Cholecystokinin-decreased food intake in rhesus monkeys

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GIBBS, JAMES, JOHN D. FALASCO, AND PAUL R. McHUGH. Cholecystokinin-decreased food intake in rhesus monkeys. Am. J. Physiol. 230(1): 15-18. 1976.—Five rhesus monkeys were infused intravenously with partially purified cholecystokinin (CCK) just prior to a test meal of solid food after overnight food deprivation; CCK produced large, rapid, dose-related suppressions of feeding. The lowest dose tested (5 Ivy U/kg body wt) produced a significant inhibition of food intake (26% suppression, P < 0.05). Equivalent infusions of partially purified CCK or the synthetic COOH-terminal octapeptide of CCK (a pure fragment with all the biological activity of the full molecule) produced equivalent suppressions. In a second experiment, gastric preloads of a potent releaser of endogenous CCK, L-phenylalanine (L-Phe), and a weak releaser, D-phenylalanine (D-Phe) were compared for their relative abilities to suppress food intake at a test meal in nine rhesus monkeys after overnight deprivation. L-Phenylalanine produced large, rapid, dose-related suppressions of feeding, but D-Phe did not. The threshold dose of L-Phe was 0.5 g/kg (32% suppression, P < 0.01). Neither CCK nor L-Phe caused signs of illness in these experiments. The results demonstrate that intravenous exogenous CCK suppresses feeding in rhesus monkeys and suggest that endogenous CCK has the same effect; they are consistent with the hypothesis that CCK is a satiety signal.

satiety; feeding behavior; phenylalanine

CHOLECYSTOKININ (CCK) DECREASES INTAKE OF SOLID AND LIQUID FOODS IN INTACT RATS (4), DECREASES FOOD INTAKE OF RATS WITH OPEN GASTRIC FISTULAS CONTINUOUSLY SHAM-FED A LIQUID DIET (5, 13), AND ELICITS THE COMPLETE BEHAVIORAL SEQUENCE THAT CHARACTERIZES SATIETY IN THE RAT (1). THESE OBSERVATIONS SUGGEST THAT CCK, WHICH IS NORMALLY RELEASED INTO BLOOD BY FOOD ENTERING THE INTESTINE (12), MAY BE A PHYSIOLOGIC SATIETY SIGNAL. THIS HYPOTHESIS PREDICTS THAT 1) CCK SHOULD DECREASE FEEDING IN ANIMALS OTHER THAN THE RAT, 2) INTRAVENOUS CCK SHOULD DECREASE FEEDING AS WELL AS INTRAPERITONEAL CCK, AND 3) ENDGENOUS CCK SHOULD DECREASE FEEDING.

In the first experiment reported here, we present evidence that intravenous infusion of partially purified CCK sharply decreased feeding in rhesus monkeys, a finding that fulfills the first and second predictions. Recent findings of Meyer and Grossman (11) allowed an indirect test of the third prediction: they showed that the intestinal perfusion of the L-isomer of phenylalanine (L-Phe) in dogs produced a marked pancreatic protein secretion, but that an identical perfusion of D-phenylalanine (D-Phe) produced very little secretion of protein. These authors provided convincing indirect evidence that this difference was due to the more potent release of CCK by L-Phe. If CCK is a satiety signal, and if L-Phe is a more potent releaser of CCK than D-Phe, then gut preloads of L-Phe should produce a more potent suppression of food intake than equivalent preloads of D-Phe. The second experiment reported here fulfills this prediction.

METHODS

Intravenous cholecystokinin infusions. Five adult male rhesus monkeys (Macaca mulatta, 4.5-6.5 kg) were maintained on a 12-h (7 A.M.-7 P.M.) light-dark cycle. They were surgically equipped with chronic inferior vena caval catheters (Silastic, Dow Corning: 0.040 inch ID, 0.085 inch OD), adapted to chronic restraint in primate chairs and maintained in individual booths. Details of preparation and care of macaques under these conditions were published previously (14). After surgery, the monkeys were placed on a daily feeding schedule that was maintained throughout the experimental period: a standard chow (Teklad primate diet, 3.6 kcal/g) was offered only during a 3-h period (11 A.M.-2 P.M.); tap water was always available. No experiments were performed until the monkeys appeared comfortable and were eating and drinking amounts equivalent to preoperative intakes. This recovery period usually lasted 7-10 days.

On experimental days, partially purified porcine CCK (20% pure, wt/wt, lot no. 27481 from GII Research Unit, Karolinska Institutet, Stockholm, Sweden) in doses of 5, 10, or 20 Ivy dog U/kg body wt or the synthetic COOH-terminal octapeptide of CCK (Squibb Institute for Medical Research, Princeton, N. J.) in a dose of 20 Ivy dog U/kg (0.91 μg/kg) and dissolved in 5 ml of 0.15 M NaCl was infused intravenously by pump (Harvard Apparatus Co.) at a rate of 1 ml/min, followed by a flushing infusion of 3 ml of 0.15 M NaCl at a rate of 3 ml/min. A measured amount of food was immediately presented to each monkey for 180 min. At 15, 30, 60, and 120 min of the test period, the uneaten chow was collected, weighed, and returned to the monkey. After the
180-min measurement, all food was removed until the following day at 11 a.m. On control days, the same procedure was followed except that isovolumetric 0.15 M NaCl infusions replaced CCK infusions. At least 2 days of saline infusions or no infusions intervened between test days of CCK infusions; CCK infusions were never given more than twice in 1 wk. All solutions were warmed to 37°C before infusion. The amount of food eaten during each period after CCK infusion was calculated and compared with the amount of food eaten after saline infusion; statistical comparisons were made with a paired-observations t test (Hewlett-Packard Co.).

Intragastric phenylalanine infusions. Two of the monkeys used in the intravenous CCK experiment and seven additional adult male rhesus monkeys (4.5–11.0 kg) were equipped with chronic intragastric catheters and maintained in standard primate cages on a 12-h (7 a.m.–7 p.m.) light-dark cycle. The catheters (Silastic, Dow Corning; 0.062 inch ID, 0.125 inch OD) were prepared before surgery by bonding a small (0.75-inch diameter) button of Silastic sheeting 5 cm from the intragastric end of the tubing with Silastic adhesive. At surgery, the catheter was inserted up to the level of the fundus. The gastric incision was closed by a purse-string suture and the button fixed to the serosal surface by interrupted polyethylene monofilament sutures (Marlex, Davol); this serosal attachment prevented dislodgment of the catheter from the stomach. The free end of the catheter was brought through a small incision on the greater curvature of the fundus. The gastric incision was closed by a purse-string suture and the button fixed to the serosal surface by interrupted polyethylene monofilament sutures (Marlex, Davol); this serosal attachment prevented dislodgment of the catheter from the stomach. The free end of the catheter was brought through the posterior abdominal wall at the level of the 12th thoracic or 1st lumbar vertebra and led subcutaneously to emerge between the scapulae. Each monkey was fitted with a soft, lightweight suede vest. A flexible, hollow steel hose (Anaconda, 0.275 inch ID) was fastened to the back of the vest and secured to the rear of the primate cage. The free end of the gastric catheter was passed through this protective hose and out the back of the primate cage. After surgery, monkeys were placed on the same feeding schedule described above for the intragastric cholecystokinin experiment and allowed the same recovery period.

On experimental days, phenylalanine (Nutritional Biochemicals Corp.) dissolved in 0.15 M NaCl in doses of 0.25, 0.50, and 1.00 g/kg body wt was infused intragastrically by pump during a 15-min period. The concentration of phenylalanine solution was always 0.02 g/ml; volume of infusate was varied for different doses. At the end of the infusion, a measured amount of the standard primate chow was immediately presented to each monkey and the amounts eaten at 30, 60, 120, and 180 minutes were calculated. On control days, isovolumetric 0.15 M NaCl infusions replaced phenylalanine. Each dose sequence on each monkey lasted 4 days: day 1, infusion of saline; day 2, infusion of D isomer of phenylalanine (D-Phe); day 3, infusion of saline; and day 4, infusion of the L isomer of phenylalanine (L-Phe). This sequence was chosen because it would eliminate any compensation for small meals eaten after L-Phe infusion. Three days intervened until another test sequence was begun. All other methodological details were as described above for the intravenous cholecystokinin experiment. The amount of food eaten during each period after L-Phe and D-Phe infusions was compared to the amount eaten after saline infusion; statistical comparisons were made with a paired-observations t test (Hewlett-Packard Co.).

RESULTS

Intravenous cholecystokinin infusions. The CCK suppressed food intake (Table 1). The suppression was large (70% decrease after the 20-U/kg dose), rapid and transient (largely limited to the first 15 min of the 3-h test period), and dose-related. The lowest dose tested, 5 U/kg, produced a statistically significant 26% suppression of food intake. In two monkeys, infusions of 20-U/kg doses of either the partially purified CCK preparation or the pure synthetic COOH-terminal octapeptide of CCK produced equivalent suppressions of food intake (73% decrease after impure CCK, 66% decrease after the octapeptide).

Intragastric phenylalanine infusions. Preloads of L-Phe inhibited food intake (Table 2). The suppression was large (63% decrease after the 1-g/kg dose) and rapid (appearing in the first 30-min interval of the 3-h test period). The threshold dose of L-Phe under these conditions is about 0.50 g/kg. Increasing doses of L-Phe produced progressively larger and more sustained (Table 2) suppressions of food intake.

In contrast to the consistent, dose-related suppressions of feeding produced by L-Phe preloads, D-Phe preloads produced a statistically significant decrease on only one occasion. This effect occurred at the lowest dose tested (0.25 g/kg) and later in the test period than the L-Phe effect (Table 2). For these reasons, we do not consider this effect significant.

DISCUSSION

Exogenous CCK reduced food intake in monkeys. This is the first evidence that exogenous CCK has a satiety effect in a primate and that the effect can be obtained by intravenous as well as intraperitoneal administration. The suppressive effect of the extract containing CCK was limited to the first 15 min after intravenous infusion of CCK. During this period, all the monkeys ate less than they would have in response to saline preload. The suppressive effect was lost after 15 min and for the remainder of the 3-h test period, the monkeys ate as much as saline preloaded controls. The only difference between the saline and CCK-infused monkeys was the time at which food was eaten; the CCK-infused monkeys ate later than the saline preloaded controls. This delayed ingestion is consistent with a reduction in food intake over the first 15 min of test period, and inactive consumption after this period, which is often seen in rhesus monkeys on a semi-elemental chow diet under similar conditions.

<table>
<thead>
<tr>
<th>Table 1. Food intake during intervals after intravenous infusions of saline or different doses of CCK</th>
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</thead>
<tbody>
<tr>
<td><strong>Dose of CCK, Ivy Dog U/kg</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td><strong>CCK</strong></td>
</tr>
<tr>
<td>(n = 3)</td>
</tr>
<tr>
<td>(n = 5)</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>(n = 5)</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>(n = 4)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Infusions were always given intravenously immediately before food presentation. All tests were performed after 21 h of food deprivation. We cannot account for unusually low control intakes during first 15-min interval at 10-U/kg dose. Significant differences (one-tailed t test) from saline control: * P < 0.05; ** P < 0.01. Significant difference (one-tailed t test) from 5-U/kg dose: $ P < 0.05.
CHOLECYSTOKININ AND FOOD INTAKE IN MONKEYS

Table 2. Food intake during intervals after intragastric preloads of saline or different doses of L-Phe or D-Phe

<table>
<thead>
<tr>
<th>Dose of Phe, g/kg</th>
<th>0–30</th>
<th>30–60</th>
<th>60–120</th>
<th>120–180</th>
<th>0–180 (Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Phe Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>93</td>
<td>107</td>
<td>100</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>±2</td>
<td>±10</td>
<td>±8</td>
<td>±14</td>
<td>±5</td>
</tr>
<tr>
<td>0.50</td>
<td>69†</td>
<td>102</td>
<td>83</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>±17</td>
<td>±20</td>
<td>±10</td>
<td>±8</td>
<td>±4</td>
</tr>
<tr>
<td>1.00</td>
<td>58††</td>
<td>97</td>
<td>91</td>
<td>12††</td>
<td>37</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>±6</td>
<td>±9</td>
<td>±14</td>
<td>±4</td>
<td>±3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Preloads were always given intragastrically immediately before food presentation. All tests were performed after 21 h of food deprivation. Significant differences (two-tailed t test) from saline control: *P < 0.05; †P < 0.01. Significant difference (two-tailed t test) from 0.5-g/kg dose: ‡P < 0.05. Significant differences (two-tailed t test) from same dose of D-Phe. $P < 0.005.

Thus the magnitude of the dose of CCK does not seem to be a reliable index of toxicity across species.

The large preloads of L- and D-Phe could be considered amino acid-imbalanced diets. Such diets decrease food intake in rats (7) but the effect never occurs before 4–6 h after ingestion (9), which is much later than the immediate effect we observed. Furthermore, weight gain in rhesus monkeys chronically fed up to 3 g/kg of L-Phe in their daily milk supply was equivalent to normals during a 3-mo period (15); this dose of L-Phe is 3 times the one used in the second experiment. All these findings make it unlikely that the suppressions of feeding in the present study were the result of illness or toxicity.

Although we have provided strong evidence that the inhibition of feeding is a biological action of CCK (4), we do not know whether the doses of exogenous CCK used in the first experiment or the levels of endogenous CCK produced by L-Phe in the second experiment are within the physiological range in the monkey. This judgment can be made when the pattern and levels of CCK produced by food intake under our test conditions are known.

Regardless of whether the doses of CCK used here are within the physiological range, the biological action of CCK in suppressing feeding may have practical applications. Note that monkeys injected with the larger doses of CCK do not compensate for early decreases in food intake by eating significantly more than control intakes later in the test period (Table 1). This observation has a therapeutic implication: if CCK can be employed to reduce appetite in humans, its effect may be sufficiently prolonged to be of practical value. It is important to note, however, that in the only reported test of this possibility, a slow intravenous infusion of CCK (3 Ivy U/kg per h) failed to reduce food intake in five subjects after an overnight fast (6). Since negative results are never decisive, the problem deserves further investigation.

L-phenylalanine was an efficient suppressor of feeding. The caloric decrements in food intake produced by the L-Phe preloads were far out of proportion to the caloric content of the preloads: at the 0.5-g/kg dose, the total preload of 14 kcal (4 kcal/g of L-Phe) produced a mean decrease in food intake of 116 kcal (3.6 kcal/g of food) during the first 30 min and 225 kcal for the total feeding period; at the 1-g/kg dose, the total preload of 28 kcal produced a decrease of 224 kcal during the first 30

venous infusion (Table 1). A very similar rapid onset and brief duration of action of CCK on feeding were seen previously in rats given intraperitoneal injections under similar conditions (4, 5).

The suppressive effect of the 20% pure extract was due to CCK and not to impurities present in the impure preparation, since an equivalent dose of the synthetic COOH-terminal octapeptide of CCK produced an equivalent suppression in two monkeys. Furthermore, we have previously shown in rats that equivalent doses of the synthetic COOH-terminal octapeptide of CCK and a 10% pure extract of CCK produced identical suppressions of food intake across a wide range of doses (4).

Gut preloads of L-Phe produced marked, dose-related suppressions of food intake in monkeys; identical preloads of D-Phe did not. These results suggest that endogenous CCK also decreased food intake. If the quantity of CCK released was directly proportional to the load of L-Phe contacting the intestinal mucosa, then larger loads of L-Phe should have produced larger suppressions of food intake. This occurred (Table 2). Furthermore, larger intragastric loads of L-Phe should produce more prolonged suppressions of food intake, because the gastric emptying times for the larger loads would be longer. This also occurred: Table 2 shows that the largest load of L-Phe (1 g/kg) significantly suppressed food intake through the 2nd h of the test period.

There was a striking absence of any consistent effect of large volumes (up to 350 ml) of D-Phe on food intake. This observation suggests that volume (distension) and tonic effect were not effective stimuli for decreasing food intake under these conditions.

Although we have interpreted the difference between L- and D-Phe as a difference in their CCK-releasing potency, we recognize that there are many other differences in the pre- and postabsorptive effects of these isomers that may be involved in their differential effect on food intake. Until these other effects are excluded, our interpretation is reasonable but not proven.

It is possible that the reduction in feeding after CCK or L-Phe was due to illness. We saw no signs of illness or distress after CCK or L-Phe. The monkeys always ate eagerly at the beginning of the daily test period; they simply ate less. Although a 0.4-U/kg dose of the synthetic COOH-terminal octapeptide of CCK has been reported to produce toxic effects in humans (10), we have failed to observe toxicity in rats given 40 U/kg (4, 8).
min and 414 kcal for the total feeding period. These marked falls after small caloric preloads of l-Phe are in contrast to the complete inability of a large (100 kcal) intragastric preload of glucose to produce any decrease in food intake under very similar conditions in rhesus monkeys (2). Our observations are consistent with the finding in humans that protein-rich meals produce greater suppressions of food intake than an equicaloric protein-poor meal (3). The results with l-Phe raise the interesting therapeutic possibility that certain foods relatively low in caloric content may be particularly potent in suppressing food intake by releasing a physiological satiety signal, such as CCK may prove to be.

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