Intraventricular insulin and leptin decrease sucrose self-administration in rats

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Abstract

Data from our laboratory and others have demonstrated an effect of the candidate adiposity signals insulin and leptin to decrease brain reward function, as assessed by lateral hypothalamic self-stimulation and food-conditioned place preference. In this study, we evaluated the effect of centrally administrated insulin or leptin to acutely decrease motivated performance for 5% sucrose, i.e., progressive ratio (PR) sucrose self-administration. Consistent with findings using other behavioral assays, both insulin and leptin significantly decreased the number of bar presses (62±7 and 76±8% of paired controls respectively), and the number of sucrose rewards obtained (87±4 and 91±4% of paired controls respectively), relative to within-subjects' control day performance on PR sucrose self-administration, whereas acute intraventricular cerebrospinal fluid had no effect. Rats fed a higher fat diet for 5 weeks were resistant to the effects of the intraventricular insulin or leptin, suggesting a central resistance to their action. Thus the findings of this study extend and support previous observations which suggest that neuroendocrine signals which regulate energy homeostasis in the CNS may also play a role in modulating reward circuitry, and specifically, food reward.

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1. Introduction

We have previously reported that the hormones insulin and leptin, which act at the hypothalamus as energy regulatory signals [1,2], also act within the central nervous system (CNS) to decrease food reward. Thus, both intraventricular (IVT) insulin and leptin prevent the expression of a place preference conditioned to a high-fat treat in rats that are not food-deprived [3]. Leptin reverses food-deprivation induced place preference conditioned by sucrose pellets [4]; and IVT insulin decreases initial lick rates for preferred sucrose solutions in a lickometer task [5]. Together these findings support the hypothesis that insulin and leptin can blunt food reward. Further, IVT insulin and leptin decrease performance in the lateral hypothalamic self-stimulation task, such that there is an increase in the electrical frequency threshold required to sustain the behavior [6,7]. Findings of insulin and leptin receptors on ventral tegmental area (VTA) neurons [8] provide some evidence that brain pathways for reward and motivation [9] may serve as targets for energy regulatory signals, in addition to the well-identified medial hypothalamic targets.

In the present study, we tested the ability of IVT insulin or leptin to decrease performance in another task which evaluates food reward, self-administration of sucrose. This task can be used to assess primary [10] or conditioned [11] motivational properties of sucrose. In the former, motivation to self-administer sucrose is assessed using the progressive ratio (PR) schedule of reinforcement wherein the response requirement for reward delivery increases following each delivered reward. The amount of responding a subject is willing to make on this schedule gives an indication of motivation to self-administer the reward [12,13]. In this study, we administered insulin or leptin into the third cerebral ventricle (IVT) in rats trained to bar press for sucrose solutions.
We observed decreased lever pressing in response to an acute injection of insulin or leptin, adding further support to our hypothesis that energy regulatory signals provide negative modulation of food reward [14], and can do so with a relatively rapid time course. Finally we demonstrated that, when rats are placed on a higher fat diet for 5 weeks prior to training—similar to a protocol that we have previously demonstrated results in CNS insulin insensitivity [15]—they are no longer responsive to insulin or leptin effects on sucrose self-administration.

2. Methods

2.1. Subjects

Subjects were male Albino rats (350–450 g) from Simonsen (Gilroy, CA). Rats were maintained on chow ad libitum except as noted for Experiments 1 and 3. They were maintained on a 12:12 h light–dark cycle with lights on at 6 AM. All procedures performed on the rats followed the NIH guidelines for animal care, and were approved by the Animal Care and Use Sub-Committee of the Research and Development Committee at the VA Puget Sound Health Care System. Rats in Experiments 2 and 3 had chronic cannulae placed in the third cerebral ventricle (IVT) following fixed ratio (FR) training (see below), according to our published methodology [5,16]. Cannula placement was functionally verified with a drinking response to angiotensin II injection (10 ng/μl, 2 μl per injection). Only rats with functional cannulae were continued in the experiments (criterion was 5 ml water consumed within 30 min). Following recovery of pre-surgical body weight, rats were re-trained for 1 day on fixed ratio (FR) responding then trained on progressive ratios (PR) responding.

2.2. Apparatus

The operant boxes, controlled by a Med Associates (Georgia, VT) system, had two levers, but only one lever (an active, retractable lever) activated the infusion pump. Presses on the other lever (an inactive, stationary lever) were also recorded. For all experiments, the number of presses on the inactive lever was very low (less than 10 presses/session) and no experimental manipulation altered inactive lever pressing. The sucrose solution was delivered into a liquid drop receptacle for oral consumption (Med Associates).

2.3. General procedures

Procedures were based upon our published methodology [11,17]. The experiment included 3–4 phases: autoshaping and FR training; surgery and recovery (Expts. 2 and 3); PR training using the PR algorithm of Richardson and Roberts [13]; and experimental procedure(s). The PR algorithm requires 1, 2, 4, 6, 9, 12, 16, 20, 28, 36, 48, 63, 83, 110, 145, 191, 251, 331, 437, 575, 759, 999, 999 (etc.) lever presses for succeeding reward deliveries within a session [18]. Table 1 lists the number of bar presses required for successive sucrose rewards relevant to this study. Rats were trained to self-administer sucrose (0.6 ml or 0.4 ml for FR or PR reward, respectively) delivered into a liquid drop receptacle. Training was conducted during 1-h sessions for 10 days under a continuous reinforcement schedule (FR1: each lever press was reinforced). PR training was carried out for 3 h/day for approximately 5 days. Each session began with the insertion of the active lever and the illumination of a white houselight that remained on for the entire session. A 5-s tone (2900 Hz, 20 dB above background)+light (7.5 W white light above the active lever) discrete compound cue accompanied each reward delivery and continued into a 20-s (FR) or 5-s (PR) time out. The active lever was retracted during the timeouts in the PR sessions. PR sessions ended after 30 min of no active lever press responding, at which point the house light was turned off and the active lever retracted.

2.4. Experimental procedures

Experiment 1. We validated previous observations that nutritional status of the rats can alter sucrose self-administration. Following FR and PR training, rats were subjected to food deprivation (no food for 24 h prior to testing) and self-administration of 10% of sucrose was measured. Another group of rats received a single intraperitoneal injection of the opioid antagonist naloxone (3 mg/kg), which has been shown to acutely decrease self-administration responding, immediately prior to being placed in the test chambers. Experiment 2. We evaluated the effect of IVT insulin or leptin on self-administration of sucrose. Following FR training, surgery/recovery, FR re-training, and PR training, rats received three IVT injections in randomized order, of artificial cerebrospinal fluid (CSF), insulin (5 mU) or leptin (0.2 μg) immediately prior to being placed in the self-administration chamber. Baseline performance (baseline days) did not differ across the experimental phase of the study. Doses of insulin and leptin were based upon our previous studies demonstrating the efficacy of these doses to decrease reward performance without significantly affecting food intake or body weight in our rats (e.g.,[15]), and these acute treatments likewise did not affect body weight in either Experiment 2 or 3. Rats were returned to 3 days of PR training in between each injection, and 3 days of PR performance was measured following the final injection. The experiment was run with two cohorts of rats (one subset run with the rats of Experiment 3 and one separate subset). Because the

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two cohorts did not differ in baseline self-administration, either prior to the start of the injection series or on the days between injections, data were combined for final analysis. Experiment 3. Chavez et al. [19] demonstrated impaired efficacy of IVT insulin to decrease food intake and body weight in rats, as a result of brief exposure to high(er) fat maintenance diets. Here, we evaluated the influence of chronic exposure to a high fat diet (Research Diets Inc #D12266B; www.researchdiets.com) on the efficacy of IVT insulin and leptin to decrease sucrose self-administration. This diet is based on the studies of Levin et al. (e.g., [20]) characterizing chronic effects of a higher fat diet on brain and metabolic parameters in Sprague–Dawley rats. It is 32% fat (as % of total kcal) and 4.4 kcal/gm, compared with the lab chow (www.labdiet.com, Rodent Diet 5001), which is 12% fat and 4.0 kcal/gm. Rats were fed for 5 weeks on the diet prior to training. We have previously observed that a more limited access to this diet for 5 weeks resulted in resistance to the action of insulin at the arcuate nucleus to decrease food intake and body weight (i.e., central insulin resistance) [15]. Other than the diet treatment, the experimental procedures were identical to those described for Experiment 2.

2.5. Statistical analyses

Active lever responses and sucrose rewards were analyzed as within-subjects’ paired Student’s t-test and Kruskal–Wallis comparison (change between paired, prior training day and treatment day). Baseline responding comparing chow-fed and high-fat diet fed rats was evaluated with ANOVA (two-way, diet×days overall; one way, individual training days). Group data are presented as the mean ± SEM in the text and Figures. Significance is defined as p ≤ 0.05.

3. Results

3.1. Experiment 1

Non-food deprived rats display relatively low performance in sucrose self-administration with a progressive ratio schedule.

![Fig. 1. 24 h food deprivation increases, and acute naloxone (3 mg/kg ip) treatment decreases, motivated response for sucrose. Within-subjects’ t-test compares PR self-administration on the day prior to, vs. the day of, treatment. Data are shown as mean±standard error of the mean (SEM).](image)

As shown in Fig. 1, active presses for 10% sucrose were approximately 50/session. Following 24-h food deprivation, the number of active presses increased significantly from 49±9 to 195±21 (p=0.0007). Conversely, acute treatment with ip naloxone (3 mg/kg) resulted in a significant decrease in active lever presses, from 63±12 to 38±5 (p=0.038). These findings are consistent with the observations of others [21–24], and further demonstrate that sucrose self-administration performance can be both increased or decreased by physiological or pharmacological manipulations in the non-food deprived rat.

3.2. Experiment 2

We tested the hypothesis that IVT insulin or leptin could acutely decrease sucrose self-administration. This regimen of IVT insulin or leptin had no effect on 24 h body weight. As shown in Fig. 2, active lever presses for 5% sucrose on training days preceding CSF, insulin, or leptin injections were comparable (91±10, 92±8, and 83±7, respectively). Acute injection of CSF had no effect on active lever pressing relative to the paired, preceding non-injection day (91±10 vs. 86±13). However both IVT injection of insulin and IVT injection of leptin resulted in significantly decreased active lever pressing compared to the paired, preceding non-injection day (91±10 vs. 86±13). However both IVT injection of insulin and IVT injection of leptin resulted in significantly decreased active lever pressing compared to the paired, preceding non-injection day (92±8 vs. 57±6, p<.005; and 83±7 vs. 63±7, p<.01, respectively). This was reflected in a decrease of sucrose rewards when rats were treated acute injections of insulin or leptin, but not CSF, compared with paired pre-injection days (CSF: 7.3±0.4 vs. 7.8±0.4; insulin: 6.5±0.3 vs. 7.5±0.4, p=.003; leptin: 6.7±0.3 vs. 7.4±0.4, p=.01). The time that rats spent engaged in PR responding until they obtained the last sucrose reward of the session was decreased significantly following insulin treatment, with a trend towards a decrease following leptin treatment (CSF: 29.8±7.1 min, range from 1.1–166.3 min; Insulin: 16.8±3.0 min [p<.05], range from 0.4–46.3 min; Leptin: 19.6±6.3 min, range from 1.1–85.2 min). The effects of insulin and leptin were acute, as post-injection day PR responding was comparable to both pre-injection day control levels, and post-CSF injection day performance (active lever

![Fig. 2. Acute intraventricular insulin (5 mU) or leptin (0.2 μg) administration decreases PR self-administration of sucrose in rats maintained on ad libitum chow. Comparisons are between each rat’s respective pre-injection training day and injection day. Artificial CSF had no effect on PR self-administration. Data are shown as mean±standard error of the mean (SEM).](image)
presses: CSF, 77±7; insulin, 88±8; leptin, 78±7; sucrose rewards: CSF, 7.2±0.4; insulin, 6.9±0.4; leptin, 6.9±0.4).

3.3. Experiment 3

We tested the hypothesis that IVT insulin or leptin would be less effective, or ineffective, at decreasing sucrose self-administration in rats fed a diet regimen that we have previously demonstrated to result in CNS resistance to (exogenous) insulin. Similar to baseline sucrose self-administration in chow-fed rats, active lever presses for 5% sucrose on training days preceding CSF, insulin, or leptin injections were comparable (157±33, 147±29, and 121±20, respectively). Intriguingly, overall baseline self-administration was greater in the high fat diet-fed rats compared with that of chow-fed rats. This became evident during the PR training phase (Fig. 3): active lever pressing was identical between chow-fed and high fat diet-fed rats during FR training (as was the number of sucrose rewards, 26±2 vs. 26±1 for chow- vs. high fat diet-fed rats, respectively). Although lever pressing increased in both groups when switched from FR to PR training (as would be expected), lever pressing was significantly enhanced in the high fat fed rats (overall ANOVA, \( p = 0.04 \) for effect of diet; with \( p < 0.05 \) for days 1, 3, and 4 of PR training). This was consistent during the experimental phase as well. Mean active presses from the three pre-injection days were 141±22 (\( n = 13 \)) for the high fat diet-fed rats, vs. 89±6 (\( n = 31 \)) for the chow-fed rats (\( p = .003 \)). Likewise, the number of sucrose rewards from the three pre-injection training days was higher in the high fat diet-fed rats vs. chow-fed rats (9.0±0.4 vs. 7.5±0.3, \( p = .015 \)).

Comparable to what we have previously observed, this diet regimen did not result in significant weight gain in the high fat diet fed rats compared with the chow-fed cohorts (370±7 vs. 379±8 \( n = 7, p = 0.47 \) or 360±4 \( n = 31, p = 0.28 \) gm) at the start of the experimental phase of the study (and throughout). Nonetheless, the high fat diet rendered the rats insensitive to the effects of acute IVT insulin and leptin (Fig. 4). Active lever presses were 157±33 vs. 136±32 for the paired, preceding non-injection day vs. CSF injection day; 147±29 vs. 129±42 for the paired, preceding non-injection day vs. insulin injection day; and 121±20 vs. 100±19 for the paired, preceding non-injection day vs. leptin injection day (\( p = n s \) for all treatment conditions). The number of sucrose rewards was not altered by CSF or leptin treatments (9.2±0.6 vs. 8.5±0.7, or 8.6±0.5 vs. 7.8±0.6, respectively) but was decreased by IVT insulin (9.2±0.6 vs. 8.3±0.7).

![Fig. 3](https://example.com/fig3.png)

**Fig. 3.** Progressive ratio responding is increased in rats fed a high fat diet vs. rats maintained on chow. FR = fixed ratio training days. FR-RE = day of FR retraining post-surgery. PR = progressive ratio training days. Vertical dashed line reflects interval for surgery, post-surgical recovery, and angiotensin II testing. Overall ANOVA for PR responding between chow and high fat diet fed rats = 0.04; **p** represents post hoc comparisons and statistical significance (\( p < 0.05 \)) for individual days.

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** Acute intraventricular insulin (5 mU) or leptin (0.2 \( \mu g \)) administration do not decrease PR self-administration of sucrose in rats fed high fat diet. Comparisons are between each rat’s respective pre-injection training day and injection day. Data are shown as mean±standard error of the mean (SEM).

4. Discussion

In this study we observed that acute intraventricular insulin or leptin treatment decreases motivation for sucrose in rats maintained on (low fat) chow; but insulin and leptin lose their efficacy when rats are fed a higher fat diet for a period of several weeks. This finding extends our previous observations and provides additional support for our hypothesis that these energy regulatory signals can decrease food reward in the CNS [14], here, as assessed by the self-administration motivational task. Our previous studies have demonstrated the effectiveness of IVT insulin or leptin on food-conditioned place preference [3]. IVT insulin also synergizes with a dopamine receptor antagonist to decrease acute sucrose lickrates [5], a task which assesses the (initial) hedonic impact of a solution or food [25]. Additionally, IVT insulin and leptin have been shown to decrease performance in lateral hypothalamic self-stimulation, an alternative type of reward task [6,7]. The effects in our studies are relatively rapid and acute, occurring in a timeframe of minutes to hours. Thus, in the current study, insulin and leptin decreased PR responding within 60 min, but had no effect on PR responding the subsequent day. Expression of food-conditioned place preference is blocked by IVT insulin or leptin within a timeframe of less than an hour [3]. IVT insulin modulation of sucrose lickrates likewise occurs within a timeframe of less than 30 min [5]. Given the rapidity of these effects and the fact that these doses of IVT insulin or leptin in our rats do not decrease
body weight or 24-h food intake, the effects may reflect a primary CNS function of insulin and leptin—to decrease food reward or motivation—that is, in turn, a contributing mechanism to their action to decrease food intake.

As with the energy regulatory actions of insulin and leptin, the influence of insulin and leptin on food reward is blunted when rats are maintained for a chronic period on a high(er) fat diet. This may be interpreted as insulin or leptin resistance at the CNS (see Ref. [26] for review and discussion). High fat diets are very effective at inducing peripheral resistance to insulin and leptin, even in paradigms where there are no large weight gains [27]. Further, the diet regimen not only changed responsivity to exogenous insulin and leptin, but also resulted in altered baseline performance (increased pressing on the active lever and number of rewards). Specifically, we observed increased motivation for sucrose when rats were switched to PR self-administration, suggesting that rats maintained on the chow diet were less motivated for sucrose when they were required to work for it. One interpretation of this finding is that responsivity to endogenous insulin and leptin is altered as well. Both the change of baseline PR responding, and the lack of efficacy of exogenous insulin or leptin, are consistent with a resistance to insulin and leptin action. The mechanism(s) underlying this can only be speculated upon, at this point. Studies of responding for sucrose pellets [28] or food [29] demonstrate altered efficacy of dopaminergic agents on performance, dependent upon the baseline response rate. Thus, agents which either decreased or increased responding for 10% sucrose pellets were more efficacious when animals were responding at a low, vs. a high rate, although this in turn was modulated by both schedule and the choice of the reinforcing food [28]. Whether the apparent decrease of insulin and leptin efficacy in our study was a cause or an effect of the increased PR responding remains to be determined.

How exogenous insulin and leptin decrease PR responding also remains to be elucidated. It is possible, but somewhat unlikely, that the decreased PR responding could be due to a shift in the valuation of sucrose. Although exogenous leptin has been reported to decrease taste cell response to sweet tastants in mice [30], leptin was given IP and its actions have been ascribed to direct effects on taste sensory neurons, something unlikely to occur with our very low dose of leptin in the third cerebral ventricle. Further, in our study of IVT insulin effects on sucrose lickrates [5], this dose of insulin was ineffective on its own, but only effective in the concomitant presence of a dopaminergic receptor antagonist.

There are several CNS sites which may be anatomical targets for insulin and leptin effects on food reward. The ventral tegmental area (VTA) has been identified as a critical CNS site for motivational or rewarding aspects of stimuli [9,31,32], and an anatomical substrate for reinforcement for a number of pharmacological agents and addictive drugs [33–36]. We have reported the expression of insulin and leptin receptors there [8]. DiLeone and colleagues have further identified the VTA as a behavioral target for leptin, as leptin administration in the VTA decreases 24-h chow intake (unpublished observations; Society for Neuroscience 2004). Thus, this may be one CNS target site. Alternatively, insulin and leptin may modulate food reward indirectly via interaction with receptors in the medial hypothalamus, and altered signaling relayed to reward circuitry via the lateral hypothalamus. We are currently investigating these hypothetical scenarios.

In conclusion, this study provides further evidence of the capacity for energy regulatory signals to have crosstalk with CNS reward circuitry. Whereas most studies have evaluated the effect of food deprivation (a circumstance in which insulin and leptin concentrations would be low) and its reversal on food reward, this study suggests that circumstances where insulin and leptin are low (Experiment 1, food deprivation) as well as circumstances where insulin and leptin are elevated can impact on food reward. Thus, this may indeed be a physiological action of insulin and leptin in the CNS, and it appears that insulin and leptin decrease ‘reward’ evaluated several ways: free feeding paradigms; learned associations with food; and motivated performance for food [14]. The effects of insulin and leptin on sucrose self-administration in the food-replete rat are relatively modest, whereas the effect of food deprivation is large, suggesting that, as has been considered for the regulation of body weight [37], modulation of food reward is ‘asymmetric’ in favor of enhancement of the rewarding or motivational properties of food. This concept is speculative, but consistent with our findings. Recent studies from others, such as the report that the orexigenic peptide ghrelin can stimulate food intake directly within the VTA [38], further substantiate the significant physiological role of energy regulatory signals in the modulation of food reward.

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