately 3 months of age at the commencement of the treatment. Rats with *ad libitum* food access increase body weight throughout most of their life-span, but this age was chosen because weight gain is substantially slower than in younger rats. Male and female rats were tested separately. Peptides were infused intravenously for 4 weeks, using osmotic minipumps, and body weight and food intake were monitored daily. We chose to infuse for 4 weeks to allow both acute and chronic effects to be detected.

As controls, some rats were infused with phosphate-buffered saline (PBS), and some rats received leptin peptide 83–108, which we have shown not to be taken up by the brain [13]. We also tested peptide 15–32, which was shown to have an intermediate level of brain uptake.

Peptides 1–33 and 61–90 were found to produce increased food intake and weight gain in female rats, while peptide 15–32 produced a similar but smaller effect. In male rats, however, the same peptides failed to increase food intake and weight gain to a statistically significant extent. This was due, at least in part, to the masking effect of the large background weight increases that occurred in male rats during the experimental period. The results are consistent with antagonist actions by leptin peptides that are able to be taken up by the brain, at least in female rats.

2. Methods

2.1. Study design

Two studies were performed. In each study, peptides or PBS were administered intravenously for 28 days, using Alzet osmotic minipumps. All peptides were infused at a constant rate of 20 μg/kg/day in the first study, and 60 μg/kg/day in the second study. Body weight was measured daily in both studies, and food intake was measured daily in the second study. Sprague–Dawley rats were used, and male and female rats were analysed separately. There were 5–6 rats in most treatment groups, the variable number being due to the exclusion of several rats due to catheter displacement or incomplete infusion (see under Peptide Infusions). Rats were assigned to each treatment group in such a way as to make average starting weights identical, or very close to it, across the groups. Rats were housed in groups of 2 or 3 per cage in experiment 1, and in individual cages in study 2. They were maintained on a 12 hour light:dark schedule, and allowed water and rat chow *ad libitum*. At the commencement of each study, the female Sprague–Dawley rats were between 12 and 15 weeks old. Their average starting weight was 289 g in study 1, and 284 g in study 2. The male rats were between 11 and 13 weeks old. Their average starting weight was 380 g for study 1, and 387 g for study 2. Animals were treated as humanely as possible at all times, and the study was approved by the University of Melbourne Animal Ethics Committee.

2.2. Peptides

Leptin peptides 1–33, 15–32, 61–90 and 83–108 were used. The numbering is based on the sequence of mature human leptin, excluding the pro sequence. Peptides were synthesized by Ezbiolab (Westfield, IN, USA) and Mimotopes Pty Ltd (Clayton, Australia), and were HPLC purified by the suppliers. All peptides had structure and purity confirmation by HPLC and mass spectrometry. We also checked peptide integrity by HPLC, prior to loading the osmotic minipumps. Recombinant murine leptin was obtained from Phoenix Pharmaceuticals (Burlingame, CA, USA).

2.3. Peptide infusions

Alzet 2004 minipumps (Cupertino, CA, USA) were used to deliver the test solutions continuously for 28 days, at 0.25 μl per hour. Minipumps were filled with 200 μl of the appropriate peptide solutions in PBS (pH 7.4), or with PBS alone. Prior to filling, solutions were filter-sterilized through 0.2 μm syringe filters. Rats were anaesthetized with Xylazil (6 mg/kg) and Ketamine (100 mg/kg), and the jugular vein was exposed and catheterized with a polyethylene catheter. The catheter was connected to an Alzet 2004 minipump which had been primed before the operation to ensure prompt onset of infusion after implantation. The catheter was routed subcutaneously to the back of the neck, and the minipump was implanted subcutaneously behind the base of the neck. After 28 days, rats were anaesthetized again, and the Alzet minipumps were removed and checked to ensure complete delivery of their contents. In the few cases in which pump emptying was incomplete, or there was detachment of displacement of the catheter, the rats were excluded from the study. All rats remained apparently healthy throughout the experiment, and there did not appear to be any toxic effects from the peptides or minipump insertion.

2.4. Body weight and food intake

Rats were weighed daily throughout each 28 day experimental period. In the second study, food intake was also monitored. To assess food intake, rats were housed in individual cages. 400 grams of standard rat chow pellets (4.6% fat content, Specialty Feeds, Glen Forrest, Western Australia) were added to each hopper at 10 am daily, and the remainder of the previous day's allotment was removed and weighed. The amount consumed was used to calculate the amount of food consumed per day per rat. Rats were allowed free access to water throughout the study.

2.5. Statistical analysis

The effects of peptides on body weight were analysed by a mixed design two-way ANOVA with repeated measures, with peptide treatment and time as the variable factors. Repeated measures were used because data was gathered on each rat over multiple time-points. This was followed by post-hoc individual comparisons using Tukey's test. All analyses were performed using the Minitab 15 software package (State College PA, USA). The daily food intake data were analysed by two-way ANOVA with repeated measures, with peptide treatment and time as the variable factors. The food intake data were further analysed by subjecting each time-point to one-way ANOVA, with peptide treatment as the variable factor. Food intake data were additionally presented as averages over a weekly period, with the aim of reducing crowding and revealing overall trends. The weekly averages were analysed in the same way as the daily intake.

3. Results

3.1. Leptin administration

To verify the experimental model, as well as provide a basis for comparison for the effects of the peptides, we tested the effects of 4 weeks intravenous infusion of leptin. Control rats had an average initial weight of 251 g, and were given PBS by osmotic minipump. The leptin group had an average starting weight of 255 g. The concentration of leptin in the minipumps was adjusted to provide a constant daily dosage of 125 μg/kg/day, based on their starting weight. After 4 weeks the average weight of the PBS group increased to 282 g. The mean weight increase of this group was 31 ± 4.7 g (Fig. 1). The group that received leptin lost weight in the first week, but thereafter gained weight. Their mean weight after 4 weeks was 270 g, and the mean weight increase was 15 ± 4.1 g. The effect of leptin was significantly