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Central infusion of GLP-1, but not leptin, produces conditioned taste aversions in rats

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Thiele, Todd E., Gertjan Van Dijk, L. Arthur Campfield, Francoise J. Smith, Paul Burn, Stephen C. Woods, Ilene L. Bernstein, and Randy J. Seeley. Central infusion of GLP-1, but not leptin, produces conditioned taste aversions in rats. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R726–R730, 1997.—Leptin (ob protein) and glucagon-like peptide-1-(7—36) amide (GLP-1) are peptides recently proposed to be involved in the regulation of food intake. Although the ability of exogenous leptin and GLP-1 to modulate consummatory behavior is consistent with the suggestion that these peptides are endogenous regulatory agents, central administration of these peptides may have aversive side effects, which could explain the anorexia. In the present experiment, exposure to a saccharine taste was immediately followed by central administration of leptin or GLP-1 to determine if these drugs could produce a conditioned taste aversion (CTA) in rats. At doses equated for producing comparable reductions in short-term food intake, GLP-1, but not leptin, generated a robust CTA. Although leptin caused no aversion, this peptide was the only drug to cause relatively long-term reductions in food consumption (16 h) and body weight (24 h). Hence, the results indicate that central GLP-1 produces aversive side effects, and it is argued that these nonspecific effects may explain the anorectic actions of GLP-1.

food intake; body weight; satiety; peptides; lithium chloride

EVIDENCE SUGGESTS THAT FOOD intake and body weight regulation are mediated, in part, by neuropeptides acting at the level of the central nervous system (CNS). Leptin, secreted from white adipose tissue (9, 20), and glucagon-like peptide-1-(7—36) amide (GLP-1), secreted from the distal portion of the gut (8) and by neurons within the CNS (6, 7), are peptides that have each recently been suggested to function as metabolic regulatory factors. Central infusion of exogenous GLP-1 reduces food intake in rats (16, 18), and central administration of exogenous leptin reduces food intake and body weight in mice and rats (1, 11, 18).

Before a peptide that causes anorexia can be considered a naturally occurring satiety factor, it must be determined that it does not induce other states that could account for reduced food intake (2, 19). Although the ability of exogenous leptin and GLP-1 to modulate consummatory behavior and/or adiposity is consistent with the suggestion that these peptides are endogenous regulatory agents, central administration of these peptides may have aversive side effects. Such nonspecific effects would likely cause reductions in eating and could explain the anorectic actions associated with central leptin and GLP-1 administration.

To determine if infusion of leptin or GLP-1 into the third ventricle (i3vt) at doses that reduce short-term food intake is associated with aversive reactions, the present experiment examined the ability of these drugs to support the development of a conditioned taste aversion (CTA). When consumption of a novel taste is followed by intraperitoneal injection of the toxin lithium chloride (LiCl), rats avoid subsequent consumption of the taste, indicating that they have learned a CTA (14, 15). In the present study, rats were given intraoral infusion of a saccharine taste and were then immediately given a i3vt infusion of leptin or GLP-1. These peptides were administered at doses found to produce similar reductions in food intake over a 4-h period after infusion (see Table 1; data previously published in Ref. 18). As a positive control to show that this general procedure could promote CTA learning, a third group of rats were given intraperitoneal injection of LiCl after saccharin exposure. The development of a CTA would suggest that administration of the drug that produced the CTA also produces aversive side effects.

MATERIALS AND METHODS

Animal preparation. Subjects were male Long-Evans rats weighing between 300 and 400 g at the onset of the experiment. They were individually housed in stainless steel cages and maintained on a 12:12-h light-dark cycle. Laboratory rat chow and water were provided ad libitum (except where noted). Under Equithesin (3.3 ml/kg ip) anesthesia, rats to be infused with leptin or GLP-1 or their vehicle were implanted with 21-gauge stainless steel cannulas (Plastics One, Roanoke, VA) aimed at the third ventricle. With bregma and lambda at the same vertical coordinate, the sagittal venous sinus was carefully displaced laterally with a metal probe, and the cannulas were placed directly on the midline, 2.2 mm posterior to bregma and 7.4 mm ventral to dura, and fixed to the skull with anchor screws and dental acrylic. The cannulas were fitted with removable obturators that extended 0.5 mm beyond the tip of the guide cannula. All rats were implanted unilaterally with intraoral cannulas constructed of PE-100

Table 1. *Four-hour food intake in rats after central infusion of drug*

Drug ($n = 6$ each)	Food Intake, g
Leptin (3.5 μg)	2.67 + 0.68*
GLP-1 (10.0 μg)	2.23 ± 0.48*
sCSF	5.82 ± 0.55

Values are means ± SE. *$P < 0.05$ for 3rd ventricular (i3vt) drug [leptin and glucagon-like peptide-1-(7—36) (GLP-1)] vs. i3vt synthetic cerebrospinal fluid (sCSF). These data were previously reported in Ref. 18.

tubing. The cannulas were placed anterolateral to the first maxillary molar and threaded subcutaneously to exit the top of the head. Each rat was given 0.15 ml each of Chloromycetin (100 mg/ml sc) and gentamicin (40 mg/ml ip) prophylactically. When rats regained their preoperative body weights after surgery (2–3 wk), placement of i3vt 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 of water within 60 min were not used in the study.

Taste aversion training. Rats were initially habituated to a Plexiglas observation chamber and intraoral fluid infusion by being placed in the chamber for 20 min/day over 4 days. On the last 2 days of habituation, rats received an intraoral infusion of 5 ml distilled water (0.5 ml/min) during the first 10 min in the chamber. The rats were then assigned to one of three drug treatment conditions (leptin, GLP-1, or LiCl), and each treatment condition was further divided into two subgroups (all subgroups were equated for body weight). On each of 2 conditioning days (separated by 1 day), rats were placed in the observation chamber for 5 min and intraorally infused with 2.5 ml of 0.15% saccharin delivered by infusion pump at a rate of 0.5 ml/min. This amount of saccharin is readily ingested by rats (15). During the infusion, rats were videotaped, and the amount of time (s) that passed before the rat rejected fluid (passive dripping or active fluid expulsion) was scored. Immediately after taste exposure, one of the subgroups within each treatment condition was given i3vt infusion (3.5 μl manually infused with a Hamilton syringe over 60 s; see Ref. 18 for details) of either leptin [Paired-Leptin; 3.5 μg dissolved in synthetic-cerebro-spinal fluid (sCSF; see Ref. 17 for composition of sCSF; $n = 5$)] or GLP-1 (Paired-GLP-1; 10.0 μg dissolved in sCSF; $n = 5$) or an intraperitoneal injection of LiCl (Paired-LiCl; 127 mg/kg; 0.15 M solution dissolved in sterile water; $n = 4$). Thus experience with the taste and the drug occurred at approximately the same time. Rats from the other subgroup of each treatment condition were given control solutions (i3vt sCSF or intraperitoneal isotonic saline) immediately after taste exposure (Unpaired-Leptin, $n = 5$; Unpaired-GLP-1, $n = 5$; and Unpaired-LiCl, $n = 5$).

After each conditioning day, unpaired rats were given i3vt infusion of leptin or GLP-1 or given intraperitoneal injection of LiCl (same doses described above). Paired rats were infused with sCSF or injected with saline. Animals were treated with the drug or the control solution, without exposure to the taste, and immediately returned to their home cages. With this experimental design, paired and unpaired subgroups of each treatment condition had similar exposure to drugs and to saccharin; however, in the paired subgroups, the drug was presented immediately after saccharin exposure, whereas unpaired subgroups received the taste and the drug on separate days.

Assessment of conditioned taste aversions. Two independent measures were used to examine CTAs. First, on the second conditioning day (before drugs were administered) rejection of saccharin was used as an index of CTA. Immediately after the second drug administration, two animals developed infections and were killed. Three days after the second conditioning trial, a second measure of CTA learning was assessed by measuring 24-h consumption of rats from two available bottles, one containing 0.15% saccharin and one containing distilled water. The saccharine preference ratio was calculated as the amount of saccharin consumed divided by total consumption of both fluids.

Assessment of food consumption and body weight. On each day, the weight of rats and food hoppers was taken at ~1000. In addition, hoppers were weighed at 1800 (to assess overnight 16-h food intake). Sixteen-hour food intake and 24-h body weight change of rats were assessed after the first administration of drug (leptin, GLP-1, or LiCl) and after the first administration of control solution (sCSF or isotonic saline). It should be noted that subjects received the i3vt or intraperitoneal injection of solutions (drug treatments presented in a counterbalanced order) at various times of the day during the taste aversion procedure. Thus the 16-h consumption data and the 24-h body weight data represent the effects of the drugs during the measured periods but do not represent the time interval immediately after drug exposure.

Data analyses. Data from each CTA measure (fluid rejection and two-bottle preference ratio) were analyzed with two-way, 2 × 3 (subgroup × drug) analyses of variance (ANOVA). The subgroup factor examined differences between paired and unpaired subgroups, whereas the drug factor examined differences between groups exposed to leptin, GLP-1, or LiCl. Data from consumption and body weight measures were analyzed with mixed-factor, 2 × 3 (exposure × drug) ANOVA. The within-subjects exposure factor examined differences after subject exposure to drug versus control solution, whereas the between-subjects drug factor examined differences between groups exposed to either of the three drugs. When significant differences were found, post hoc analyses were conducted using *t*-tests. In all cases, $P < 0.05$ (2-tailed) indicated statistical significance.

RESULTS

Conditioned taste aversions. On the first conditioning day, saccharin was ingested throughout all or most of the 5-min infusion period, and there were no differences among groups. Fluid rejection data collected after CTA training are presented in Fig. 1*A*, and saccharin preference data collected during the two-bottle test are presented in Fig. 1*B*. Relative to unpaired subgroups, rats that received saccharin paired with either GLP-1 or LiCl rejected saccharin earlier in the test session. Furthermore, rats had a decreased preference for saccharin if that taste had been paired with i3vt GLP-1 or intraperitoneal injection of LiCl. With both measures, pairing with leptin did not alter the acceptability of the saccharine taste. These results are confirmed by the following analyses.

An ANOVA performed on the fluid rejection data revealed a significant subgroup effect [$F(1,23) = 25.15$] and a significant interaction effect between the subgroup and drug factors [$F(2,23) = 6.53$]. Post hoc tests revealed that, although subgroups Paired-GLP-1 and Paired-LiCl differed from their control subgroups (Unpaired-GLP-1 and Unpaired-LiCl, respectively), subgroups Paired-Leptin and Unpaired-Leptin did not differ significantly. ANOVA performed on the saccha-

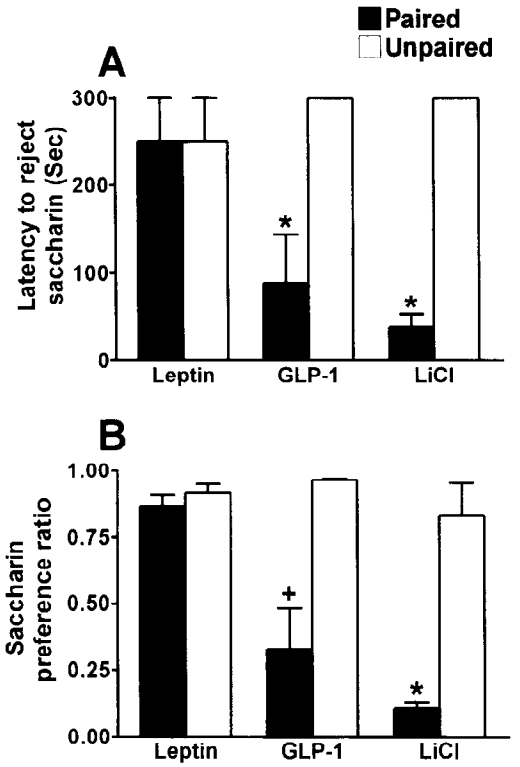

Fig. 1. Mean (+SE) latency to reject intraorally infused saccharin (*A*) and preference for saccharin in a consumption test (*B*) after conditioned taste aversion training. Preference was defined as consumption of saccharin divided by total fluid (saccharin plus water) consumption. Filled bars represent paired subjects that received saccharin immediately followed by drug [leptin (3.5 μg), glucagon-like peptide-1-(7—36) (GLP-1) (10.0 μg), or LiCl (127 mg/kg)], and open bars represent unpaired subjects that received saccharin and drug on separate days. + Significant at $P < 0.05$ relative to unpaired; *$P < 0.001$ relative to unpaired.

rin preference data revealed a significant subgroup effect [$F(1,21) = 29.09$], a significant drug effect [$F(2,21) = 9.11$], and a significant interaction effect [$F(2,21) = 6.13$]. Post hoc tests showed that paired subgroups treated with GLP-1 and LiCl demonstrated significant reductions in saccharin preference ratio relative to controls; again, subgroups Paired-Leptin and Unpaired-Leptin did not differ significantly.

Drug effects on food consumption and body weight. Overnight 16-h food consumption after the first drug or control treatment is presented in Fig. 2*A*, and data representing 24-h change in body weight are presented in Fig. 2*B*. Only i3vt leptin reduced overnight 16-h food consumption relative to the control solution (sCSF); consumption after i3vt GLP-1 infusion or LiCl injection was not reduced when compared with that after the control drug (sCSF and saline, respectively). The same trend is visible with the body weight data; only leptin caused reduction of body weight during the 24-h measurement.

ANOVA performed on the consumption data revealed a significant exposure effect [$F(1,26) = 17.26$] and a significant interaction between the exposure and drug effects [$F(2,26) = 3.52$]. Tests revealed that the only drug to cause a significant difference relative to the control infusion was leptin; neither GLP-1 nor LiCl differed from its respective controls. Likewise, ANOVA run on the body weight data revealed significant drug [$F(2,26) = 7.19$], exposure [$F(1,26) = 7.12$], and interaction [$F(2,26) = 4.21$] effects. Again, tests revealed that only leptin-treated animals differed from the control treatment.

DISCUSSION

At doses that produce similar reductions of short-term food intake (see Table 1; Ref 18), i3vt infusion of GLP-1, but not leptin, generated a robust CTA. Furthermore, these results were obtained using two independent measures of CTA learning (latency to reject intraoral saccharin and a two-bottle, saccharin versus water preference test). Because the development of a CTA implies that the drug supporting the aversion produces aversive, nonspecific effects, an alternative explanation for the previously observed short-term anorectic actions of GLP-1 is that aversive effects of the drug reduce food intake. It may be argued that, whereas GLP-1 produced relatively immediate aversive effects, a delay in the onset of aversive side effects produced by leptin infusion may have prevented CTA learning. However, because strong taste aversions can be obtained even when the toxin is presented 6-h after the taste (13), any aversive effects produced by leptin would presumably occur more than 6 h after drug infusion. These effects could not explain the reductions in food intake found within the first few hours after leptin infusion (e.g., Ref. 18). Thus, although the doses of both leptin and GLP-1 administered in the present study produce similar reductions in short-term food intake, only leptin does so

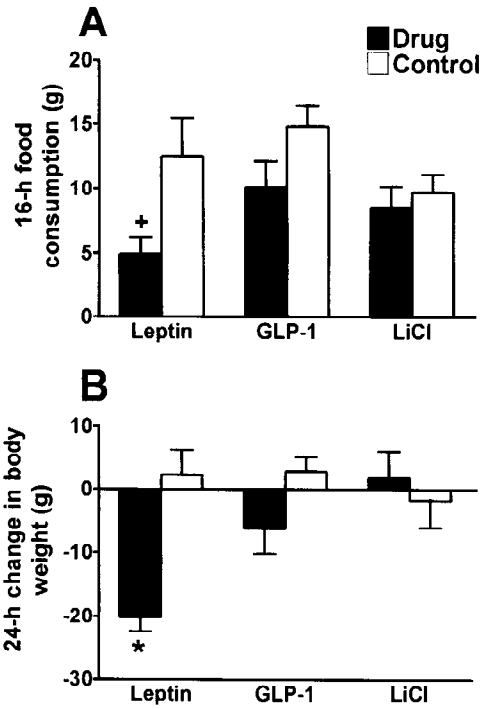

Fig. 2. Mean (+SE) 16-h food consumption (*A*) and 24-h change in body weight (*B*) in rats on first day of exposure to drug (filled bars) and to control solution (synthetic cerebrospinal fluid or saline; open bars). + Significant at $P < 0.05$ relative to control; *$P < 0.001$ relative to control.

via a mechanism apparently unrelated to effects that support CTAs. Additionally, although it may be argued that a larger dose of centrally infused leptin may produce a CTA, the present results demonstrate that a dose exists that produces robust reductions in food intake and body weight without inducing aversive side effects. Finally, because peripheral injection of leptin causes reductions in food intake and adiposity (1), the possibility remains that peripheral administration of leptin may be associated with aversive side effects.

Although the present results argue that caution is necessary when interpreting the anorectic actions of GLP-1, the data do not necessarily exclude the possibility that GLP-1 is an endogenous satiety factor. Exendin, a GLP-1 receptor antagonist, produces small but significant increases in food intake in satiated rats, supporting the possibility that GLP-1 is an endogenous satiety agent (16). A similar pattern of results has been observed with the proposed anorectic agent cholecystokinin octapeptide (CCK-8); CCK-8-receptor antagonists increase food consumption in satiated rats (5, 12). Furthermore, like GLP-1, high doses of CCK-8 reduce food intake and produce a CTA (3). On the other hand, at lower doses, CCK-8 produces anorexia without supporting a CTA. Finally, both CCK-8 and GLP-1 activate c-Fos expression in similar brain stem areas (4, 10, 18). Given the similarities between CCK-8 and GLP-1, it seems possible that lower doses of central GLP-1 (i.e., <10.0 μg) could reduce acute eating in the absence of aversive side effects. It is also possible that the aversive side effects associated with GLP-1 are due to i3vt administration, but that injection of the drug into specific sites (e.g., hypothalamic nuclei) could reduce food intake in the absence of aversion.

The anorectic actions of GLP-1 have typically been examined over the first several hours after drug administration (16), and we have previously found comparable results using the same dose of GLP-1 as used in the present experiment (see Table 1; Ref. 18). In the present experiment, however, the effects of GLP-1 on food intake over a relatively long postinfusion interval (i.e., 16 h or longer) were insignificant. Thus GLP-1-induced anorexia appears to be relatively short lived. The effects of leptin on consummatory behavior are, therefore, different from those of GLP-1, as leptin reduced food intake and body weight over the longer interval in the present experiment.

Using c-Fos immunoreactivity as an indication of neural activity, i3vt infusion of either leptin or GLP-1 has been found to induce neuronal activity in hypothalamic and limbic forebrain regions involved in the regulation of feeding behavior (16, 18). In addition, GLP-1, but not leptin, was found to stimulate neuronal activity in several brain stem areas, including the nucleus of the solitary tract, lateral parabrachial nucleus, and area postrema (18), regions also activated by the toxin LiCl (14, 15). Thus, although it is possible that both leptin and GLP-1 have satiating properties, central GLP-1 infusion also produces aversive side effects that may be caused by this peptide's stimulation of brain stem regions. Together, c-Fos immunoreactivity data (18) and the present CTA and consumption data suggest that leptin and GLP-1 produce their anorectic actions via different mechanisms.

Perspectives

Over the last several years, our ability to identify putative anorectic compounds that are involved in the regulation of food intake and body weight has increased dramatically. However, reduced food intake is not sufficient evidence that a compound is an endogenous regulatory agent; it may produce anorexia by inducing a variety of side effects, including aversive states (e.g., visceral illness or malaise, fear, pain), motor impairment, sleepiness, or behavioral disorganization (2, 19). As reported here, CTA procedures can provide important screens for the ability of a compound to induce aversive states. We suggest that multiple measures of the effects of an agent (including but not limited to its effect on food intake and its ability to support a CTA) need to be employed before ascribing a role for that agent as an endogenous regulator of food intake and body weight.

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REFERENCES

1. **Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos, and P. Burn.** Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science Wash. DC* 269: 546–549, 1995.
2. **Deutsch, J. A.** Food intake. In: *Handbook of Behavioral Neurobiology: Neurobiology of Food and Fluid Intake*, edited by E. M. Striker. New York: Plenum, 1990, vol. 10, p. 151–182.
3. **Ervin, G. N., L. S. Birkemo, M. F. Johnson, L. K. Conger, J. T. Mosher, and J. A. Menius.** The effects of anorectic and aversive agents on deprivation-induced feeding and taste aversion conditioning in rats. *J. Pharmacol. Exp. Ther.* 273: 1203–1210, 1995.
4. **Fraser, K. A., and J. S. Davidson.** Cholecystokinin-induced c-fos expression in the rat brain stem is influenced by vagal nerve integrity. *Exp. Physiol.* 77: 225–228, 1992.
5. **Hewson, G., G. E. Leighton, R. G. Hill, and J. Hughes.** The cholecystokinin receptor antagonist L346.718 increases food intake in the rat by attenuation of the action of endogenous cholecystokinin. *Br. J. Pharmacol.* 93: 79–84, 1988.
6. **Jin, S. L. C., V. K. M. Han, J. E. Simmons, A. C. Towle, J. M. Lander, and P. K. Lund.** Distribution of glucagon-like peptide-1 (GLP-1), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J. Comp. Neurol.* 271: 519–532, 1988.
7. **Kreymann, B., M. A. Ghatei, P. Burnet, G. Williams, S. Kanse, A. R. Diani, and S. R. Bloom.** Characterization of glucagon-like-peptide-1(7–36) amide in the hypothalamus. *Brain Res.* 502: 325–331, 1989.
8. **Kreymann, B., Y. Yiangou, S. Kanse, G. Williams, M. A. Ghatei, and S. R. Bloom.** Isolation and characterization of GLP-1 7–36 NH_2 from rat intestine. *FEBS Lett.* 242: 167–170, 1988.
9. **Masuzaki, H., Y. Ogawa, N. Isse, N. Satoh, T. Ozazaki, M. Shigemoto, K. Mori, N. Tamura, K. Hosoda, H. Jingami, Y. Yoshimasa, H. Jingami, T. Kawada, and K. Nakao.** Human

obese gene expression. Adipocyte-specific expression and regional differences in the adipose tissue. *Diabetes* 44: 855–858, 1995.

10. **Olson, B. R., G. E. Hoffman, A. F. Sved, E. M. Striker, and J. G. Verbalis.** Cholecystokinin induces c-fos expression in hypothalamic oxytocinergic neurons projecting to the dorsal vagal complex. *Brain Res.* 569: 238–248, 1992.
11. **Schwartz, M. W., R. J. Seeley, L. A. Campfield, P. Burn, and D. G. Baskin.** Identification of hypothalamic targets of leptin action. *J. Clin. Inv.* 98: 1101–1106, 1996.
12. **Shillabeer, G., and J. S. Davidson.** The cholecystokinin antagonist, proglumide, increases food intake in the rat. *Regul. Pept.* 8: 171–176, 1984.
13. **Smith, J. C., and D. L. Roll.** Trace conditioning with x-rays as an aversive stimulus. *Psychon. Sci.* 9: 11–12, 1965.
14. **Swank, M. W., G. E. Schafe, and I. L. Bernstein.** c-Fos induction in response to taste stimuli previously paired with amphetamine or LiCl during taste aversion learning. *Brain Res.* 673: 251–261, 1995.
15. **Thiele, T. E., M. F. Roitman, I. L. Bernstein.** c-Fos induction in rat brainstem in response to ethanol- and lithium chloride-induced conditioned taste aversions. *Alcohol. Clin. Exp. Res.* 20: 1023–1028, 1996.
16. **Turton, M. D., D. O'Shea, I. Gunn, S. A. Beak, C. M. Edwards, K. Meeran, S. J. Choi, G. M. Taylor, M. M. Heath, P. D. Lambert, J. P. Wilding, D. M. Smith, M. A. Ghatei, J. Herbert, and S. R. Bloom.** A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature Lond.* 379: 69–72, 1995.
17. **Van Dijk, G., A. E. Bottone, A. B. Steffens, and J. H. Strubbe.** Hormonal and metabolic effects of paraventricular hypothalamic administration of neuropeptide Y during rest and feeding. *Brain Res.* 660: 96–103, 1994.
18. **Van Dijk, G., T. E. Thiele, J. C. K. Donahey, L. A. Campfield, F. J. Smith, P. Burn, I. L. Bernstein, S. C. Woods, and R. J. Seeley.** Central infusion of leptin and GLP-1-(7—36) amide differentially stimulate c-FLI in the rat brain. *Am. J. Physiol.* 271 (*Regulatory Integrative Comp. Physiol.* 40): R1096–R1100, 1996.
19. **Woods, S. C., M. Chavez, C. R. Park, C. Riedy, K. Kaiyala, R. D. Richardson, D. P. Figlewicz, M. W. Schwartz, D. Porte, and R. J. Seeley.** The evaluation of insulin as a metabolic signal controlling behavior via the brain. *Neurosci. Biobehav. Rev.* 58: 1237–1241, 1995.
20. **Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman.** Positional cloning of the mouse obese gene and its human homologue. *Nature Lond.* 372: 425–432, 1994.