Intraventricular insulin potentiates the anorexic effect of corticotropin releasing hormone in rats

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Richardson, Ralph D., Koichi Omachi, Rasoul Kermani, and Stephen C. Woods. Intraventricular insulin potentiates the anorexic effect of corticotropin releasing hormone in rats. Am J Physiol Regul Integr Comp Physiol 283: R1321–R1326, 2002; 10.1152/ajpregu.00521.2001.—Intraventricular corticotropin releasing hormone (CRH) suppresses food intake and body weight as a stress response. Insulin, acting within the brain, also suppresses food intake and body weight, and this suppression is related to caloric homeostasis. We determined if increased insulin within the brain potentiates the anorexic effects of intraventricular CRH. Rats were food deprived for 17 h each day and then given 30-min access to Ensure. One-half received continuous third ventricular infusion of synthetic cerebrospinal fluid via osmotic minipumps, and one-half received insulin (0.6 mU/day). During the infusion, rats also received 0, 0.1, 1.0, or 5.0 μg of CRH into the lateral ventricle just before access to Ensure. Insulin alone had no effect on Ensure intake or body weight. CRH dose dependently reduced Ensure intake in both groups, and the reduction was greater in the insulin group. Hence, central insulin potentiates the ability of centrally administered CRH to suppress food intake. These findings suggest that stress-related influences over food intake, particularly those mediated via CRH, interact with relative adiposity as signaled to the brain by central insulin signaling. The anorectic site of action of CRH has been suggested to be in closely linked nuclei in the ventral hypothalamus, including the paraventricular and ventromedial hypothalamic nuclei (35, 36) and the medial preoptic area (23). When CRH is administered on a daily basis, it reduces body weight of rats. This occurs when CRH is given as a daily intraventricular bolus (38) as well as when it is infused 24 h/day (4, 5). Although the effect is apparent in normal (lean) rats (3–5), it is accentuated in several models of obesity, including rats with lesions of the ventromedial nuclei of the hypothalamus (4) and genetically obese Zucker (fatty) rats (3, 59). In fact, CRH has been reported to prevent hyperphagia and weight gain in young, rapidly growing fatty Zucker rats (59). That same group later provided evidence for an altered CRH system in the brain (16) and hypothalamo-pituitary-adrenal axis (33) in fatty Zucker rats. The important points are that CRH powerfully suppresses food intake under several natural conditions and that obesity might be related to alterations of CRH levels and/or sensitivity in the central nervous system.

The pancreatic hormone insulin is also involved in the normal control of body weight. Insulin is secreted in direct proportion to adiposity (6, 51, 52) and a receptor-mediated transport system passes it from the plasma into the brain (7, 15, 62, 67). Within the brain, insulin interacts with specific receptors on neurons (10, 12, 22, 27) and dose dependently reduces food intake and body weight in several species (17, 43, 50, 76, 77). The hypothesis emanating from this work is that insulin signals the amount of fat present in the body to brain centers important in the regulation of food intake. If adiposity increases, the brain “sees” more insulin and food intake is reduced; if adiposity decreases, a reduction of endogenous insulin in the brain enables the animal to eat more. Hence, insulin is considered to provide a stabilizing influence that minimizes perturbances and prevents extremes of adiposity (see Ref. 69, 76, 78 for reviews).

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Insulin, acting within the brain, interacts in predictable ways with other controllers of food intake. For example, intraventricular administration of subthreshold amounts of insulin, levels that by themselves have no effect on food intake or body weight, enhances the meal-size reducing properties of peripherally administered CCK (29, 56). Likewise, centrally acting corticosterone appears to be necessary for rapid weight regain in underweight rats (32), and this activity is counteracted by insulin acting within the brain (21). Analogously, the synthesis of neuropeptide Y, a potent stimulant of food intake and body weight, is inhibited by insulin acting within the brain (64, 68). The conclusion is that many signals that influence food intake interact in predictable ways with central insulin. We therefore hypothesized that insulin, acting within the brain, would potentiate the anorexic action of CRH. The present experiment was designed to test this hypothesis.

METHODS

Animals. Subjects were 16 naive male Long Evans rats obtained from the colony maintained by the Department of Psychology of the University of Washington. On transfer to the lab they were housed in individual hanging stainless steel cages in a temperature (21°C)- and light-controlled vivarium (lights on at 0700; lights off at 1900). Before surgery, all rats had ad libitum access to pelleted chow. After surgery they were maintained on a schedule in which they were deprived of food for 17 h each day (food removed at 1700). They were then given 30-min access to the liquid diet Ensure followed by 6.5-h access to chow until the next day. Water was freely available at all times.

Surgeries. Each animal received two intraventricular stainless steel guide sleeves, one aimed at the right lateral ventricle and one aimed at the third ventricle. The two were implanted in one operation during which the animal was anesthetized with Equithesin (3 ml/kg ip). Rats were affixed to a Kopf stereotaxic instrument with the incisor bar initially set slightly lower than the interaural line. Final adjustment of the incisor bar was made to equalize the vertical coordinates of lambda and bregma. A small hole for passage of the lateral ventricular guide sleeve (22 gauge) was drilled 1 mm posterior to bregma and 2.3 mm right of the midline. The guide sleeve was angled 14 degrees (lateral to medial) and lowered 3.7 mm from the outer surface of the skull. A second small hole for passage of the third ventricular guide sleeve was drilled 1 mm posterior to bregma and 1.5 mm left of midline. This guide sleeve was lowered 8.6 mm from the outer surface of the skull at a 10-degree angle (lateral to medial). Obdurators (26 gauge), which extended 1 mm beyond the guide sleeves, were inserted into each guide sleeve at the time of surgery. The guide sleeves were held in place via anchor screws and dental acrylic. All animals were injected prophylactically with 0.2 ml im Gentamicin at the time of surgery. The guide sleeves were held in place via anchor screws and dental acrylic. All animals were in-

RESULTS

Two rats were terminated in the initial part of the experiment due to malfunctioning of the cannula system (1 each in the s-CSF and insulin conditions). The dose of insulin used (0.6 mU/day) had no obvious effect in and of itself. Body weights of the control (s-CSF into the third ventricle) rats averaged 415 ± 5 g (means ± SE) on day 1 and 395 ± 18 g on day 8. Body weights of the experimental rats (insulin into the third ventricle) averaged 396 ± 9 g on day 1 and 381 ± 11 g on day 8. Neither the absolute weights nor the change across the 8 days of the experiment was significantly different by t-test between the two groups (Ps > 0.05).

Intake of Ensure after lateral ventricular s-CSF or CRH during the initial phase of the experiment is depicted in Fig. 1. The intakes after s-CSF alone (i.e., on days 1, 2, 4, 6, and 8) did not differ reliably from one another and were therefore averaged for each rat; the
was 19.0 + 1.0 ml (t-test; P > 0.05, not significant). Repeated measures analysis of variance revealed a significant main effect for CRH dose and a significant CRH by third ventricular compound interaction (P< 0.05). Planned analyses revealed that, in the control animals, the decrease of 30-min Ensure intake was not significant at the lowest dose by t-test (0.1 μg/rat; 3.0 ± 1.9 ml, P > 0.05) but was after 1.0 (−8.6 ± 0.8 ml, P < 0.01) and 5.0 μg/rat (−9.7 ± 1.1 ml, P < 0.01). In the insulin-infused animals, the reduction of 30-min intake was significant at every dose of CRH by individual paired t-tests (0.1 μg = −6.3 ± 1.4 ml; 1.0 μg = −12.0 ± 2.6 ml; 5.0 μg = −15.9 ± 0.7 ml; all Ps < 0.01). The reduction of 30-min Ensure intake by CRH was significantly greater in rats infused with insulin than in rats infused with s-CSF at both the 1 and the 5 μg/rat doses (both Ps < 0.05). In both the control and the insulin-infused rats there was a significant effect of dose of CRH, with higher doses causing greater suppression of Ensure intake (both Ps < 0.01).

Chow intake over the subsequent 6.5 h after the various doses of CRH did not differ among groups, although there was a trend toward less intake as the dose of CRH was increased (Table 1). Likewise, there were no significant changes of body weight among groups.

Intake after the three doses of CRH in the control (s-CSF infused) rats during the initial and subsequent replication of the identical injection protocol is listed in Table 2. There were no reliable differences at any dose between the first and second replications.

**DISCUSSION**

The anorexic effect of the acute administration of CRH intraventricularly was enhanced in the presence of intraventricularly administered exogenous insulin. It is important to note that this dose of insulin had no effect on Ensure intake, total daily food intake, or body weight in and of itself. This result is therefore consistent with our previous findings that rats normally do not reduce their body weight significantly until doses of third ventricular insulin of >1 mU/day are infused (56). Comparable findings have been reported by others (17). The dose of insulin used (0.6 mU/day) was therefore considered to be subthreshold with regard to reducing food intake.

The important observation in the present experiment is that the ability of intraventricularly administered CRH to reduce acute food intake was both dose dependent and enhanced by the presence of a subthreshold dose of insulin. Hence, both control rats receiving a continuous infusion of synthetic cerebrospinal fluid into the third ventricle, and experimental rats receiving an infusion of insulin had a dose-dependent reduction of Ensure intake. The reductions were comparable to what has been reported previously for CRH over a similar dose range (18, 39, 53, 54), and they were also comparable across the two replications in the control rats, indicating that lateral-ventricular CRH is equally effective whether an infusion is simultaneously occurring in the third ventricle or not. More to the point, the suppression of Ensure intake by CRH was significantly greater when a subthreshold dose of insulin was being infused into the brain. As depicted in Fig. 1, the maximal suppression of Ensure intake by CRH in the control rats was ~50% at these doses and under these conditions. In rats receiving insulin, on the other hand, CRH reduced Ensure intake by >80%, although it had no effect on total daily caloric intake. Hence, the maximal suppression of Ensure intake appears to be increased when insulin is increased in the brain.

CRH is a potent suppressant of meal size (18, 31, 49, 55, 60), and it has been implicated as a mediator of

**Table 1. Mean intake of chow over the 6.5 h after Ensure consumption in each treatment condition**

<table>
<thead>
<tr>
<th>Dose of CRH</th>
<th>s-CSF</th>
<th>0.1 μg</th>
<th>1 μg</th>
<th>5 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-CSF in 3rd V</td>
<td>18.2 ± 3.7</td>
<td>19.1 ± 4.1</td>
<td>17.9 ± 3.8</td>
<td>15.2 ± 4.3</td>
</tr>
<tr>
<td>Insulin in 3rd V</td>
<td>22.0 ± 3.5</td>
<td>18.6 ± 2.5</td>
<td>18.0 ± 3.9</td>
<td>18.1 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE in g. CRH, corticotropin releasing hormone; s-CSF, synthetic cerebrospinal fluid; 3rd V, third ventricle.

**Table 2. Intake of Ensure over 30 min after the acute intraventricular injection of s-CSF or 0.1, 1.0, or 5.0 μg/rat of CRH**

<table>
<thead>
<tr>
<th>Dose of CRH</th>
<th>s-CSF</th>
<th>0.1 μg</th>
<th>1 μg</th>
<th>5 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial series</td>
<td>19.7 ± 1.9</td>
<td>16.6 ± 2.3</td>
<td>11.0 ± 0.8</td>
<td>9.9 ± 0.6</td>
</tr>
<tr>
<td>Second series</td>
<td>19.4 ± 2.2</td>
<td>18.0 ± 1.8</td>
<td>13.2 ± 1.2</td>
<td>9.0 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml. The 2 series were initiated 14 days apart, and the animals were receiving 3rd ventricular s-CSF during the first but not the second series.

**Fig. 1.** Mean (+SE) 30-min intake of Ensure in ml for synthetic cerebrospinal fluid (s-CSF)- and insulin-infused rats after the indicated doses of corticotropin releasing hormone (CRH). *P < 0.01 relative to a dose of 0 CRH for that group of rats; #P < 0.05 relative to the intake in s-CSF-infused rats.
the anorexic action of leptin (30, 47, 73). Because leptin and insulin both meet the criteria for being circulating adiposity signals to the brain (69, 78) and because their intracellular signaling pathways overlap (9, 34, 71), the present results add to the growing evidence that insulin and leptin have complementary actions in the control of energy homeostasis. Furthermore, CRH would appear to be an integral part of the downstream signaling mechanisms for this control system. It is not known whether insulin would enhance other effects of CRH.

Perspectives
The present results are consistent with a growing literature suggesting that signals important in the regulation of body adiposity interact at the level of the brain with other factors that control food intake. Insulin is the most studied of these adiposity related signals (see Refs. 63, 76, 78). In this regard, subthreshold doses of central insulin have been found to enhance the efficacy of intraperitoneally administered CCK in reducing meal size in rats (56) and intravenously or intraventricularly administered CCK in baboons (28, 29). Likewise, subthreshold doses of insulin, when administered into the third ventricles of adenalec-tomized rats, have increased potency and cause a reduction of food intake and body weight (21). These findings are consistent with a meal size-stimulating action of corticosterone in the brain (32). Doses of insulin that reduce body weight of rats also decrease the synthesis of mRNA for preproNPY in the arcuate nucleus, thus perhaps contributing to a decrease of food intake by reducing a major stimulant of feeding (64, 68). Lower doses of insulin, which do not reduce food intake, have no effect on preproNPY mRNA (68).

Similar to insulin, the adipose tissue hormone leptin is secreted in direct proportion to adiposity and is transported into the brain (see Refs. 1, 24, 69, 78). As occurs with insulin, receptors for leptin are located in several brain areas important in the regulation of energy homeostasis, including the arcuate nucleus (11, 13, 14, 66), and, also similar to insulin, leptin reduces the synthesis of preproNPY in the arcuate nucleus (61). Finally, and also analogous to what occurs with insulin, low doses of leptin administered into the brain potentiate the anorexic and brain stem-activating actions of CCK (8, 25, 26, 40–42) and leptin deficiency is associated with reduced ability of CCK to reduce meal size (44). It is not known whether intraventricular leptin potentiates the anorexic action of CRH, but it is a strong possibility because expression of the CRH-2 receptor gene is induced after intraventricular leptin administration (35, 65) and because CRH antagonists reportedly inhibit the anorexia elicited by leptin (30, 47). In summary, the strong conclusion from all of these experiments is that the regulation of body fat content is superimposed on control systems involved in the size of individual meals by means of the hormonal signals, insulin and leptin. Hence, the regulation of body fat can be efficiently accomplished by allowing individuals to eat whenever their environment dictates so long as meal size if regulated.

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REFERENCES


