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Intraventricular Insulin Reduces Food Intake and Body Weight of Marmots During the Summer Feeding Period

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FLORANT, G. L., L. SINGER, A. J. W. SCHEURINK, C. R. PARK, R. D. RICHARDSON AND S. C. WOODS. *Intraventricular insulin reduces food intake and body weight of marmots during the summer feeding period.* PHYSIOL BEHAV **49**(2) 335–338, 1991.—The study presented below describes experiments that investigate the ability of insulin to inhibit food intake in awake, active marmots during the summer season. Our results suggest that increasing intraventricular insulin concentration during the summer active feeding period will cause a decrease in food intake and body weight of marmots. When infused with insulin into their lateral ventricles (Alzet #2002 minipumps), animals had significantly lower food intake as compared to their food intake during the control period. In addition, these animals lost body weight during the period of the insulin infusion. We suggest that during the summer when marmots are not hibernating and are actively feeding, brain insulin levels may play a role in regulating food intake.

Hibernation Marmot Body weight Food intake Insulin

THE amount of adipose tissue in the body of mammals appears to be precisely regulated (25). Evidence supporting this concept derives from the fact that (a) the adipose tissue mass is relatively stable in adult mammals over time (6,25); (b) there is a rapid return to basal conditions following forced or voluntary changes of adiposity (6,25) and (c) hyperphagia and lipogenesis occur in lipectomized animals until presurgical fat content is restored (9). The primary mechanism by which this regulation is achieved is behavioral, i.e., via changes of food intake (22). Animals below their preferred body adiposity eat more food than normal, and animals above their preferred body mass eat less food than normal (13). The implication is that information concerning the amount of fat present in the body must be signalled to the central nervous system (CNS) and integrated with other controllers of food intake.

There is considerable evidence that the pancreatic hormone insulin is important in this process (23,27). Insulin is secreted into the blood in direct proportion to adiposity in mammals (3, 5, 25). Because this is true under both basal and stimulated conditions (13), plasma insulin concentration is a reliable indicator of the amount of fat present in the body. There is also evidence that plasma insulin gains access to the CNS and that specific insulin receptors are found in discrete brain areas, many of which are important in the control of food intake (4).

As such, it is reasonable to suggest that the amount of insulin detected by certain brain areas may indicate the level of adiposity and be a determinant of food intake (27). Consistent with this hypothesis, administration of exogenous insulin directly into the CNS causes a dose-dependent reduction of food intake and body mass in baboons (26) and rats (1, 7, 14). Likewise, administration of insulin antibodies into the CNS causes an increase of food intake (20) and body mass (18) of rats. The latter studies argue strongly that endogenous brain insulin is an important mediator of food intake and body mass.

Marmots (*Marmota flaviventris*) are large rodents that undergo profound circannual rhythms in feeding, body mass and metabolism (2,22). In the late spring and summer they eat food and gain weight in the form of body fat. By late autumn and winter, they hibernate and food intake drops to zero (22). Although the mechanisms and causes of this circannual metabolic and behavioral pattern have been extensively studied, the precise controllers of food intake in these mammals are unknown.

In previous studies, we determined the relationship between plasma insulin and body weight (10) as well as plasma insulin and brain insulin of marmots at different points in their seasonal cycle (12). We found that during specific times of the year when marmots are feeding (spring), elevating endogenous plasma insulin (i.e., by infusing glucose) raises insulin levels within the cerebrospinal fluid (CSF) of the brain (12). However, during midwinter when the animals are hibernating, increased plasma insulin causes little or no change in CSF insulin concentrations (12). These data suggest that insulin acting within the brain is an unlikely candidate to be responsible for the suppression of feeding that occurs in these animals during the winter months because CSF insulin is highest when the animals are eating at the greatest rate. As such, since insulin appears to have better access to the brain when marmots are feeding, the purpose of the present experiment was to determine if insulin suppresses feeding and body mass in marmots during the summer when they are feeding and do not enter bouts of hibernation.

METHOD

Four yellow-bellied marmots which had been trapped in the West Elk Mountains of Colorado were used. The animals were shipped to Seattle in the late summer and maintained in individual cages in an outdoor enclosure throughout the ensuing autumn, winter and spring. They had ad lib food (pelleted Purina rodent chow and fresh vegetables) and water until November, when food was removed since they had stopped eating. All animals hibernated during the winter, and in spring (April) food was returned.

The present study was conducted in June, i.e., approximately one month after the initiation of feeding by the animals and at a time when food intake is high (22). During the study, the animals had ad lib access to laboratory chow and water. Vegetables were removed from the diet throughout the experiment so that food intake could be more accurately assessed by daily weighing of the pellets.

Prior to the experiment, each animal was tranquilized with ketamine (35 mg/kg) and anesthetized with halothane. Its head was then positioned in a Kopf small animal stereotaxic holder such that the top of the head was parallel to the horizontal plane of the apparatus. The top of the skull was then exposed, a small hole was drilled, and a 21-gauge stainless steel cannula fitted with a 26-gauge obturator was lowered into the brain. It was aimed at the lateral cerebral ventricle, the coordinates being 6 mm anterior to the interaural line, 5 mm lateral to the mid-line, and 8 mm ventral to the dura (8). Anchor screws were placed in the skull surrounding the cannula and the cannula was secured with dental acrylic.

Nine days later, the animals were again anesthetized and a small incision was made in the dorsum of the neck. The obturator was removed from the cannula and an osmotic minipump (Alzet #2002) containing synthetic CSF was attached to a 26-gauge injector via PE-60 tubing and the injector was inserted into the cannula. The pump was affixed to subcutaneous tissue in the neck and the wound closed. The pump delivered synthetic CSF at a rate of 0.55 μl/h for four days.

After the fourth day, the animals were again anesthetized and the minipump was removed and replaced with a similar pump containing porcine insulin in synthetic CSF at a concentration calculated to deliver 20 mU of porcine insulin/day into the ventricle. The insulin infusion continued for seven days. Food intake was determined on a daily basis over the entire experimental period following implantation of the intraventricular cannulae. Body weights were obtained when the animals were tranquilized for the various surgical procedures as well as at the end of the experiment. A paired *t*-test or Student's *t*-test was used to determine statistical differences.

RESULTS

Food intake and body weight data for the four animals during the final 5 days prior to the infusion of synthetic CSF are depicted in Fig. 1 (baseline period). The average intake (in grams) for each marmot was calculated over this interval and served as a baseline. Intake on each day during the infusion of synthetic CSF or of insulin was taken as the change from this baseline. Baseline daily intakes ranged from 56.6 to 134.8 g for the four individual marmots. At the end of the baseline interval, body weight averaged 4.07 kg.

During the control infusion period (Fig. 2), food intake increased steadily over days as the animals recovered from the pump insertion procedure. At the end of this interval, food intake was not reliably different (paired *t*-test) from what it had been at the end of the baseline period. Likewise, body weight had not changed, averaging 4.06 kg at the end of this interval.

As shown in Fig. 3, intraventricular infusion of insulin at 20

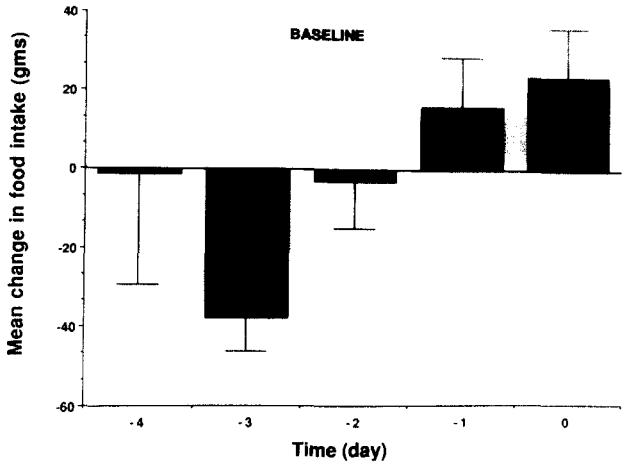

FIG. 1. Baseline food intake values for marmots prior to intraventricular injection of synthetic CSF or porcine insulin. Each bar presents the mean ± SE of the 4 animals. Food intake was determined for 4 days prior to injection (day zero).

mU/day resulted in a different pattern of food intake. On the first two days following implantation of the pump containing insulin, food intake rose slightly and was comparable to baseline values, suggesting that the animals had recovered from the procedure. On the subsequent five days, however, food intake decreased and remained below baseline until the end of the experiment. Daily intakes during these five days averaged −30.2 g/day relative to the baseline and −40.6 g/day relative to the control infusion period. Mean food intake was calculated for each animal during the final 5 days of the insulin infusion interval (i.e., after the effects of the surgical treatment were gone). In spite of the small number of animals, food intake during this interval was significantly reduced ($p<0.05$) relative to the synthetic CSF infusion interval, and approached significance ($p<0.10$, two-tailed paired *t*-test) relative to the baseline period. When only the final 2 days of the synthetic CSF infusion period were considered for the analyses, the decrease in food intake by marmots during the final 5 days of insulin infusion was −63.3 g/day ($p<0.01$, paired *t*-test). Body

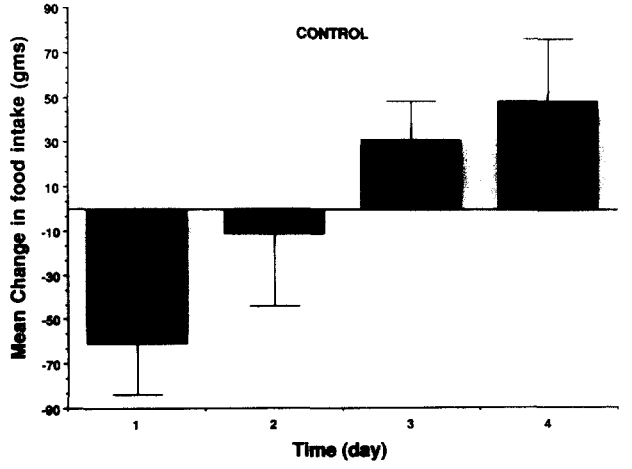

FIG. 2. Mean (±SE) change in control food intake measurements in marmots beginning on day 1 to day 4 after injection of synthetic CSF and continuing until day 4.

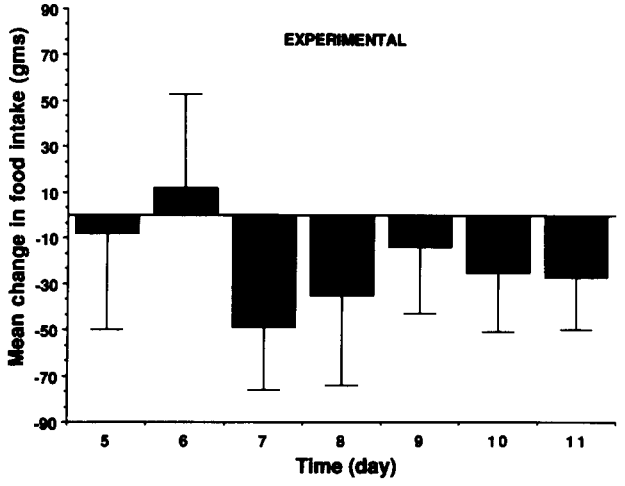

FIG. 3. Mean change in food intake after porcine insulin injection in marmots. Each bar represents the mean ± SE of 4 animals.

weight at the end of the insulin infusion period averaged 3.79 kg, and every animal had decreased its weight relative to the end of the control infusion period ($p<0.05$, sign test). The animals all appeared normal and in good health throughout the infusion interval.

DISCUSSION

Intraventricular infusion of insulin resulted in decreased food intake and body weight of marmots during their summer feeding period. In summer, when the marmot is actively feeding, it responds to exogenously infused insulin like that reported in baboons (26) and rats (7,14). The dose of insulin chosen (20 mU/marmot/day) is comparable on a units/kg/day basis to what is effective in rats (7,14).

Anytime a reduction of food intake is observed consequent to an experimental procedure, it cannot be concluded with certainty that nonspecific factors were not the cause. In the present experiment, we tried to control for the stress associated with surgery and accompanying procedures by subjecting the animals to an identical protocol but with synthetic CSF infused without added insulin. Food intake in that condition did not differ from baseline and the animals maintained their weight. When insulin was added to the infusate, the animals ate less food and lost weight, and appeared healthy and normal. The lag of several days for the insulin effect to become apparent is consistent with an initial recovery period (from the stress of the pump implantation) followed by a period of insulin action. Previous studies in which insulin was infused intraventricularly into rats (7,14) and baboons (26) also reported a lag of several days before food intake was reduced. It is noteworthy that the decrease in food intake that occurred was sufficient to account for the relatively small decrease in body weight. This suggests that marmots, like baboons (26), need not invoke other metabolic adaptations to achieve weight loss. Ideally, food intake would have been determined following the completion of the insulin infusion. Unfortunately, food intake measurements could not be obtained following the termination of the experiment, but casual observations suggested that it had returned to normal. Both rats (7,14) and baboons (26) demonstrate hyperphagia and rebound of lost weight following termination of intraventricular insulin.

One implication from these findings is that, during the summer feeding season, food intake in hibernators may be controlled by the same mechanisms as in nonhibernators. Marmots are lipogenic during the summer as they store fat for the subsequent winter's hibernation (11). Only after they enter hibernation do they become essentially lipolytic for a prolonged interval such that many tissues, presumably including the CNS, rely upon lipids for much of their energy (11). The sensitivity of marmots to intraventricular insulin during the summer lipogenic period is in agreement with the data of others. Arase et al. (1) reported that whereas rats on a normal (i.e., relatively lipogenic) diet reduce their food intake in response to intraventricular insulin, rats maintained on a high-fat diet do not. The high-fat diet animals were presumably deriving a relatively large percent of their calories from lipids. Similarly, Nagai et al. (16) reported that the ability of intraventricular insulin to reduce feeding in rats was greater during the night when the animals are lipogenic, and is reduced when they are lipolytic. One inference from these experiments is that whereas insulin is effective at reducing feeding in the lipogenic marmot, it might not be effective at reducing food intake in the lipolytic marmot. Such a contention is rather difficult to investigate since marmots in the lipolytic state are already hypophagic and cannot reduce their food intake further. A test of the hypothesis would therefore require administering insulin antibodies intraventricularly into hypophagic lipolytic marmots in the autumn and looking for an increase of food intake.

Related to this, we have found in other experiments that insulin is an unlikely candidate for the mechanism which elicits hypophagia and induces hibernation in marmots in the autumn (12). In those experiments, the amount of insulin detected within the CSF of marmots as hibernation began, as well as during hibernation, was disproportionately low. The inference is that insulin is unlikely to be controlling behavior by acting within the CNS at that time. Contrary to this, during the spring when the marmots were initiating feeding, insulin far more readily penetrated into the CSF. This suggested to us that if insulin has a role in the control of food intake and body weight in the marmot, it is most likely to be manifest in the summer when it readily gains access to the central nervous system. The present results support this contention.

In conclusion, the intraventricular infusion of insulin into feeding marmots caused a reduction of food intake and a decrease of body mass over a 7-day interval. The results are consistent with the hypothesis that insulin acts within the CNS to reduce feeding when animals are in a lipogenic condition.

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