ELSEVIER

Pharmacology, Biochemistry and Behavior 72 (2002) 423–429

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Acute third ventricular administration of insulin decreases food intake in two paradigms

Ellen L. Air[a,b,*,1], Stephen C. Benoit[c,1], Kathleen A. Blake Smith[c], Deborah J. Clegg[c], Stephen C. Woods[c]

[a]*Department of Biomedical Sciences, University of Cincinnati, Cincinnati, OH 45267, USA*
[b]*Department of Cell Biology, Neurobiology, and Anatomy, University of Cincinnati, Cincinnati, OH 45267, USA*
[c]*Department of Psychiatry, University of Cincinnati, Cincinnati, OH 45267, USA*

Received 20 July 2001; received in revised form 31 October 2001; accepted 29 November 2001

Abstract

The pancreatic hormone, insulin, has been hypothesized to be an important regulator of food intake. Consistent with this hypothesis is the finding that exogenous insulin, in doses that do not affect blood glucose, reliably suppresses food intake and body weight. However, previous experiments have utilized a long-term delivery paradigm, in which insulin is administered via osmotic minipump and changes in body weight and food intake are measured across days. In separate experiments, we report that acute central injections of insulin can reduce food intake. In Experiment 1, injection of insulin (8 mU) into the third cerebral ventricle reliably suppressed intake of pelleted rat chow beginning at onset of the rats' dark phase. In Experiment 2, central insulin reliably and dose dependently suppressed intake of a 1-h 15% sucrose meal in the middle of the light phase. These data suggest that insulin can reduce food intake in acute delivery paradigms and provide another means by which to assess the roles of other central systems in the mediation of insulin's effects on energy homeostasis.

Keywords: Test meal; Food intake; Sucrose; Body weight; Adiposity signal

1. Introduction

The pancreatic hormone, insulin, has been hypothesized to be an important regulator of food intake and energy balance (Woods, 1996; Woods et al., 1979, 1995). Like the more recently discovered hormone, leptin, insulin fulfills many of the requirements for a putative adiposity signal to the brain. Plasma insulin is positively correlated with body weight, and with adipose mass in particular (Bagdade et al., 1967; Polonsky et al., 1988a,b). Further, the brain expresses insulin receptors in nuclei long recognized to be important in the control of food intake and energy balance (Baskin et al., 1986, 1993, 1994), and insulin enters the brain from the plasma via a saturable, receptor-mediated transport mechanism (Baura et al., 1992, 1993). Finally, and consistent with a role as an adiposity signal, exogenous insulin reduces food intake when administered locally into the brain in a number of species under different experimental paradigms (Woods et al., 1979; Brief and Davies, 1984; Florant et al., 1991). In most of these experiments, the insulin was administered chronically either by use of implanted osmotic minipumps or multiple daily intraventricular injections, and the general conclusion has been that when insulin is given chronically, it is highly efficacious at reducing food intake and body weight. A secondary conclusion from this literature is that insulin is a long-term regulator of body fat stores.

An important and, as yet, unanswered question about insulin's role as an adiposity signal concerns the specific mechanism(s) through which insulin influences energy homeostasis. Numerous brain circuits and neuropeptides have recently been identified that alter one or another aspect of food intake and energy expenditure (see reviews in Woods et al., 1998; Schwartz et al., 2000). One way to assess how these circuits and neuropeptides may mediate the actions of insulin, or interact with insulin, is to co-inject agonists or antagonists that may act at various locations in

* Corresponding author. Physician Scientist Training Program, University of Cincinnati, PO Box 670555, Cincinnati, OH 45267-0555, USA. Tel.: +1-513-558-3504; fax: +1-513-558-8990.
E-mail address: aire@ucmail.uc.edu (E.L. Air).
[1] These authors contributed equally to this work.

0091-3057/02/$ – see front matter
PII: S0091-3057(01)00780-8

the brain and study the resulting effects on food intake and body weight. Unfortunately, the chronic paradigms previously used to study insulin are not readily conducive to the study of potential interactions with many of these acutely acting compounds. We therefore sought an easy-to-apply paradigm in which potential interactions of "chronically acting" and "acutely acting" signals could be assessed. Here, we report the results of two experimental paradigms in which acute third ventricular (icv) infusions of insulin reliably suppress food intake relative to control infusions.

2. Experiment 1

The purpose of this experiment was to determine the effect of a single intracerebroventricular injection of insulin on food intake starting at the beginning of the dark phase of the day/night cycle when rats eat their largest meals. In addition, we varied the interval prior to food availability that insulin was administered.

2.1. Animals and materials

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. Male Long–Evans rats (200–250 g) were individually housed in plastic tub cages and maintained on a 12:12 light/dark cycle. Laboratory chow (Harlan-Teklad, Indianapolis, IN) and water were provided ad libitum (except where noted) during the experiments. Seven days after arrival in the laboratory, rats were anesthetized with 1.0 ml/kg ketamine/xylazine (10.0:6.5 ratio, respectively) and implanted with a 21-gauge stainless-steel cannula (Plastics One, Roanoke, VA) aimed at the third cerebral ventricle (icv) 2.2 mm posterior to bregma and 7.5 mm ventral to dura with bregma and lambda at the same vertical coordinate (see (Seeley et al., 1996) for details). Placement and patency of cannulas were confirmed by administration of 10 ng angiotensin II in saline, while the animals were water replete. Animals that did not drink at least 5 ml water within 60 min were considered to have failed cannula placement and were not used in the experiments.

2.2. Procedure

After recovery from intracerebroventricular cannulation (around 1 week), food intake was measured to establish a baseline. Once intake was stable, food intake over the final 3 days served as a baseline. Rats were assigned to groups matched with respect to body weight and food intake during

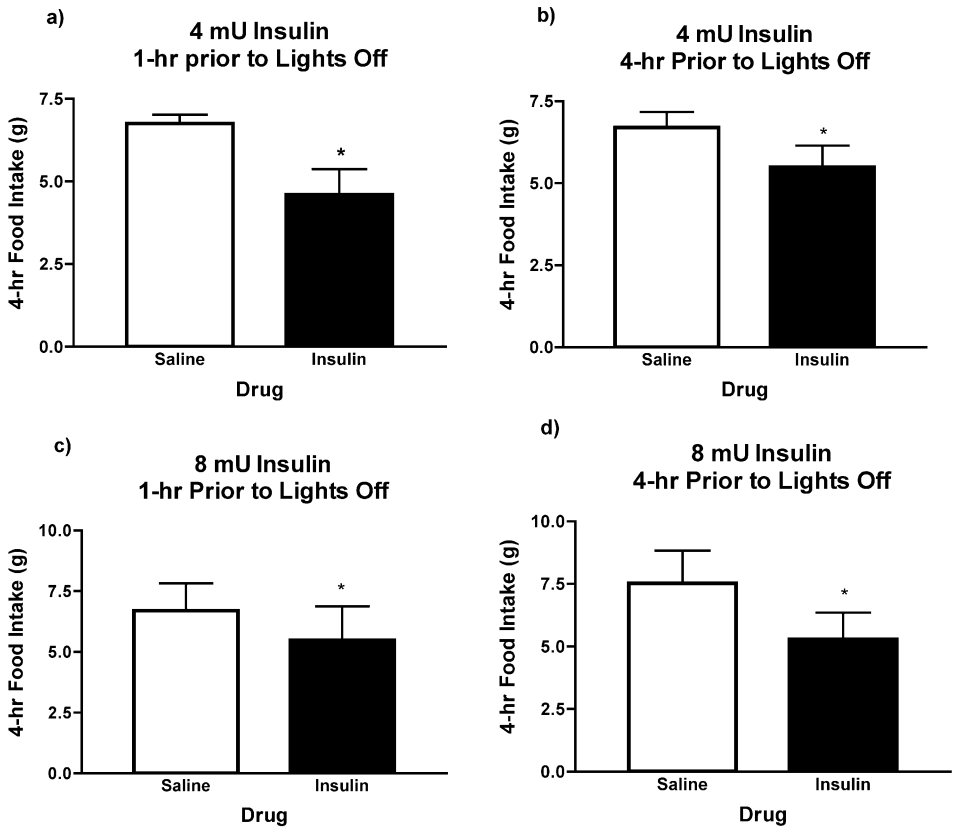

Fig. 1. Mean 4-h intake ±S.E.M. of pelleted chow following intracerebroventricular saline or insulin [4 mU (a and b) or 8 mU (c and d)] administration. Injections were given either 1 h (a and c) or 4 h (b and d) prior to lights off. In each condition, insulin significantly reduced food intake relative to saline ($P < .05$).

the baseline period. One group received intracerebroventricular injections 1 h prior to lights off, and the other group received intracerebroventricular injections 4 h prior to lights off. Half of each group was randomly assigned to receive 8 mU insulin (Iletin II Regular pork insulin, Eli Lilly, Indianapolis, IN) or its vehicle, and the other half to receive 4 mU insulin or its vehicle.

On test days, rats were weighed and food hoppers were removed 4 h prior to lights off. Each rat received the vehicle (physiological saline in a volume of 2 μl delivered over 60 s) and insulin on separate test days in random order. At least 3 days intervened between successive tests. Food was returned at the onset of the dark and intake was recorded 4 and 24 h later. Body weight was recorded 24 h postinfusion.

3. Results

3.1. Food intake

Rats in both the 4- and 8-mU groups consumed less lab chow after administration of insulin than after infusion of saline. However, the time of administration (1 or 4 h prior to lights off) did not significantly influence the magnitude of the effect. Fig. 1 depicts cumulative food intake data 4 h after the onset of dark. The top panels depict data for rats receiving 4 mU insulin, and the bottom panels depict data for rats in the 8-mU group. The left-hand panels depict data for rats infused 1 h before dark onset, and the right-hand panels depict data from rats infused 4 h before dark onset. Fig. 2 depicts food intake over 24 h. These data reveal that both doses of insulin (4 and 8 mU), at both infusion times (1 and 4 h prior to lights off), reduced food intake relative to saline.

To assess the statistical validity of our conclusions, we subjected the data to two three-way ANOVAs using Infusion Time (1 or 4 h before dark), Drug (saline or insulin), and Dose (4 or 8 mU) at each food intake measurement time point. For the 4-h food intake, ANOVA revealed a main effect of Drug [$F(1,22)=4.49$, $P<.05$]. No other main effects or interactions were statistically reliable. For the 24-h food intake, ANOVA also revealed a main effect of Drug [$F(1,22)=6.74$, $P<.05$], but no other reliable main effects or interactions. Thus, these data support the hypothesis that intracerebroventricularly administered insulin reduces food intake acutely.

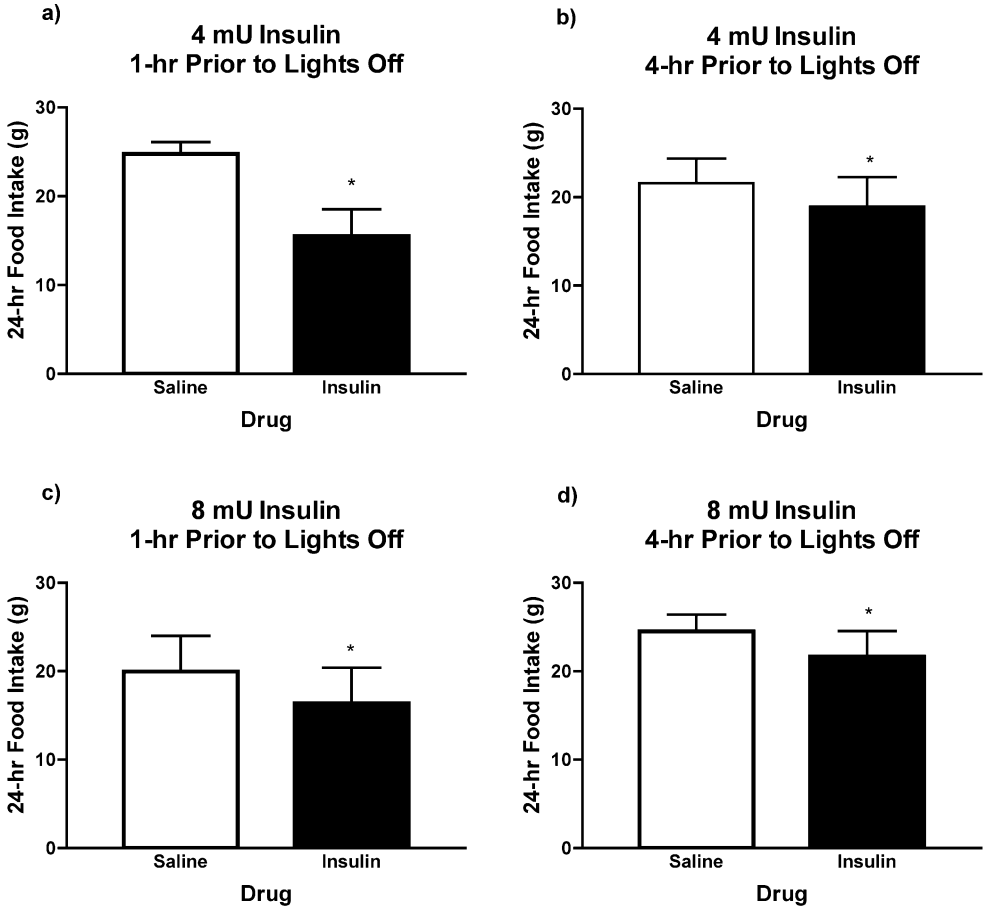

Fig. 2. Mean 24-h intake ±S.E.M. of pelleted chow following intracerebroventricular saline or insulin [4 mU (a and b) or 8 mU (c and d)] administration. Injections were given either 1 h (a and c) or 4 h (b and d) prior to lights off. In each condition, insulin significantly reduced food intake relative to saline ($P<.05$).

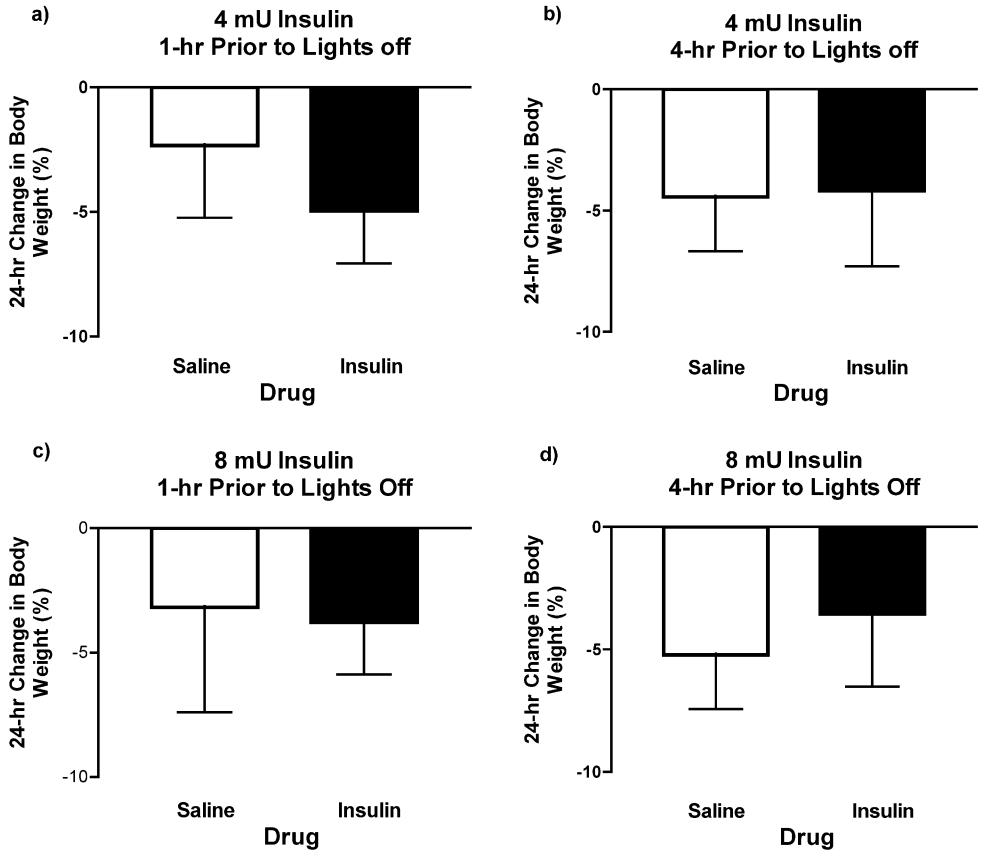

Fig. 3. Mean change in body weight ±S.E.M. over the 24-h period following intracerebroventricular saline or insulin[4 mU (a and b) or 8 mU (c and d)] administration. Injections were given either 1 h (a and c) or 4 h (b and d) prior to lights off. No significant change in body weight was seen in any condition.

3.2. Body weight

Body weight was reduced 24 h following both insulin and saline injections (Fig. 3), and there was no reliable difference between the groups. ANOVA using Infusion Time (1 or 4 h before dark), Drug (saline or insulin), and Dose (4 or 8 mU) as factors yielded no reliable main effects or interactions (P's > 0.1).

4. Discussion

These results support the hypothesis that an acute intracerebroventricular administration of insulin significantly reduces food intake relative to saline administration. These data therefore are consistent with the possibility that acute delivery paradigms can be used effectively to assess the food intake effects of insulin. However, the paradigm is not ideal. While insulin reliably reduced food intake over time and while the analysis did not yield any reliable interactions including time of measurement, the pattern of data suggested that the insulin exerted its effects from 8 to 28 h following injection in this paradigm. We therefore sought a different paradigm, one in which an effect might be observed earlier in time.

5. Experiment 2

The purpose of this experiment was to assess the acute effects of a bolus intracerebroventricular injection of insulin on a sucrose test meal, as well as on the 24-h food intake and body weight change. We were guided by a series of experiments by Riedy et al. (1995) in which a test meal was used to study the ability of insulin to potentiate the actions of CCK-8. Rats were habituated to receive a 30-min sucrose meal at the same time each day. We opted to provide the sucrose during the light portion of the day when chow intake would not be impacted too greatly.

5.1. Animals and materials

Male Long–Evans rats (Harlan, Indianapolis, IN) were implanted with third ventricular cannulas and maintained as described in Experiment 1.

5.2. Procedure

Animals were habituated to a feeding schedule (Fig. 4) adapted from Matson and Ritter (1999). Specifically, at lights on, the animals were weighed, and 1 h later, chow was removed from the cages. Four hours into the light

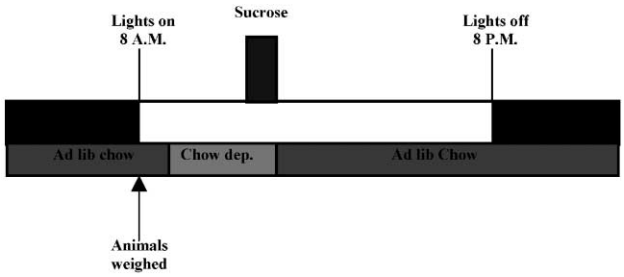

Fig. 4. The diagram represents one 24-h period. The light phase is represented by the open black rectangle and the dark phase is represented by the closed black rectangle. Chow was removed from the cages 1 h after lights on, and the rats remained without chow for 4 h (light gray bar). The 20-h access to chow is indicated by the dark gray bar. The period of the sucrose meal is indicated by the vertical gray bar.

cycle, the rats were presented with a 15% sucrose solution in a second water bottle adjacent to the one containing water. Following 1-h access to the sucrose solution, the sucrose bottle was removed and the chow replaced. One-hour sucrose intake and 20-h chow intake were recorded daily. The experiment began once daily sucrose intake for individual animals stabilized, approximately 7–10 days. Rats were then divided into four groups matched for daily sucrose intake on the three prior days. Baseline group means did not differ by more than 0.5 ml. On the test day, different groups received a 2-μl intracerebroventricular injection of either 0, 1, 2, or 8 mU insulin (Iletin II Regular pork insulin, Eli Lilly) in 0.9% saline 3 h prior to the sucrose meal. Chow was returned to the cages following the sucrose meal, and food intake and body weight were measured the following morning.

6. Results

Rats increased their daily sucrose intake during the training period until reaching a plateau after around 10 days. While the plateau volume differed among individual rats, it was relatively constant from day to day within rats. Saline injection decreased sucrose intake slightly relative to non-injection days, though not significantly (data not shown). Sucrose intake on the test day is depicted in Fig. 5a. Insulin decreased sucrose intake in a dose-dependent manner. While the 1-mU dose had little effect relative to saline, sucrose intake was decreased by 30% and 48% at doses of 2 and 8 mU, respectively. Chow intake over the rest of the day was also decreased following intracerebroventricular insulin administration, with each of the two higher doses reducing intake by nearly 50% (Fig. 5b). As might be expected when both sucrose and chow intake are decreased, body weight was also reduced following insulin (Fig. 5c). Sucrose intake and chow intake returned to their baseline levels during the subsequent 24 h. Body weight increased during the 24- to 48-h period following insulin, but full recovery to baseline weight required an additional day.

Given the results of Experiment 1, we specifically hypothesized that food intake, sucrose intake, and body weight would be decreased to a greater extent following insulin than following saline and therefore used planned t tests to analyze these data. These analyses revealed a significant difference between saline and 8 mU insulin on all three variables (sucrose intake $P < .001$; food intake and body weight change $P < .05$). The 2-mU dose of insulin signific-

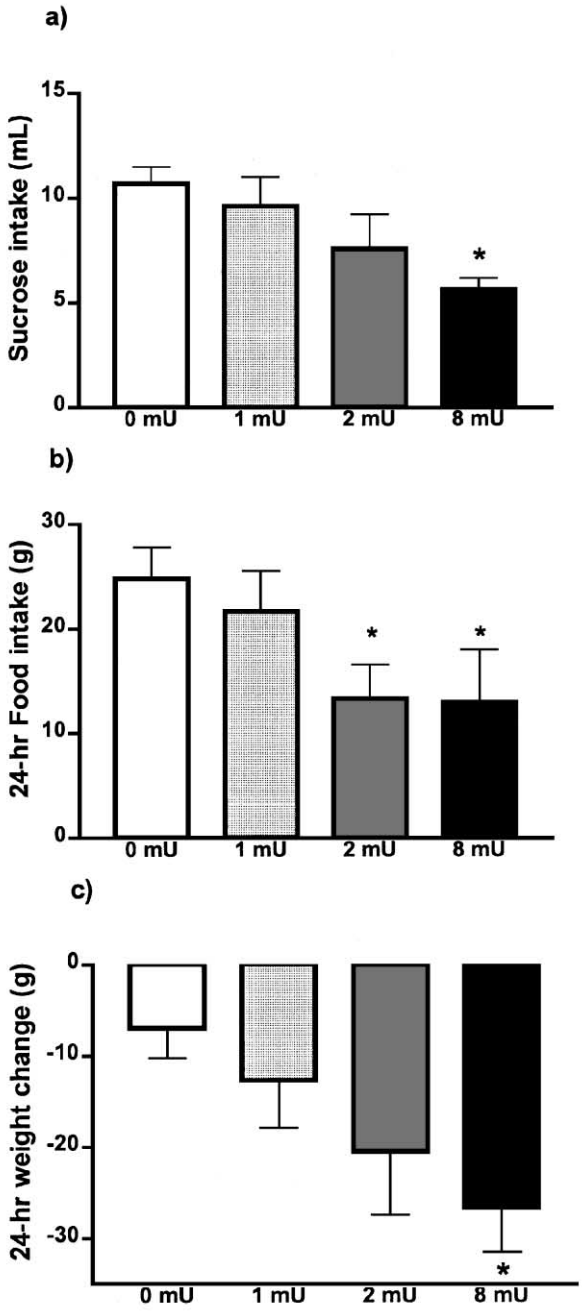

Fig. 5. Mean ± S.E.M. (a) 1-h sucrose intake, (b) 20-h chow intake, and (c) 24-h body weight change following intracerebroventricular saline or insulin injection. Both sucrose intake (a) and body weight were significantly decreased by 8 mU insulin, while chow intake was significantly reduced by the 2- and 8-mU doses. * $P < .05$.

antly reduced 20-hr chow intake ($P < .05$) but not sucrose intake or body weight. The 1-mU dose of insulin did not significantly alter any of the three measures.

7. Discussion

These results indicate that when a habitual test meal is included, a single intracerebroventricular injection of insulin acutely reduces food intake. Hence, the data are consistent with and extend the observations of Riedy et al. (1995) who found that intracerebroventricularly administered insulin interacts with intraperitoneally administered CCK-8 to reduce meal size with comparable temporal parameters. Placing rats on a meal-feeding schedule in which a daily period of food deprivation is followed by return of food at a precise time each day is a well-established way to ensure that rats will eat a relatively constant amount of food at that time (Wiley and Leveille, 1970; Woods et al., 1977). Assessing the size of these daily test meals has become a commonly employed strategy when evaluating potentially anorexic or orexigenic compounds (McKay et al., 1981; Seeley et al., 1993; Covasa et al., 2000; Gibson and Booth, 2000; McMinn et al., 2000; Smith, 2000). In this study, we successfully used the size of a 1-h 15% sucrose meal to demonstrate the acute effects of insulin on food intake. In addition, the paradigm demonstrated effects of the same injection of insulin over a longer span, with both body weight and 20-h chow also reduced. All of these effects were greater when more insulin was administered.

8. General discussion

Insulin has been found to act as an adiposity signal when administered over periods of days to weeks in several species (Woods et al. 1979; Porte and Woods, 1981; Brief and Davis, 1984; Florant et al., 1991). That is, when chronically administered into the brain, insulin elicits a reduction of both food intake and body weight. The demonstration that rats whose body weights were reduced prior to receiving intracerebroventricular insulin injection were not hypophagic, and rather that they simply maintained their already-reduced weights in the presence of insulin, provides the most compelling argument that insulin provides a long-acting signal to the brain to reduce body weight (Chavez et al., 1995). Our goal in the present experiments was to determine if a comparable conclusion could be reached with a more acute paradigm, one that required only a single, bolus injection of insulin. A second and equally important feature of the paradigm is that the size of the test meal is very consistent from day to day. This allows assessment of potential interactions of compounds such as insulin with others thought to act in relatively narrow window of time. The success of the strategy was initially demonstrated by Riedy et al. in that intracerebroventricular insulin administration was found to enhance the ability of an acutely given dose of CCK to reduce meal size.

We have presented two paradigms here. While both of these paradigms offer substantial advantages over the long duration and technical difficulties of chronic infusion, including the opportunity for easy injection of additional compounds, some disadvantages must be acknowledged. The paradigm used in Experiment 1, while demonstrating that insulin is effective, resulted in a large window (20 h) within which effects are seen. This problem was circumvented in Experiment 2, but that paradigm is limited by the long training period required to ensure that the rats eat a meal of constant size at the same time each day. Nonetheless, modifications of these paradigms will undoubtedly provide useful tools with which to study the role of insulin and other adiposity signals in the regulation of energy homeostasis.

Acknowledgments

This work was supported by grants from the American Diabetes Association (Physician Scientist Training Award to ELA) and the National Institutes of Health (DK17844 to SCW and DK10032 to SCB) and Procter & Gamble.

References

Bagdade JD, Bierman EL, Porte D. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. J Clin Invest 1967;46:1549–57.

Baskin DG, Brewitt DA, Corp E, Pacquette T, Figlewicz DP, Lewellen TK, Graham MK, Woods SC, Dorsa DM. Quantitative autoradiographic evidence for insulin receptors in the choroid plexus of the rat brain. Diabetes 1986;35:246–9.

Baskin DG, Sipols AJ, Schwartz MW, White MF. Immunocytochemical detection of insulin receptor substrate-1 (IRS-1) in rat brain: colocalization with phosphotyrosine. Regul Pept 1993;48:257–66.

Baskin DG, Sipols AJ, Schwartz MW, White MF. Insulin receptor substrate-1 (IRS-1) expression in rat brain. Endocrinology 1994;134: 1952–5.

Baura GD, Kahn SE, Taborsky GJ, Porte D, Foster D, Bergman RN, Schwartz MW. Evidence for saturable transport of insulin from plasma into cerebrospinal fluid in vivo. Clin Res 1992;40:55A.

Baura G, Foster D, Porte D, Kahn SE, Bergman RN, Cobelli C, Schwartz MW. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo: a mechanism for regulated insulin delivery to the brain. J Clin Invest 1993;92:1824–30.

Brief DJ, Davis JD. Reduction of food intake and body weight by chronic intraventricular insulin infusion. Brain Res Bull 1984;12:571–5.

Chavez M, Kaiyala K, Madden LJ, Schwartz MW, Woods SC. Intraventricular insulin and the level of maintained body weight in rats. Behav Neurosci 1995;109:528–31.

Covasa M, Ritter RC, Burns GA. Reduction of food intake by intestinal macronutrient infusion is not reversed by NMDA receptor blockade. Effects of intraduodenal glucose and fructose on antropyloric motility and appetite in healthy humans. Am J Physiol Regul Integr Comp Physiol 2000;278:R345–51.

Florant GL, Singer L, Scheurink AJ, Park CR, Richardson RD, Woods SC. Intraventricular insulin reduces food intake and body weight of

marmots during the summer feeding period. Physiol Behav 1991;49: 335–8.
Gibson EL, Booth DA. Food-conditioned odour rejection in the late stages of the meal, mediating learnt control of meal volume by aftereffects of food consumption. Appetite 2000;34:295–303.
Matson CA, Ritter RC. Long-term CCK–leptin synergy suggests a role for CCK in the regulation of body weight. Am J Physiol 1999;276: R1038–45.
McKay LD, Kenney NJ, Edens NK, Williams RH, Woods SC. Intracerebroventricular beta-endorphin increases food intake of rats. Life Sci 1981;29:1429–34.
McMinn JE, Sindelar DK, Havel PJ, Schwartz MW. Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. Endocrinology 2000;141:4442–8.
Polonsky BD, Given E, Carter V. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. J Clin Invest 1988a;81:442–8.
Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, Beebe C, Galloway JA, Frank BH, Karrison T, Van-Cauter E. Quantitative study of insulin secretion and clearance in normal and obese subjects. J Clin Invest 1988b;81:435–41.
Porte D, Woods SC. Regulation of food intake and body weight in insulin. Diabetologia 1981;20:274–80.
Riedy CA, Chavez M, Figlewicz DP, Woods SC. Central insulin enhances sensitivity to cholecystokinin. Physiol Behav 1995;58:755–60.
Schwartz MW, Woods SC, Porte DJ, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404:661–71.
Seeley RJ, Kaplan JM, Grill HJ. Effects of interrupting an intraoral meal on meal size and meal duration in rats. Appetite 1993;20:13–20.
Seeley RJ, van Dijk G, Campfield LA, Smith FJ, Nelligan JA, Bell SM, Baskin DG, Woods SC, Schwartz MW. The effect of intraventricular administration of leptin on food intake and body weight in the rat. Horm Metab Res 1996;28:664–8.
Smith JC. Microstructure of the rat's intake of food, sucrose and saccharin in 24-hour tests. Neurosci Biobehav Rev 2000;24:199–212.
Wiley JH, Leveille GA. Significance of insulin in the metabolic adaptation of rats to meal ingestion. J Nutr 1970;100:1073–80.
Woods SC. Insulin and the brain: a mutual dependency. Recovery of function following damage to central catechoamine-conating neurons: a neurochemical model for the lateral hypothalamic syndrome. J Comp Physiol Psychol 1996;16:53–81.
Woods SC, Vasselli JR, Kaestner E, Szakmary GA, Milburn P, Vitiello MV. Conditioned insulin secretion and meal feeding in rats. Absence of satiety during sham feeding in the rat. Progress in Psychobiology and Physiological Psychology 1977;91:128–33.
Woods SC, Lotter EC, McKay LD, Porte D. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 1979;282:503–5.
Woods SC, Chavez M, Park CR, Riedy C, Kaiyala K, Richardson RD, Figlewicz DP, Schwartz MW, Porte D, Seeley RJ. The evaluation od insulin as a metabolic signal controlling behavior via the brain. Neurosci Biobehav Rev 1995;20:139–44.
Woods SC, Seeley RJ, Porte DJ, Schwartz MW. Signals that regulate food intake and energy homeostasis. Science 1998;280:1378–83.