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Insulin acts at different CNS sites to decrease acute sucrose intake and sucrose self-administration in rats

Dianne P. Figlewicz,1,2 Jennifer L. Bennett,2 Sepideh Aliakbari, Aryana Zavosh,2 and Alfred J. Sipols3

1VA Puget Sound Health Care System (151), Seattle, Washington; 2Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, Washington; and 3Faculty of Medicine, University of Latvia, Riga, Latvia

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Figlewicz DP, Bennett JL, Aliakbari S, Zavosh A, Sipols AJ. Insulin acts at different CNS sites to decrease acute sucrose intake and sucrose self-administration in rats. Am J Physiol Regul Integr Comp Physiol 295: R388–R394, 2008. First published June 4, 2008; doi:10.1152/ajpregu.90334.2008.—Findings from our laboratory and others have demonstrated that the hormone insulin has chronic effects within the CNS to regulate energy homeostasis and to decrease brain reward function. In this study, we compared the acute action of insulin to decrease intake of a palatable food in two different behavioral tasks—progressive ratios sucrose self-administration and mu opioid-stimulated sucrose feeding—when administered into several insulin-receptive sites of the CNS. We tested insulin efficacy within the medial hypothalamic arcuate (ARC) and paraventricular (PVN) nuclei, the nucleus accumbens, and the ventral tegmental area. Administration of insulin at a dose that has no chronic effect on body weight (5 μU) into the ARC significantly suppressed sucrose self-administration (75 ± 5% of paired control). However, although the mu opioid DAMGO, [d-Ala2, N-MePhe4, Gly5-ol]-enkephalin acetate salt, stimulated sucrose intake at all four CNS sites, the ventral tegmental area was the only sensitive site for a direct effect of insulin to antagonize acute (60 min) mu opioid-stimulated sucrose feeding: sucrose intake was 53 ± 8% of DAMGO-induced feeding, when insulin was coadministered with DAMGO. These findings demonstrate that free feeding of sucrose, and motivated work for sucrose, can be modulated within unique sites of the CNS reward circuitry. Further, they support the interpretation that adiposity signals, such as insulin, can decrease different aspects of ingestion of a palatable food, such as sucrose, in an anatomically specific manner.

WE HAVE PREVIOUSLY REPORTED that insulin can act within the central nervous system (CNS) to decrease food reward, in addition to providing an energy regulatory signal at the medial hypothalamus (3, 11, 14, 31). Intraventricular (IVT) insulin administration prevents the expression of a place preference conditioned to a high-fat food treat (11), decreases initial lick rates for preferred sucrose solutions in a lickometer task (42), and decreases sucrose self-administration (13) in rats that are not food deprived. Further, IVT insulin decreases performance in the lateral hypothalamic self-stimulation task, such that there is an increase in the electrical frequency threshold required to sustain the behavior (7). Collectively, these findings support the hypothesis that insulin can blunt brain reward activity, including the rewarding attributes of food. However, the specific CNS sites that mediate the actions of insulin in different behavioral paradigms evaluating food reward have not been determined.

Both the caloric and the motivating aspects of a palatable food such as sucrose might contribute to its ingestion, or overingestion, and the CNS circuitry implicated in the intake of palatable food are also candidate sites for modulation by insulin. The medial hypothalamus has been identified as a target for the energy-regulatory actions of insulin (3, 10, 31). Mesocorticolimbic dopamine circuitry has been implicated in motivational properties of stimuli, including food, and in effort-based responses for food (6, 19–22, 30, 40, 44, 45). Self-administration of sucrose is a behavioral paradigm that can be used to assess motivational properties of sucrose, utilizing the progressive ratio (PR) schedule of reinforcement, whereby the response requirement for reward delivery increases following each delivered reward (18). The amount of responding a subject is willing to make on this schedule gives an indication of motivation for the reward (18, 37). Our observation that insulin receptors are expressed on ventral tegmental area (VTA) neurons, including dopamine neurons (13), provides a rationale for the hypothesis that the VTA may be one CNS target site for the insulin-induced suppression of sucrose self-administration, which we have reported previously. Finally, discrete populations of opioidergic neurons and opioid-receptive sites within the CNS have been shown to mediate spontaneous feeding (5), with activation of certain opioid pathways being associated specifically with the mediation of palatable food intake, independent of caloric need (16, 32, 43). We have demonstrated that insulin administration can blunt opiate-stimulated sucrose intake in sated rats, and in an acute free-feeding paradigm, whether administered IVT, or directly into the VTA, where it functionally antagonizes the effects of κ- or μ-opioid agonists, respectively (10, 41).

In the present study, we systematically compared the acute efficacy of insulin to decrease motivated responding for sucrose, or μ-opioid-stimulated sucrose feeding, at anatomical locations within the medial hypothalamus and mesolimbic...
circuitry that are insulin responsive (3, 13, 31, 33) and that are opiate responsive for feeding (25, 26, 29, 36). We hypothesized that insulin might have overlapping, but not identical, anatomical targets for any effects on these two different paradigms, which evaluate motivation for, or free-feeding of, the palatable food, sucrose. In fact, we observed specific effects of insulin at the arcuate nucleus (ARC) to decrease motivated responding for sucrose, and at the VTA, to decrease opioid-stimulated sucrose intake. These findings thus begin to identify specific anatomical targets for endocrine adiposity signals, such as insulin, to modulate specific aspects of food intake.

**MATERIALS AND METHODS**

**Subjects.** Subjects were male Albino rats (325–425 g) from Simonsen (Gilroy, CA). Rats were maintained on chow ad libitum. They were maintained on a 12:12-h light-dark cycle with lights on at 6 AM and were trained and tested between 7 AM and noon. All procedures performed on the rats followed the National Institutes of Health guidelines for animal care and were approved by the Animal Care and Use Subcommittee of the Research and Development Committee at the VA Puget Sound Health Care System. Body weight data for rats at time of experimental treatments are shown in Tables 1 and 2.

**Cannula placements.** Rats had chronic unilateral or bilateral cannulas placed in the neuropil, as indicated for each experiment. Rats were anesthetized with ketamine/xylazine (86/12.9 mg/kg ip) or isoflurane (4% induction, 2.5% maintenance in O2, at 0.5–1 l/min) and with 0.1 ml lidocaine/marcaine/epinephrine infiltrate as a topical anesthetic into the skin overlying the skull before incision. Each rat received 26-gauge stainless-steel guide cannulas (Plastics One, Roanoke, VA). For experimental injections, obturators were removed from the guide cannulas, and infusion cannulas were inserted that extended 1 mm beyond the tip of the guide cannula. The intracranial cannulas were held in place by acrylic cement (Lang Dental, Wheeling, IL) and skull screws (Small Parts, Miami Lakes, FL). Animals received periocular artificial tears for eye lubrication (AKWA, Akorn; Buffalo Grove, IL), subcutaneous injections of 3 cc lactated Ringers solution (Baxter Pharmaceutical Products, New Providence, NJ), and intramuscular 0.2 cc gentamicin antibiotic (40 mg/ml; Bayer AG, Leverkusen, Germany). They were maintained on a circulating-water heating pad until recovery from anesthesia. They received buprenorphine (50 µg/kg sc) at finish of surgery for postoperative pain (every 12 h as needed for 48 h). All animals were allowed to recover at least 1 wk and had to have regained weight to at least the presurgical level and be on a positive weight gain trajectory before undergoing the experimental procedure. For self-administration studies, surgery and recovery followed fixed ratio (FR) training (see Experimental procedures: sucrose self-administration), according to our published methodology (12). For sucrose pellet intake studies, rats underwent surgeries and recovery initially, followed by the sucrose intake protocol (see Experimental procedures: sucrose self-administration).

**Target cannula locations** were determined in pilot rats and are shown in Fig. 1 (34). They included the hypothalamic arcuate (ARC; bilateral, AP = −2.2 to −2.5, ML ±0.4, DV = −9.8 mm, with limits of AP = −2.12 to −2.56, ML ±0.4 [no shift tolerated], and DV = −9.2 to −9.8 mm) and paraventricular (PVN, bilateral, AP = −2.0, ML ±0.6, DV = −7.2 mm, with limits of AP = −1.8 to −2.2, ML ±0.2 −0.6, and DV = −7.8 to −8.4 mm) nuclei; the nucleus accumbens (NAc; unilateral or bilateral, AP = +1.7, ML 0.8, DV = −6.4 mm from skull with limits of AP1.2 to 2.2, ML 0.6 to 1.0, and DV = −6.8 to −7.4 mm); and the ventral tegmental area (VTA; unilateral or bilateral, AP = −5 mm; DV = −8.0 mm; lateral = −1.0 mm, angled at 10° to target the VTA at −4.8 to −5.3 mm from bregma). The specific NAc location was based on the report of MacDonald et al. (29) that mu-opioid injection elicited robust feeding there. Studies from Zhang and Kelley (46) and Pecina and Berri`3de (35) have identified this site as being a “hot spot” for mu-opioid-induced feeding. Cannula placements were verified histologically post hoc. Following the termination of infusions, each animal was overdosed with pentobarbital sodium (Nembutal, Abbott Laboratories, Chicago, IL) or isoflurane. Brains were removed and placed in 4% paraformaldehyde at 4°C for 7 days. Brains were then submerged in 20% sucrose, then 30% sucrose, followed by freezing at −80°C in embedding media (Fisher, Pittsburgh, PA), until sectioning at 50 µm. Tissue sections were mounted on slides and viewed under low-power magnification to verify cannula placement. Only data from rats with correctly placed cannulas were analyzed.

**Experimental procedures: sucrose self-administration.** Procedures were based upon our published methodology (12). The experiment included 4 phases: autoshaping and FR training; surgery and recovery; PR training using the PR algorithm of Richardson and Roberts (37); 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 (37). Rats were trained to self-administer 5% sucrose (0.5 ml reward) delivered into a liquid-drop receptacle. 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. As we have observed previously, the number of presses on the inactive lever was very low (less than 10 presses/session) and intraneuropil injections had no effect on inactive lever pressing (data not shown). The sucrose solution was delivered into a liquid drop receptacle for oral consumption (Med Associates). Initial training was conducted during 1-h sessions for 10 days under a continuous reinforcement schedule (FR1: each lever press was reinforced), with a maximum possible of 50 sucrose rewards delivered per session. 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 (2,900 Hz, 20 dB above background) + light (7.5

### Table 1. Body weight data for sucrose self-administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>Pre</th>
<th>CSF</th>
<th>Post</th>
<th>Pre</th>
<th>Insulin</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARC (bi)</td>
<td>22</td>
<td>401±5</td>
<td>404±6</td>
<td>399±6</td>
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<td>403±4</td>
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<tr>
<td>NAc (bi)</td>
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<td>410±4</td>
<td>412±4</td>
<td>412±3</td>
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<td>413±3</td>
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<tr>
<td>NAc (bi)</td>
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<tr>
<td>VTA (uni)</td>
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<td>390±3</td>
<td>393±3</td>
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<td>388±3</td>
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<tr>
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<td>24</td>
<td>384±5</td>
<td>386±5</td>
<td>387±5</td>
<td>384±5</td>
<td>386±5</td>
<td>389±5</td>
</tr>
</tbody>
</table>

Within-subjects data are presented for brain region; body weight is given in grams. ARC, arcuate nucleus; PVN, paraventricular nucleus; NAc, nucleus accumbens; VTA, ventral tegmental area.

### Table 2. Body weight data for sucrose pellet intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CSF</th>
<th>Insulin</th>
<th>DAMGO</th>
<th>DAMGO/Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARC (bi)</td>
<td>374±10 (14)</td>
<td>374±7 (11)</td>
<td>375±6 (12)</td>
<td>374±7 (9)</td>
</tr>
<tr>
<td>PVN (bi)</td>
<td>401±18 (10)</td>
<td>400±8 (12)</td>
<td>402±7 (9)</td>
<td>400±7 (10)</td>
</tr>
<tr>
<td>NAc (uni)</td>
<td>383±5 (16)</td>
<td>376±4 (18)</td>
<td>379±5 (16)</td>
<td>378±5 (15)</td>
</tr>
<tr>
<td>VTA (uni)</td>
<td>408±6 (10)</td>
<td>419±9 (5)</td>
<td>409±12 (5)</td>
<td>417±6 (5)</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Between-subjects data are presented for brain region and treatment; body weight is given in grams. DAMGO, [D-Ala²,N-MePhe²,Gly⁵-ol]-enkephalin acetate salt. n = number within parentheses.
We evaluated the effect of localized injections of insulin on self-administration of sucrose. Following recovery of presurgical body weight, rats were retrained for 1 day on FR responding then trained on progressive ratios (PR) responding. Subsequent to FR training, surgery/recovery, FR retraining, and PR training, rats received two intraneuropil injections of artificial cerebrospinal fluid (aCSF) for habituation to the injection procedure. Then, on the following days, they received in randomized order, aCSF or insulin (5 mU), immediately prior to being placed in the self-administration chamber (t − 10 min). They were returned to 2 or 3 days of PR training between injection days, and data were also collected 2 or 3 days after the second test injection, which verified that the injection procedures did not modify baseline performance (training days) across the experimental phase of the study (data not shown). The dose of insulin was based upon our previous studies demonstrating the efficacy of this dose to decrease reward performance without significantly affecting food intake or body weight in our rats (e.g., Ref. 15), and these acute treatments likewise did not affect body weight, as shown in Tables 1 and 2 (before and after aCSF or insulin treatment day body weights). Additionally, it is the dose of insulin used in our previous self-administration study: administered into the third cerebral ventricle, it significantly suppressed sucrose self-administration in chow-fed rats.

**Experimental procedures: acute sucrose pellet intake.** Rats were acclimated to the housing facility for ~1 wk prior to study. Cannulas were implanted, and the rats recovered from surgeries. Rats were maintained on ad libitum rat chow. An initial habituating session with no injection was carried out to acclimate the animals to the sucrose and reduce neophobia. On the morning of experimental sessions, rats were placed in a clean empty cage without food and received intraneuropil injections (0.2 μl in 1 min). Injectors were removed, and rats received a supply of sucrose pellets (Research Diets, New Brunswick, NJ) for two contiguous 30-min periods. Rats were returned to their home cages after the sucrose feeding session. Sucrose feeding session days were separated by 3 or 4 days with no treatment. For the day 1 session, all rats received a aCSF infusion and for the day 2 session, all rats received one of the following treatments: CSF; 5 mU insulin; 3 nmol of the μ-opioid agonist DAMGO ([d-Ala2,N-MePhe4,Gly5-ol]-enkephalin acetate salt, Sigma); or insulin + DAMGO. The dose of DAMGO has been shown to stimulate chow intake by others (29), and we have demonstrated its efficacy given unilaterally into the VTA in a preliminary study. For confusional conditions, insulin was mixed with the DAMGO, and a single volume of infusion was administered. Rats were subsequently anesthetized and euthanized, and brains were removed for verification of cannula site. Data from rats with misplaced cannulas were discarded.

**Statistical analyses.** For both experiments, group data are presented as means ± SE in the text, tables, and figures. Significance is defined as P ≤ 0.05. For the self-administration experiment, active lever presses were analyzed by ANOVA for brain region × treatment interaction for the bilateral infusion groups. Additionally, active lever responses and “stop time” (time from start of session to last lever press) were analyzed as a within-subjects, paired Student’s t-test comparison. For sucrose intake, absolute number of sucrose pellets ingested was analyzed by overall ANOVA as between-subjects comparisons for treatment effect (aCSF/aCSF, DAMGO/aCSF, aCSF/insulin, DAMGO/insulin) within one brain area. Specific treatment differences were established by post hoc t-test. Additionally, the day 2 to day 1 difference was calculated for each rat, since the intake of sucrose pellets between individual rats is variable. This within-subjects change of intake reflected the effect of day 2 treatments and was analyzed for treatment effect, comparing treatment subgroups for insulin effect.
RESULTS

Sucrose self-administration. As shown in Table 3 (raw data) and Fig. 2 (data normalized with aCSF condition set to 100% for each respective brain region), acute insulin treatment immediately before sucrose self-administration was effective in decreasing sucrose self-administration only when administered bilaterally into the ARC. For the aCSF bilateral infusions, there was no interaction with brain region (PVN, ARC, NAc, or VTA) (F_{3,88} = 1.835, P = 0.147); however, there was a significant interaction between insulin treatment and brain region (F_{3,88} = 4.623, P = 0.005). Active lever presses were significantly decreased (aCSF vs. insulin, within-subjects comparison, P = 0.026) following ARC insulin treatment. Additionally, the time from the beginning of the session until the last lever press of the session (“stop time”), was also significantly decreased when insulin was infused into the ARC (Fig. 3).

Insulin had no effect on sucrose self-administration when infused bilaterally into the PVN, unilaterally into the NAc, or unilaterally or bilaterally into the VTA (Fig. 2). When infused bilaterally into the NAc, insulin administration had a border-line effect to increase sucrose self-administration, with a trend toward increasing the number of active lever presses, and a significant increase in the time that the rats were engaged in the self-administration task (stop time) (Fig. 3). Taken together, these data are consistent with the interpretation that our previous observation of IVT insulin-induced suppression of self-administration of sucrose can be accounted for by specific action of insulin directly at the ARC.

Sucrose pellet intake. We then evaluated these identical CNS regions for their sensitivity to mu-opioid stimulation of sucrose intake and to insulin-induced suppression of the opioid-stimulated feeding. As shown in Table 3 (raw data) and Fig. 4, DAMGO significantly stimulated 60-min sucrose pellet intake, when administered into all four areas tested (ARC-bi, PVN-bi, NAc-uni, and VTA-uni; overall ANOVA vs. aCSF-treated rats, F_{7,54} = 5.649, P < 0.001; for each area, P < 0.05). In comparing the efficacy of DAMGO in the first vs. second half-hour of the 60-min feeding test, we observed that DAMGO was effective in the 30- to 60-min interval in the ARC and PVN (P < 0.05 vs. aCSF). DAMGO stimulated sucrose intake predominantly in the first 30 min in the NAc (P < 0.05 vs. aCSF). Within the VTA, DAMGO treatment significantly stimulated sucrose intake during both the initial (0–30 min) and second halves (30–60 min) of the feeding test (P < 0.05 vs. aCSF for both 30-min time bins).

Insulin infusion on its own did not decrease sucrose pellet intake below control intakes (insulin vs. CNS comparison, P = ns for all brain regions), comparable to what we have observed and reported previously for sucrose pellet intake when tested IVT (41) or unilaterally into the VTA (14). Co-administration of insulin with DAMGO into the VTA reduced sucrose pellet intake, such that intake no longer differed from that of aCSF-treated controls (insulin/DAMGO vs. aCSF, P = ns). This effect was due to a combination of decreased intake during both the 0–30 min time bin (127 ± 17 vs. 76 ± 16 pellets) and the 30–60 min time bin (40 ± 15 vs. 13 ± 15 pellets) for DAMGO vs. DAMGO/insulin. Importantly, since sucrose intake is somewhat variable between rats, we also compared each rat’s control aCSF treatment day (day 1) with intake on the

Table 3. Effect of insulin on sucrose pellet intake and sucrose self-administration

<table>
<thead>
<tr>
<th></th>
<th>CSF</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5% Sucrose Self-Administration, Active Lever Presses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARC (bi) (n = 22)</td>
<td>57±7</td>
<td>43±3</td>
</tr>
<tr>
<td>PVN (bi) (n = 23)</td>
<td>82±10</td>
<td>81±10</td>
</tr>
<tr>
<td>NAc (uni) (n = 22)</td>
<td>69±7</td>
<td>73±7</td>
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<tr>
<td>VTA (uni) (n = 21)</td>
<td>87±9</td>
<td>87±13</td>
</tr>
<tr>
<td>NAc (bi) (n = 23)</td>
<td>56±6</td>
<td>71±9</td>
</tr>
<tr>
<td>VTA (bi) (n = 24)</td>
<td>73±10</td>
<td>65±5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CSF</th>
<th>Insulin</th>
<th>DAMGO</th>
<th>DAMGO/Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>60-min Sucrose Pellet Intake, Number of Pellets Consumed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARC (bi)</td>
<td>39±3</td>
<td>43±5</td>
<td>68±12</td>
<td>67±12</td>
</tr>
<tr>
<td>PVN (bi)</td>
<td>54±9</td>
<td>48±6</td>
<td>99±18</td>
<td>86±11</td>
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<td>NAc (uni)</td>
<td>33±5</td>
<td>48±4</td>
<td>87±11</td>
<td>82±10</td>
</tr>
<tr>
<td>VTA (uni)</td>
<td>66±7</td>
<td>73±21</td>
<td>167±29</td>
<td>89±14</td>
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</tbody>
</table>

Values are presented as means ± SE.
second treatment day (i.e., day 2 to day 1 comparison within subjects), hypothesizing that DAMGO treatment should result in enhanced intake over individual baseline intake, whereas aCSF or insulin should have negligible effects. Consistent with this hypothesis, DAMGO stimulated sucrose intake 72 ± 13 pellets over paired baseline intake (P ≤ 0.05), whereas neither aCSF (3 ± 6 pellets), insulin (2 ± 3 pellets), nor DAMGO/insulin (8 ± 15 pellets) stimulated intake above each rat’s respective baseline intake. Co-administration of insulin with DAMGO in the ARC, PVN, or NAc had no effect on sucrose pellet feeding, whether evaluated as between-subjects treatment groups (Table 3 and Fig. 4) or comparing individual rats’ change from baseline (data not shown). In the NAc, insulin blunted the effect of DAMGO (35 ± 9 vs. 64 ± 10 pellets for DAMGO/insulin vs. DAMGO, P < .043) during the 0–30-min time interval. However, the DAMGO/insulin-treated rats ate more during the 30–60 min time interval (48 ± 9 vs. 17 ± 5 pellets, P < .05), resulting in no overall effect of insulin in the NAc during the 60-min test.

DISCUSSION

In this study, we compared the efficacy of insulin, administered acutely in several CNS locations, to decrease performance in two different sucrose intake paradigms: progressive ratios sucrose self-administration, which reflects motivated performance and effort-related decision making, for a reward (6, 8, 17, 23, 40); and acute opioid-stimulated sucrose intake, which reflects intake of a palatable food (16, 32). Because both studies were carried out in nondeprived rats after a night of typical feeding and used a dose of insulin that does not decrease chronic food intake and body weight, our data suggest that both the VTA and medial hypothalamic ARC are targets for a very acute action of insulin to decrease intake of (a palatable) food.

We observed that acute insulin injection directly into the ARC decreased sucrose self-administration and can account almost quantitatively for the ability of insulin given IVT to decrease self-administration, which we reported previously (12). Activation of VTA dopamine neurons and dopaminergic action in the NAc have been identified as neural mechanisms underlying self-administration of drugs of abuse (19–21, 30). Further, NAc dopamine release correlates with approach behavior for sucrose self-administration (38). Given our prior observations of insulin receptor expression (13), and insulin-induced cell signaling within the VTA (14), the lack of insulin effect there on sucrose self-administration was somewhat surprising. Likewise, the lack of efficacy of insulin within the NAc was surprising, given our previous observation of rapid and physiological effects of insulin on striatal tissue in vitro (33). It is possible that our cannula placements within the NAc in this study were not in an insulin-sensitive area. In fact, insulin administered bilaterally into the NAc appeared to increase the amount of time during which rats were active in the sucrose self-administration task. Baskin and colleagues (3) have observed mRNA for insulin receptors in ARC neurons that project to the PVN, and we have identified insulin receptor immunoreactivity within the PVN (both observations unpublished). The PVN has been demonstrated to be a target site for NPY-induced self-administration of ethanol (24); and insulin has been identified as a regulator of the medial hypothalamic NPY neurons (3, 31). We thus tested this region as a potential anatomical target for insulin-induced suppression of self-administration but observed that insulin had no effect. Taken together, these findings suggest that the effect of insulin on sucrose self-administration occurs primarily at the ARC and would engage the VTA or NAc limbic circuitry via multisynaptic activation. Further, the data suggest that circuitry that engages ARC neurons but does not include the PVN is important for sucrose self-administration: direct synaptic connections between the ARC and LH, relaying to other reward circuitry (4), could be one candidate.

Our second study suggested a role for insulin in the VTA, in an acute sucrose feeding paradigm. Numerous studies have documented the ability of opioids, particularly mu opioids, to stimulate feeding in a variety of feeding paradigms and with numerous potential loci within the CNS, including the central nucleus and basolateral region of the amygdala, the lateral hypothalamus, the ARC, the PVN, the VTA, and the NAc (2, 5, 16, 25–29, 36, 43). Evidence suggests that opioids play a critical role in the maintenance of feeding, and in the ingestion of palatable foods, particularly in situations in which choice between foods of varying palatability or preference is available (16, 23, 32, 39, 43). There is dopaminergic/opioidergic communication in the mediation of the different aspects of feeding, including interaction in the VTA/NAc circuitry (1, 6). Specifically, feeding stimulated by DAMGO injection into the VTA is dependent upon dopaminergic transmission in the NAc, as concomitant treatment with dopaminergic antagonists in the NAc decreases VTA DAMGO-stimulated feeding in rats (29). We speculate that insulin in the VTA must oppose mu-opioid-initiated processes by mechanisms that have not yet been determined. The lack of effectiveness of insulin in the medial hypothalamus (PVN and ARC) on this task suggests that there is local synaptic circuitry within the VTA that is a joint target there for mu opioids and insulin, perhaps the GABAergic neurons, which impinge on the dopamine (DA) neurons and express mu-opioid receptors, or perhaps via mu opioids effects on GABA neurons and insulin action on DA neurons. Further, it emphasizes the importance of insulin action within the VTA for acute feeding in circumstances independent of caloric need. The effect of insulin in the VTA to decrease DAMGO-stimu-
lated feeding was across the two 30-min time bins, for an overall suppressive effect. In the NAc, the rapid effect of insulin to decrease DAMGO-stimulated feeding (0–30 min) was followed by enhanced feeding in the second 30-min time bin. Because opiate effects to enhance feeding include an increased intake and a prolongation of feeding time, it is possible that insulin was having a very rapid effect in the NAc, via a different mechanism than its effects in the VTA. Our study design does not allow us to conclude that hedonic-based feeding per se has been decreased by insulin but demonstrates a rapid effect of insulin on free feeding, which is also consistent with the effects recently reported for leptin to decrease chow-feeding on a longer-term basis, when administered into the VTA (see Ref. 14 for discussion).

In conclusion, the two experiments emphasize that differential modulation of feeding and reward circuits occurs with these two different behavioral tasks, one of which evaluates motivation, reinforcement, and effort-related decision making (18, 22, 37, 40), and one which measures acute free-feeding. It should be noted that functionally connected circuitry, linking multiple nodes for reward and palatability-based feeding, has been identified in other studies (16, 26, 27, 29, 36), and thus insulin action within the ARC or the VTA might also decrease feeding stimulated by opioids at some other CNS sites. This more complex type of interaction remains to be studied. Additionally, under physiological circumstances, insulin would presumably be available to all of the sites studied here simultaneously. The possibility of resultant synergistic effects of insulin cannot be eliminated: Thus, insulin might simultaneously act to decrease motivation or effort to seek out a palatable food and also to decrease ingestion of already available palatable food. At a minimum, the results from this study identify two CNS regions that are sufficient in themselves as targets for insulin action to decrease food intake.

Perspectives and Significance

One contributor to the high rate of obesity in developed countries is thought to be the ready availability of highly palatable foods that have high caloric density or high sugar content (9). We have proposed that peripheral adiposity signals, such as insulin, provide a brake on food intake, in part, by decreasing the rewarding aspects of food intake. Importantly, in these studies, we have identified two critical anatomical targets for an action to decrease food reward and intake of palatable sucrose, that is, the medial hypothalamic ARC and the VTA. These studies simulate a “dessert” or “between-meal snack” experience, and emphasize the potential for endocrine signaling to blunt food reward in animals that are not calorie deprived. Further, they lay the groundwork for the identification of specific neural pathways and signals that are targets for modulation by metabolic signals, such as insulin. Identifying the circuitry that is engaged by behavioral paradigms that include both free feeding, and having to work for, a rewarding food may provide insight into human behavioral strategies for modification of feeding habits driven by the availability of high-energy density, highly palatable foods.

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