

UC Davis

UC Davis Previously Published Works

Title

Leptin in the Anterior Piriform Cortex Affects Food Intake in Rats

Permalink

<https://escholarship.org/uc/item/4986m0k2>

Journal

Nutritional Neuroscience, 2(5)

ISSN

1028-415X

Authors

Blevins, J Ernie
Havel, Peter J
Beverly, J Lee
[et al.](#)

Publication Date

1999

DOI

10.1080/1028415x.1999.11747290

Peer reviewed

Leptin in the Anterior Piriform Cortex Affects Food Intake in Rats

J. ERNIE BLEVINS^b, PETER J. HAVEL^a, J. LEE BEVERLY^c and DOROTHY W. GIETZEN^{b,*}

^aDepartment of Nutrition, ^bDepartment of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, and Food Intake Laboratory, University of California, Davis, CA 95616, USA; ^cDepartment of Animal Science, University of Illinois, Urbana, IL 61801, USA

(Received 8 February 1999)

Leptin administered intracerebroventricularly (ICV) or intrahypothalamically inhibits food intake (FI), however, to our knowledge the effects of leptin administration have only been examined in one extrahypothalamic site (dorsal raphe nucleus). Our objectives were to (1) determine the FI effects of leptin administration into the anterior piriform cortex (APC), an area linked to the control of FI in amino acid (AA) deficiency, (2) examine leptin action during short term anorexia that develops in response to AA deficiency. Bilateral injections of leptin (0.25 µg) into the APC suppressed FI of a balanced diet between 6 and 12 h by 36% ($p < 0.01$) and over the first 12 and 24 h by 15% ($p < 0.05$). Bilateral administration of leptin (0.1 µg) inhibited FI between 12 and 24 h by approximately 48% ($p < 0.05$) on a threonine-imbalanced diet without significantly affecting FI on a threonine-corrected diet. The increase of plasma leptin concentrations in response to feeding a threonine-basal diet was greater than that following an AA imbalanced diet, suggesting that suppression of FI by an AA imbalanced diet is not mediated by an increase of leptin. Our results suggest that (1) administration of leptin into a brain area outside the hypothalamus suppresses FI, and (2) leptin is unlikely to play a selective role in the short term anorectic response to AA deficiency. These data are consistent with the hypothesis that endogenous leptin can act within the APC to modulate FI.

Keywords: Appetite regulation, Amino acid imbalance, Extrahypothalamic, Rat

INTRODUCTION

Leptin, a hormone produced by adipocytes, has an important role in the regulation of energy balance and body adiposity by acting at the level of the brain to modulate both FI and energy expenditure. Defects in the *ob* gene (*ob/ob* mice) or in the leptin receptor (*db/db* mice and *fa/fa* rats) cause leptin deficiency (Chen *et al.*, 1996) or resistance (Caro *et al.*, 1996), respectively. Both mutations result in overeating, reduction in energy expenditure, and severe obesity in animals (Campfield *et al.*, 1995; Seeley *et al.*, 1996; Stephens *et al.*, 1995) and in humans (Clement *et al.*, 1998; Montague *et al.*, 1997). Exogenous administration of leptin into *ob/ob* mice corrects the deficiency in leptin, and thus normalizes food intake (FI) and body weight (Campfield *et al.*, 1995; Tang-Christensen *et al.*, 1999). Thus, normal leptin production and

*Corresponding author. Tel.: +530 752 9211. Fax: +530 752 7690. E-mail: dwgietzen@ucdavis.edu.

sensitivity to the effects of leptin are necessary to maintain normal body weight homeostasis (Havel, 1998). Furthermore, central or peripheral administration of leptin into normal mice and rats suppresses FI and increases energy expenditure, resulting in a loss in body weight (Campfield *et al.*, 1995; Seeley *et al.*, 1996).

Evidence suggests that leptin acts within the brain to inhibit FI. Leptin injection into either the lateral or third ventricles (intracerebroventricularly, ICV) (Schwartz *et al.*, 1997; Seeley *et al.*, 1996; Thiele *et al.*, 1997), or into discrete hypothalamic areas (ARC (arcuate nucleus (Satoh *et al.*, 1997)), LH (lateral hypothalamus (Satoh *et al.*, 1997)), VMH (ventromedial hypothalamus (Satoh *et al.*, 1997))) suppresses feeding at doses that are ineffective when administered peripherally (Campfield *et al.*, 1995; Schwartz *et al.*, 1996; Stephens *et al.*, 1995). These hypothalamic areas are linked to the control of food intake and express leptin receptors (Couce *et al.*, 1997; Hakansson *et al.*, 1998; Schwartz *et al.*, 1996). Moreover, leptin decreases the synthesis and release of NPY (Schwartz *et al.*, 1996; Stephens *et al.*, 1995; Wang *et al.*, 1997), a neuropeptide shown to cause a robust stimulation of FI following intrahypothalamic administration (Stanley *et al.*, 1993). Likewise, leptin increases expression of pro-opiomelanocortin (Mizuno *et al.*, 1997; Schwartz *et al.*, 1996), which is the precursor of the melanocortin peptides that act in the brain to inhibit feeding.

It is not clear, however, which sites located outside the hypothalamus may be targets for endogenous leptin action. The piriform cortex has been reported to express leptin receptor mRNA (Schwartz *et al.*, 1996) and importantly, the anterior piriform cortex (APC), is a site linked to the control of FI and associated with the anorectic response to amino acid (AA) deficient diets (Leung and Rogers, 1971; Meliza *et al.*, 1981). In order to determine whether APC administration of leptin may modulate FI more generally on a balanced diet, leptin was injected bilaterally into the APC into animals fed a diet with casein as the protein source. In order to examine whether

the anorexia induced by feeding a diet with an imbalance of AA composition might be in part, mediated through leptin action within the APC, the effects of bilateral administration of leptin on FI were measured in rats fed either a threonine-deficient diet or a corrected diet in which a normal level of threonine was replaced. Lastly, acute plasma leptin concentrations in response to consumption of low protein and AA imbalanced diets were examined.

MATERIALS AND METHODS

Animals

Adult, male Sprague-Dawley rats from Simonsen Laboratories, Gilroy, CA (experiments 1 and 2) or Harlan Sprague-Dawley, Indianapolis, IN (experiment 3) weighing 220–260 g were used. Animal care was according to the National Institutes of Health guidelines. Animal protocols were approved by the University of California, Davis Animal Use and Care Committee (experiments 1 and 2) and the Animal Care Advisory Committee of the University of Illinois (experiment 3). The animals were housed individually in hanging wire-mesh cages or plexiglass cages (30 × 30 × 30 cm (experiment 3)) in a temperature-controlled room (22 ± 2°C, 26 ± 2°C (experiment 3)) with a 12/12 h light-dark cycle [lights off 1100, 1300 h (experiment 3)].

Surgical Preparations

Surgical Implantation of APC Cannulas (Experiments 1 and 2)

Following adaptation to a low protein baseline diet (7–10 days), rats were surgically implanted with APC cannulas according to methods previously described (Beverly *et al.*, 1990). Each rat was anesthetized with a ketamine cocktail (1 ml/kg body weight, i.p., containing 5 ml ketamine hydrochloride [100 mg/ml, Fort Dodge Lab, Fort Dodge, IA], 1.25 ml xylazine [20 mg/ml, Mobay Corp., Shawnee, KA], 0.75 ml acepromazine

maleate [10 mg/ml, Avco, Fort Dodge, IA]. The animals were placed in a stereotaxic apparatus with the incisor bar positioned 2.8 mm below the ear bar. Bilateral guide cannulas (10 mm, stainless steel, 24 gauge, Small Parts, Miami Lakes, FL) were stereotaxically directed to a position 3 mm dorsal to the intended area of the APC [anteroposterior = +2.0, lateral = 3.6, dorsal = 3.0], using the coordinates of Paxinos and Watson (Paxinos and Watson, 1986). The cannula was fastened to the surface of the skull with dental acrylic (Hygenic, Akron, OH) and stainless steel screws. A 30 gauge stylet was inserted into the guide cannulas in order to maintain patency. An antibiotic [penicillin (Aquacillin, Vedco Inc, St. Joseph, MO, 300,000 Units/ml); 60,000 Units, i.m.] was administered at the completion of surgery.

Surgical Implantation of Jugular Vein Cannulas (Experiment 3)

Rats were anesthetized with a mixture of ketamine HCl, xylazine HCl, and acepromazine (30 : 6 : 1 mg/kg, i.m.). These animals were implanted with jugular vein catheters using procedures previously described (Beverly *et al.*, 1995). Following insertion and stabilization of the catheter, it was then filled with a 40% polyvinylpyrrolidone solution containing 500 U heparin per ml and capped with a sealed piece of Tygon tubing to maintain patency. The ends of venous catheters were fixed in position with dental acrylic cement and anchored to the skull with 4 stainless steel screws (Small Parts Inc., Miami Beach, FL). Following surgery, rats were monitored until they had completely recovered from the anesthetic. Post-surgical analgesia was provided by butorphenol (0.5 mg/kg, s.c.; Torbuterol, Aveco Co., Fort Dodge, IA).

Diet Composition

Experimental diets, which have been described previously (Beverly *et al.*, 1990; 1993) (Table I), and water were available *ad libitum*. These diets, with the exception of the casein containing diet,

TABLE I Composition of diets (% of diet by weight)

Ingredient	CAS	BAS	IMB	COR	DEV
Dispensable AA	—	7.53	7.53	7.53	8.08
Indispensable AA	—	4.18	4.18	3.32	4.18
Imbalanced AA	—	—	9.18	10.10	9.10
L-Threonine	—	0.20	0.20	0.45	—
Casein	20.30	—	—	—	—
Vitamins	1.00	1.00	1.00	1.00	1.00
Salt mix	5.00	5.00	5.00	5.00	5.00
Corn oil	5.00	5.00	5.00	5.00	5.00
Sucrose	22.87	25.66	22.60	22.50	22.51
Cornstarch	45.73	51.33	45.21	45.00	45.03
Choline Cl	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00

CAS, casein; BAS, basal; IMB, imbalanced; COR, corrected; DEV, devoid diet.

included free L-AAs as the protein source, with cornstarch and sucrose (2 : 1) as the carbohydrate source, 5% corn oil as the fat source, 1% vitamin mix, and 5% salt mix. The threonine-basal diet was a low protein diet, with threonine as the growth-limiting AA, present at about 50% of the requirement. The threonine-imbalanced diet contained an excess of essential AAs other than threonine, thus creating an imbalance in the dietary AA content. For the addition of the excess AAs, a proportionate amount of carbohydrate was removed. Animals were placed on the threonine-basal diet for 7–10 days after surgery to decrease their endogenous stores of free AA and protein, which is necessary for a complete expression of the threonine-imbalanced diet-induced anorexia (Leung *et al.*, 1968). The threonine-corrected diet was identical to the threonine-imbalanced diet except it contained a sufficient amount of threonine to correct the imbalance (Gietzen *et al.*, 1989). The threonine-devoid diet, was essentially identical to the imbalanced and corrected diets, but did not contain any threonine.

Experimental Protocols

Effects of APC Injections of Leptin on FI (Experiments 1 and 2)

Injections were made by lowering the 30-gauge injector with its tip extending 3 mm beyond the

guide cannula into the APC. Each injection needle was connected via PE-10 tubing (O.D. (0.024") I.D. (0.011") Plastics One, Roanoke, VA) to a 10 μ l syringe fitted into a microinjection pump (CMA/100, Bioanalytical systems, West Lafayette, IN). Bilateral injections (0.5 μ l/side) of either saline or human recombinant leptin (Linco, St. Charles, MO) were delivered simultaneously at a constant rate of 0.1 μ l/min within 2 h prior to the start of the dark cycle. Note: Human recombinant leptin has been shown to have equal efficacy with mouse recombinant leptin in *ob/ob* mice (Halaas *et al.*, 1995) and lean rats (Seeley *et al.*, 1996). Also, recent studies report that rat recombinant leptin suppresses FI and increases energy expenditure similarly to that of other leptin sources (Qian *et al.*, 1998). The volume of injectate was verified by movement of an air bubble (between the vehicle and H₂O) to a pre-calibrated distance along the tubing. Injection needles were left in place for approximately 60 s after the injection was completed before being removed slowly. Following each injection, stylets were replaced and the animal was returned to its cage. Food bowls were returned to the respective cages at the completion of all injections.

The two studies were divided into two phases in which there was at least one week between injections. Following implantation of the APC cannulas, rats were fed a diet with casein as the protein source (20%, experiment 1) or threonine-basal (experiments 2 and 3) diet for 7–10 days prior to the day of the injection, during which time they were adapted to handling and a mild 2 h fast prior to the start of the dark cycle. The mean FI measured during the 3 consecutive days prior to the experimental day was used to establish baseline intake. FI was determined by weighing food bowls to the nearest 0.1 g and correcting for spillage.

Effects of Leptin Injections in Rats Fed a Balanced Diet (Experiment 1)

Rats ($n = 19$) fed the balanced casein diet received bilateral injections of human recombinant leptin

(0 ($n = 8$), 0.25 μ g ($n = 11$) in 0.5 μ l) in saline into the APC. FI was measured at 6, 12, and 24 h following the start of the dark cycle, and then daily for an additional 6 days. These same animals were then reinjected in a counterbalanced order with either leptin or saline followed by the presentation of the diet consisting of 20% casein. FI was measured as previously described.

Effects of Leptin Injections in Rats Fed Threonine-imbalanced or Threonine-corrected Diet (Experiment 2)

Rats ($n = 24$) fed a threonine-basal diet received bilateral injections of human recombinant leptin (0, 0.1 μ g in 0.5 μ l) in saline into the APC and were then given either a threonine-imbalanced ($n = 10$) or threonine-corrected diet ($n = 10$). FI was measured at 1, 2, 3, 4, 5, 6, 9, 12, and 24 h following the start of the dark cycle, and then daily for an additional 6 days. These additional FI measurements were recorded to elucidate the time of onset of leptin action. Animals fed the threonine-imbalanced or threonine-corrected diet initially were then adapted to the threonine-corrected or threonine-basal diets, respectively (1 week). Following the adaptation period, using a counterbalanced design, animals were then reinjected with leptin or saline and presented with the threonine-corrected or threonine-imbalanced diet. FI was measured as previously described.

Effects of Threonine-basal and Threonine-imbalanced Diet Intake on Plasma Leptin Values (Experiment 3)

Rats were initially adapted to a rodent stock diet prior to surgery (Harlen Tek labs, Madison, WI). They were implanted with jugular vein cannulas ($n = 12$) and adapted to a threonine-basal diet for approximately 14 days prior to blood collection. FI was measured at 6 and 24 h to the nearest 0.1 g and corrected for spillage on experimental days.

Blood was collected initially at 15 minutes prior to the start of the dark cycle (days 2 and 4) and 0, 1, 2, 3 and 6 h following exposure to threonine-basal diet from five animals (all 4 days). Each animal that received threonine-basal was fed threonine-imbalance diet two days later and blood was collected at the same time points. Blood was collected (150 μ l) into 1 ml syringes and then immediately transferred into ice-chilled tubes containing 3 μ l of a 0.01% EDTA, heparin (500 U/ml), and aprotinin (1 mg/ml) cocktail. Samples were then centrifuged and plasma (75 μ l) was transferred into tubes and frozen (-80°C) until time of assay.

Histological Verification of Cannula Placement

Upon completion of the experiments, animals were sacrificed by ether inhalation, bilaterally injected with 0.5 μ l Evans blue dye, using the injection procedure described above, and immediately decapitated. This was followed by subsequent removal of the guide cannula, cannula crown, and the brain, respectively. Brains were stored in 10% formalin for 24 h followed by 10% sucrose for at least 48 h prior to freezing and sectioning. Coronal sections (40 μ m) were taken through the most ventral portion of the track marked by Evans blue. Sections were mounted on microscope slides and allowed to dry for 24 h prior to staining with Cresyl violet. A projection microscope was used to project the image of the brain section showing the most ventral portion of the injected ink, defined as the injection site, onto the corresponding section from the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 1986). Data from an animal were excluded from analysis when its injection site extended beyond the boundary described in Figure 1, based on previous work indicating this to be the area of the APC that appears to be involved in the detection of AA deficiency (Gietzen and Beverly, 1992).

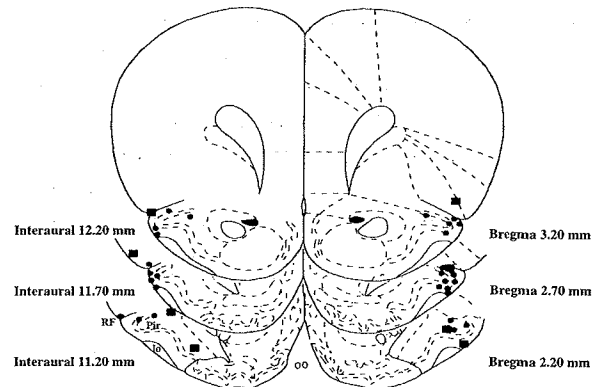


FIGURE 1 Schematic representation from Paxinos and Watson (1986) showing the injection sites corresponding to the effective area (as determined from previous studies) within the APC. The symbol ● represents sites included in analysis (having bilaterally placed cannulas within the APC or effective area). The symbol ■ is used to designate injection sites excluded from analysis.

Functional Verification of Cannula Placement

At the completion of the casein study, these animals were adapted to threonine-devoid diet (4–5 days) and received bilateral injections (0.5 μ l) of saline or L-threonine (2 nmol, Fluka Chemical Corp, Ronkonkoma, New York) and FI was measured for 90 min and 24 h following the start of the dark cycle. This paradigm was similar to that used by Monda *et al.* (1997) with the exception that the animals were not fasted for 24 h prior to receiving threonine injections. The feeding response to threonine administration into the APC has been well characterized (Beverly *et al.*, 1990). Therefore, this test serves as a means to verify both the placement of the injections in relation to the APC and the functionality of threonine sensitive uptake mechanisms pertaining to feeding within the APC.

Statistics

Data are presented as means \pm SEM. Results from experiment 1 were analyzed by a one-way ANOVA. Results from experiment 2 were

analyzed by a two-way ANOVA. Results from experiment 3 were analyzed by a one-tailed paired *t*-test. Results were considered significant if $p < 0.05$. Each animal served as their own control in a crossover design (except in experiment 2). All analyses were conducted using version 6.03 or 6.04 of PC-SAS (SAS, Cary, NC).

RESULTS

Experiment 1. Effects of Leptin Injection into the APC on Intake of a Balanced Casein Diet (20%)

Leptin (0.25 μg) suppressed FI relative to the 0 dose in the same animals (Figure 2, $p < 0.05$) during the 6–12 h interval after leptin administration. Cumulative FI during the first 12 and 24 h was also suppressed by leptin at this dose (Figure 3, $p < 0.05$). FI was not suppressed after 24 h in animals with bilateral APC cannulas, nor at any time point in animals ($n = 4$) who had one injection site placed outside the APC (illustrated in Figure 1). Also, in animals having unilateral injection sites within the gustatory neocortex ($n = 2$) leptin administration did not affect food intake.

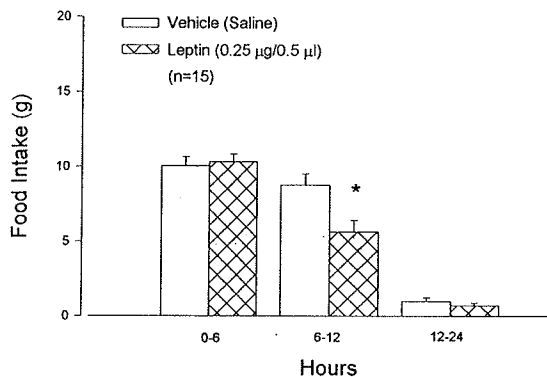


FIGURE 2 Effects of leptin injection into the APC on intake (0–6, 6–12, and 12–24 h) of a casein diet (20%). Data are presented as mean \pm SEM and were analyzed by a one-way ANOVA. * $p < 0.01$.

Experiment 2. Effects of Leptin Injection into the APC on Intake of Threonine-imbalanced and Threonine-corrected diet

Leptin (0.1 μg) did not suppress intake of either the threonine-corrected or threonine-imbalanced diet during the first 6 or 12 h, but this dose did suppress threonine-imbalanced diet intake by approximately 48% between 12 and 24 h (Figure 4, $p < 0.05$). There was no significant effect on FI during this time in animals fed the threonine-corrected diet (Figure 4); FI was approximately 33% lower after leptin on threonine-corrected diet, however this did not

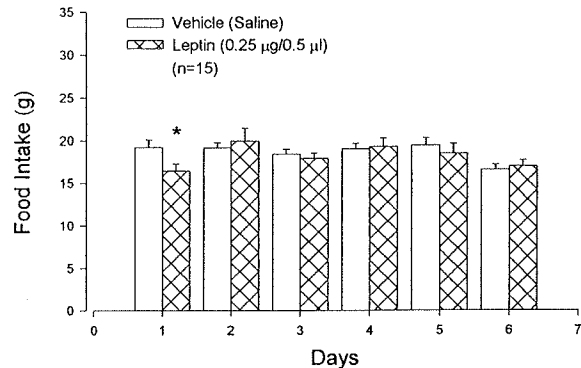


FIGURE 3 Effects of leptin injection into the APC on intake (6 days, 0–24 h) of a casein diet (20%). Data are presented as mean \pm SEM and were analyzed by a one-way ANOVA. * $p < 0.05$.

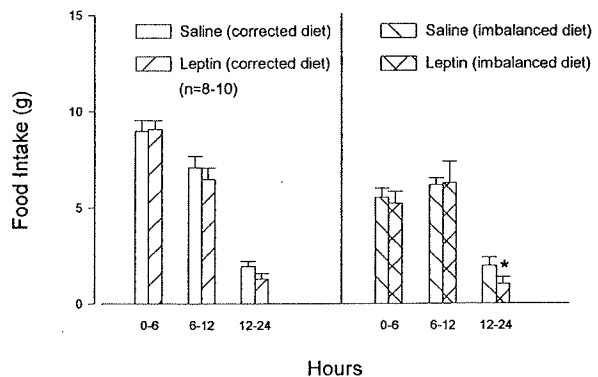


FIGURE 4 Effects of leptin injection into the APC on intake (0–6, 6–12, and 12–24 h) of a threonine-corrected/threonine-imbalanced diet. Data are presented as mean \pm SEM and were analyzed by a two-way ANOVA. * $p < 0.05$.

reach statistical significance (Figure 4, NS). By day 7, there was a significant increase in intake of the threonine-imbalanced diet (Figure 5, $p < 0.05$), with a trend towards significance day 5 ($p < 0.1$) on threonine-imbalanced diet. Again, we saw no effect of leptin on intake of the threonine-corrected diet (Figure 6, NS). The reasons for this are unclear. Compensatory mechanisms following leptin-induced inhibition of FI occurred later than we usually see, suggesting a residual effect of the leptin injections. In other models, compensatory hyperphagia occurs earlier than 5–7 days after treatment. In animals in which one cannula

was positioned outside the area depicted in Figure 1, leptin did not alter intake of threonine-corrected diet ($n = 2$) or threonine-imbalanced diet ($n = 3$) at any time.

Experiment 3. Effects of Threonine-basal and Threonine-imbalanced Diet Intake on Plasma Leptin Values

In a separate study, we utilized a well-characterized radioimmunoassay for leptin (Landt *et al.*, 1998) to determine whether an increase in plasma leptin concentrations could account for the anorectic responses to the threonine-imbalanced diet. As expected, there was a trend for suppression of FI on threonine-imbalanced diet relative to the threonine-basal group over the 6 h interval ($p < 0.1$). Thus, although animals used in this study were from a different supplier and were housed under slightly different conditions, the response to the imbalanced diet was similar between the experiments.

Changes in 3 and 6 h leptin values were compared to averaged baseline values of leptin ($-15, 0$ hr) in response to feeding threonine-basal and threonine-imbalanced diets (Figure 7). On the threonine-basal diet, leptin increased from 0.43 ± 0.12 ng/ml to 1.26 ± 0.22 ng/ml by 3 h ($\Delta = 0.83 \pm 0.25$ ng/ml, $p < 0.01$). By the 6 h time

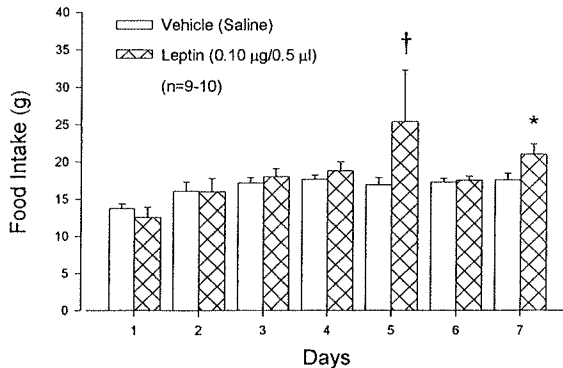


FIGURE 5 Effects of leptin injection into the APC on intake (7 days, 0–24 h) of a threonine-imbalanced diet. Data are presented as mean \pm SEM and were analyzed by a two-way ANOVA. $^{\dagger}p < 0.1$, $^*p < 0.05$.

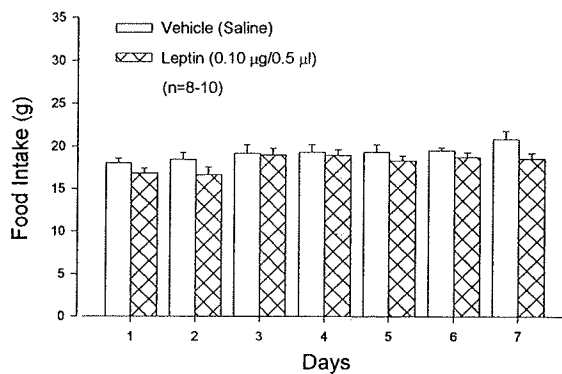


FIGURE 6 Effects of leptin injection into the on intake (7 days, 0–24 h) of a threonine-corrected diet.

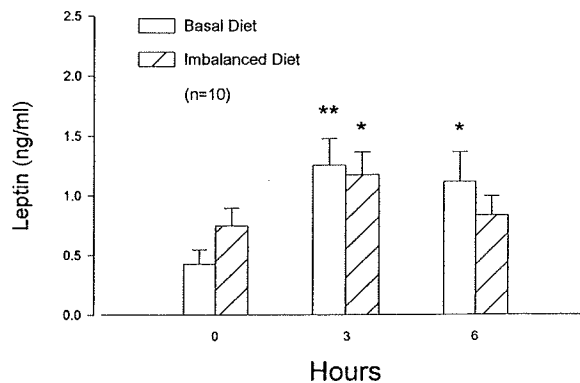


FIGURE 7 Plasma leptin concentrations (ng/ml) at 0, 3, and 6 h following presentation of a threonine-imbalanced and threonine-basal diet. Data are presented as mean \pm SEM and were analyzed by a paired *t*-test. $^*p < 0.05$, $^{**}p < 0.01$.

point, leptin had risen to 1.12 ± 0.25 ng/ml ($\Delta = 0.69 \pm 0.25$ ng/ml, $p < 0.05$). Likewise with the threonine-imbalanced diet at 3 h, leptin increased on threonine-imbalanced diet from 0.75 ± 0.15 ng/ml to 1.18 ± 0.19 ng/ml ($\Delta = 1.18 \pm 0.19$ ng/ml, $p < 0.01$), but it returned to 0.84 ± 0.16 ng/ml after 6 h ($\Delta = 0.18 \pm 0.10$ ng/ml, NS). The increases in plasma leptin concentrations during the 6 h were also compared between the diet groups. There was a greater increase (0.5 ng/ml) of plasma leptin 6 h after consuming the threonine-basal diet than after the threonine-imbalanced diet ($p < 0.05$). There were no significant correlations between threonine-basal diet intake at 6 h and either baseline leptin levels ($r = 0.17$) or changes in leptin over 3 h ($r = 0.29$) or 6 h ($r = 0.37$). Similarly on the imbalanced diet, there were no correlations between baseline leptin and FI over 6 h ($r = 0.11$) or between change in leptin over 3 h to that of subsequent intake at 6 h ($r = 0.26$). However at 6 h, there was a significant correlation between the change in leptin and threonine-imbalanced diet intake ($r = 0.68$, $p < 0.03$, data not shown).

DISCUSSION

Taken together the present results suggest that leptin would be unlikely to mediate the anorexic effect of a threonine-imbalanced diet. Thus, although administration of leptin into the APC suppressed FI with all the diets used here, we believe that leptin is unlikely to play a selective role in the short term anorectic response to AA deficiency.

Previous studies (Beverly *et al.*, 1991; Leibowitz, 1978) suggest that volumes ≤ 0.5 μ l, which are in the range of those used in these studies (0.5 μ l), should be used to minimize the distribution of substances injected into brain sites. One area, however, to which leptin could diffuse in our studies is the gustatory neocortex, located just lateral and dorsal to the APC, although it has not been established that areas within a 1 mm

diameter from the APC express leptin receptors. If the leptin-induced anorexia is a result of conditioned taste aversion, then the neocortex may be linking a nonspecific effect of the leptin with the taste of the threonine-imbalanced or balanced casein diets. Leptin, however, did not cause a reduction of FI in animals whose cannula placements were located outside the intended injection area within the APC, including the gustatory neocortex, as shown in Figure 1. Leptin also did not cause an acute suppression of FI, suggesting more of an interaction between leptin and its receptors in the APC rather than a conditioned taste aversion. Furthermore, others have demonstrated that leptin administered ICV at doses ranging from 10- to 300-fold higher than those used in this study do not cause a conditioned taste aversion in rats (Qian *et al.*, 1998; Thiele *et al.*, 1997).

Alternatively, the delay in leptin-induced suppression of FI may be a consequence of a multi-step pathway of leptin action in the APC to inhibit feeding behavior. In the hypothalamus, leptin is thought to suppress FI by acting, in part, on NPY-containing neurons in the ARC (Stephens *et al.*, 1995). However, other factors such as insulin and glucose are also thought to alter levels of NPY (Mizuno *et al.*, 1996; 1997; Schwartz *et al.*, 1992; McKibben *et al.*, 1992). Thus leptin is not the only means by which NPY levels may be altered. Mice lacking NPY which also carry the *ob/ob* mutation are not as obese and are less hyperphagic than NPY $+/+$ *ob/ob* mice (Erickson *et al.*, 1996). Furthermore, leptin receptor mRNA is found in NPY expressing neurons and leptin administered ICV decreases NPY expression and synthesis (Stephens *et al.*, 1995). In contrast, in the APC, NPY mRNA increases in animals on a threonine-deficient diet and administration of NPY into the APC decreases intake of a threonine-imbalanced diet (Cummings *et al.*, 1998). Similarly, antisense oligonucleotides to the NPY-Y1 receptor injected into the amygdala, an area which sends and receives neuronal projections to the APC (Haberly and Price, 1978) and expresses *c-fos* in

response to ICV leptin (Van Dijk *et al.*, 1996), stimulates FI, suggesting that the action of NPY in the amygdala is to inhibit FI (Heilig, 1995). Within the area of the APC, NPY-containing neurons are also found near GABA (gamma-aminobutyric acid), NE, and 5-HT containing neurons (Chronwall *et al.*, 1984; Cummings *et al.*, 1998), all of which are thought to act within the APC to mediate the effect of threonine-imbalanced diets to induce anorexia (Gietzen and Beverly, 1992; Gietzen *et al.*, 1987; Truong and Gietzen, 1997). Clearly, further studies will be necessary to determine the mechanisms by which leptin inhibits FI in the APC.

Leptin injections into the APC suppressed FI to varying degrees with respect to latency and magnitude depending on the diet and dose of administration. Leptin (0.1 μg) was not significantly effective at any time before 12 h in animals fed a threonine-imbalanced or threonine-corrected diet, whereas at 0.25 μg , it suppressed FI by approximately 36% between 6 and 12 h in animals fed a balanced casein diet. In addition, leptin did not significantly suppress FI in animals fed a threonine-corrected diet at any time, although the data suggested a trend toward a suppressive effect of leptin between 12 and 24 h. Leptin may have suppressed FI earlier in animals fed a balanced casein vs threonine-imbalanced diet as a function of the higher dose of administration. Another possibility is that leptin may be less effective at suppressing FI of more palatable diets. This is consistent with a recent study in diet induced obese rats where ICV administration of leptin inhibited FI on a standard diet but not in a more palatable high calorie diet (Widdowson *et al.*, 1997). Early after its first introduction, the casein diet (20%) may not be as palatable as the threonine-imbalanced diet. Thus, the leptin signal in the APC may be overridden by the cues associated with more palatable food.

If leptin were acting selectively to suppress FI of a threonine-imbalanced diet, then it would be expected that there would have been a greater rise in leptin in animals fed the threonine-imbalanced

diet than the threonine-basal diet. During the time at which the rapid anorexia occurs in response to the threonine-imbalanced diet, however, there was a significantly greater increase in leptin on threonine-basal than in threonine-imbalanced diet at 6 h. Furthermore, a fall in threonine-imbalanced diet intake would be expected to accompany a rise in plasma leptin. However, the FI of an threonine-imbalanced diet during the first 6 h was positively correlated with an increase in leptin suggesting that the increase in leptin would be unlikely to mediate the threonine-imbalanced diet-induced anorexia.

These results are similar to other studies using central injections of leptin on FI. The doses chosen in these studies are in the range of what others report following bilateral administration into the ARC or LH (0.125–0.5 μg), and the VMH (0.25–0.5 μg) in normal rats (Sato *et al.*, 1997). FI, however, was only measured in these animals over a 24 h period, so it is uncertain whether these doses affect short term FI following administration into specific brain sites. ICV administration of higher doses of leptin (1 μg) in both lean and *ob/ob* mice (Campfield *et al.*, 1995), and in normal rats ([1.5 μg], Cusin *et al.*, 1996, [3.5 μg] Thiele *et al.*, 1997) suppresses FI throughout the first 24 h. It is difficult, however, to compare required dose and timing of subsequent effects between ICV administration and injections into specific brain sites due to the diffusion gradient likely occurring prior to leptin binding. Thus, larger ICV doses would be expected to be required to inhibit FI compared with injections into discrete brain sites.

In summary, leptin suppressed FI when injected into the APC. Leptin reduced FI between 6 and 12 h and throughout the first 12 and 24 h in animals fed a balanced casein diet. Bilateral administration of leptin (0.1 μg) suppressed FI only between 12 and 24 h on threonine-imbalanced diet without significantly inhibiting FI on threonine-corrected diet. Plasma leptin was increased to a greater degree following intake of threonine-basal than on a threonine-imbalanced diet at 6 h. In addition, the increase of leptin

evident during this time was positively correlated with the FI response to the threonine-imbalanced diet implying that the anorexia in response to threonine-imbalanced diet is not mediated by an increase in leptin. While these results indicate that leptin administered into an area outside the hypothalamus suppresses FI, we believe that leptin is unlikely to play a selective role in the short term anorexia associated with AA deficiency. Thus, these data are consistent with the hypothesis that endogenous leptin may act outside the hypothalamus, including the APC, to modulate FI.

Acknowledgments

The authors acknowledge the assistance of Ban G. Truong, Susan M. Aja, Jennifer A. Barrett, and Kimber L. Stanhope. This work was supported by National Institute of Health Grants DK-50129, DK-35747, DK-07355, the United States Department of Agriculture Grant NRICGP 9700947, and the Juvenile Diabetes Foundation.

References

- Beverly, J.L., Beverly, M.F. and Meguid, M.M. (1995) Alterations in extracellular GABA in the ventral hypothalamus of rats in response to acute glucoprivation. *American Journal of Physiology* **269**: R1174-8.
- Beverly, J.L., Gietzen, D.W. and Rogers, Q.R. (1990) Effect of dietary limiting amino acid in prepyriform cortex on food intake. *American Journal of Physiology* **259**: R709-15.
- Beverly, J.L., Hrupka, B.J., Gietzen, D.W. and Rogers, Q.R. (1991) Distribution of dietary limiting amino acid injected into the prepyriform cortex. *American Journal of Physiology* **260**: R525-32.
- Beverly, J.L., Hrupka, B.J., Gietzen, D.W. and Rogers, Q.R. (1993) Timing and dose of amino acids injected into prepyriform cortex influence food intake. *Physiology and Behavior* **53**: 899-903.
- Campfield, L.A., Smith, F.J., Guisez, Y., Devos, R. and Burn, P. (1995) Recombinant mouse ob protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269**: 546-9.
- Caro, J.F., Sinha, M.K., Kolaczynski, J.W., Zhang, P.L. and Considine, R.V. (1996) Leptin: the tale of an obesity gene. *Diabetes* **45**: 1455-62.
- Chen, H., Charlat, O., Tartaglia, L.A., Wolf, E.A., Ellis, S.J., Lakey, N.D., Culpepper, J., Moore, K.J., Breitbart, R.E. and Duyk, G.M. (1996) Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* **84**: 491-5.
- Chronwall, B., Chase, T. and O'Donahue, T. (1984) Coexistence of neuropeptide Y and somatostatin in rat and human cortical and rat hypothalamic neurons. *Neuroscience Letters* **52**: 213-7.
- Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gormelen, M., Dina, C., Chambaz, J. and Lacorte, J.-M. (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392**: 398-401.
- Couce, M.E., Burguera, B., Parisi, J.E., Jensen, M.D. and Lloyd, R.V. (1997) Localization of leptin receptor in the human brain. *Neuroendocrinology* **66**: 145-50.
- Cummings, S.L., Truong, B.G. and Gietzen, D.W. (1998) Neuropeptide Y and somatostatin in the anterior piriform cortex alter intake of amino acid-deficient diets. *Peptides* **19**: 527-35.
- Cusin, I., Rohner-Jeanrenaud, F., Stricker-Krongrad, A. and Jeanrenaud, B. (1996) The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese fa/fa rats. *Diabetes* **45**: 1446-50.
- Erickson, J.C., Hollopeter, G. and Palmiter, R.D. (1996) Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science* **274**: 1704-7.
- Gietzen, D.W. and Beverly, J.L. (1992) Clonidine in the prepyriform cortex blocked anorectic response to amino acid imbalance. *American Journal of Physiology* **263**: R885-90.
- Gietzen, D.W., Leung, P.M.B. and Rogers, Q.R. (1989) Dietary amino acid imbalance and neurochemical changes in three hypothalamic areas. *Physiology and Behavior* **46**: 503-11.
- Gietzen, D.W., Rogers, Q.R., Leung, P.M.B., Semon, B. and Piechota, T. (1987) Serotonin and feeding responses of rats to amino acid imbalance: the initial phase. *American Journal of Physiology* **253**: R763-71.
- Haberly, L. and Price, J. (1978) Association and commissural fiber systems of the olfactory cortex of the rat. *Journal of Comparative Neurology* **178**: 711-40.
- Hakansson, M.-L., Brown, H., Ghilardi, N., Skoda, R.C. and Meister, B. (1998) Leptin receptor immunoreactivity in chemically defined target neurons in the hypothalamus. *Journal of Neuroscience* **18**: 559-72.
- Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K. and Friedman, J.M. (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269**: 543-6.
- Havel, P.J. (1998) Leptin production and action: relevance to energy balance in humans. *American Journal of Clinical Nutrition* **67**: 355-6.
- Heilig, M. (1995) Antisense inhibition of neuropeptide Y (NPY)-Y1 receptor expression blocks the anxiolytic-like action of NPY in amygdala and paradoxically increases feeding. *Regulatory Peptides* **59**: 201-5.
- Landt, M., Gingerich, R.L., Havel, P.J., Mueller, W.M., Schoner, B., Hale, J.E. and Heiman, M.L. (1998) Radioimmunoassay of rat leptin: sexual dimorphism reversed from humans. *Clinical Chemistry* **44** (3): 565-70.
- Leibowitz, S.F. (1978) Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. *Pharmacology Biochemistry and Behavior* **8**: 163-75.
- Leung, P.M.B. and Rogers, Q.R. (1971) Importance of prepyriform cortex in food-intake response of rats to amino acids. *American Journal of Physiology* **221**: 929-35.
- Leung, P.M.B., Rogers, Q.R. and Harper, A.E. (1968) Effect of amino acid imbalance in rats fed *ad libitum* interval-fed or forced. *Journal of Nutrition* **95**: 474-82.

- McKibben, P.E., McCarthy, H.D., Shaw, P. and Williams, G. (1992) Insulin deficiency is a specific stimulus to hypothalamic neuropeptide Y: a comparison of the effects of insulin replacement and food restriction in streptozocin-diabetic rats. *Peptides* 13: 721-7.
- Meliza, L.L., Leung, P.M.B. and Rogers, Q.R. (1981) Effect of anterior prepyriform and medial amygdaloid lesions on acquisition of taste-avoidance and response to dietary amino acid imbalance. *Physiology Behavior* 26: 1031-5.
- Mizuno, T.M., Bergen, H., Funabashi, T., Kleopoulos, S.P., Zhang, Y.G., Bauman, W.A. and Mobbs, C.V. (1996) Obese gene expression: reduction by fasting and stimulation by insulin and glucose in lean mice, and persistent elevation in acquired (diet-induced) and genetic (yellow agouti) obesity. *Proceedings of the National Academy of Science* 93: 3434-8.
- Mizuno, T.M., Kleopoulos, S.P., Bergen, H.T., Roberts, J.L., Priest, C.A. and Mobbs, C.V. (1997) Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting in *ob/ob* and *db/db* mice, but is stimulated by leptin. *Diabetes* 47: 294-7.
- Monda, M., Sullo, A., De Luca, V., Pellicano, M.P. and Viggiano, A. (1997) L-threonine injection into the PPC modifies food intake, lateral hypothalamic activity, and sympathetic discharge. *American Journal of Physiology* 273: R554-9.
- Montague, C.T., Farooqi, I.S., Whitehead, J.P., Soos, M.A., Rau, H., Wareham, N.J., Sewter, C.P., Digby, J.E., Mohammed, S.N. and Hurst, J.A. (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387: 903-8.
- Paxinos, G. and Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates*. 1986. Orlando, FL. Academic Press.
- Qian, H., Azain, M.J., Compton, M.M., Hartzell, D.L., Hausman, G.J. and Baile, C.A. (1998) Brain administration of leptin causes deletion of adipocytes by apoptosis. *Endocrinology* 139: 791-4.
- Satoh, N., Ogawa, Y., Katsuura, G., Hayase, M., Tsuji, T., Imagawa, K., Yoshimasa, Y., Nishi, S., Hosoda, K. and Nakao, K. (1997) The arcuate nucleus as a primary site of satiety effect of leptin in rats. *Neuroscience Letters* 224: 149-52.
- Schwartz, M.W., Seeley, R.J., Campfield, L.A., Burn, P. and Baskin, D.G. (1996) Identification of targets of leptin action in rat hypothalamus. *Journal of Clinical Investigation* 98: 1101-6.
- Schwartz, M.W., Seeley, R.J., Woods, S.C., Weigle, D.S., Campfield, L.A., Burn, P. and Baskin, D.G. (1997) Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes* 46: 2119-23.
- Schwartz, M.W., Sipols, A.J., Marks, J.L., Sanacora, G., White, J.D., Scheurink, A., Kahn, S.E., Baskin, D.G., Woods, S.C., Figlewicz, D.P. and Porte, D., Jr. (1992) Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology* 130: 3608-16.
- Seeley, R.J., Van Dijk, G., Campfield, L.A., Smith, F.J., Burn, P., Nelligan, J.A., Bell, S.M., Baskin, D.G., Woods, S.C. and Schwartz, M.W. (1996) Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats. *Hormone and Metabolic Research* 28: 664-8.
- Stanley, B.G., Magdalin, W., Seirafi, A., Thomas, W.J. and Leibowitz, S.F. (1993) The perifornical area: the major focus of (a) patchily distributed hypothalamic neuropeptide Y-sensitive feeding system(s). *Brain Research* 604: 304-17.
- Stephens, T.W., Basinski, M., Bristow, P.K., Bue-Valleskey, J.M., Burgett, S.G., Craft, L., Hale, J., Hoffman, J., Hsiung, H.M. and Kriaucinas, A. (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377: 530-2.
- Tang-Christensen, M., Havel, P.J., Jacobs, R.R., Larsen, P.J. and Cameron, J.L. (1999) Central administration of leptin inhibits food intake and activates the sympathetic nervous system in rhesus macaques. *Journal of Clinical Endocrinology and Metabolism* 84: 711-7.
- Thiele, T.E., Van Dijk, G., Campfield, L.A., Smith, F.J., Burn, P., Woods, S.C., Bernstein, I.L. and Seeley, R.J. (1997) Central infusion of GLP-1, but not leptin, produces conditioned taste aversions in rats. *American Journal of Physiology* 41: R726-30.
- Truong, B.G. and Gietzen, D.W. Gamma-aminobutyric acid (GABA) receptor in anterior piriform cortex (APC) mediate feeding responses in rats. (1997) (Abstract) *Society for Neuroscience* 23: 253.
- Van Dijk, G., Thiele, T.E., Donahey, J.C.K., Campfield, L.A., Smith, F.J., Burn, P., Bernstein, I.L., Woods, S.C. and Seeley, R.J. (1996) Central infusion of leptin and glp-1 (7-36) amide differentially stimulate c-fos-like immunoreactivity in the rat brain. *American Journal of Physiology* 40: R1096-100.
- Wang, Q., Bing, C., Al-Barazanji, K., Mossakowaska, D.E., Wang, X.-M., McBay, D.L., Neville, W.A., Tadayon, M., Pickavance, L. and Dryden, S. (1997) Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. *Diabetes* 46: 335-41.
- Widdowson, P.S., Upton, R., Buckingham, R., Arch, J. and Williams, G. (1997) Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. *Diabetes* 46: 1782-5.