LiCl and CCK inhibit gastric emptying and feeding and stimulate OT secretion in rats

MONICA J. MCCANN, JOSEPH G. VERBALIS, AND EDWARD M. STRICKER
Departments of Behavioral Neuroscience and Medicine,
University of Pittsburgh, Pittsburgh, Pennsylvania 15260

McCann, Monica J., Joseph G. Verbalis, and Edward M. Stricker. LiCl and CCK inhibit gastric emptying and feeding and stimulate OT secretion in rats. Am. J. Physiol. 256 (Regulatory Integrative Comp. Physiol. 25): R463-R468, 1989.—Systemic injection of the nauseogenic agent LiCl is known to increase neurohypophysial secretion of oxytocin (OT) in rats. The present results indicated that the induced OT secretion was related exponentially to the inhibition of food intake. A similar relation between OT secretion and food intake also was observed after systemic injection of the peptide hormone cholecystokinin (CCK). However, the effects of each agent on food intake lasted much longer than the observed increases in OT secretion. In contrast, both LiCl and CCK produced a dose-dependent inhibition of gastric emptying in rats that was closely related temporally to the inhibition of food intake. The similarity of these three responses to LiCl and CCK suggests that both agents stimulate common central mechanisms, apparently involving both magnocellular and parvocellular neurons in the paraventricular nucleus of the hypothalamus, whereby these neuroendocrine, behavioral, and autonomic functions are integrated.

Food intake; nausea; satiety

Food intake in hungry rats can be reduced markedly by systemic injection of various chemical agents such as lithium chloride (LiCl) and copper sulfate (CuSO4). Although rats lack a complete emetic reflex, the effects of LiCl and CuSO4 on food intake have been attributed to the production of nausea because their administration induces vomiting in other species (2) and learned taste aversions in rats (4, 18) and because antiemetic drugs attenuate LiCl-induced taste aversions in rats (3).

Food intake in hungry rats also can be reduced markedly by systemic administration of the peptide hormone, cholecystokinin (CCK). This effect has led to the hypothesis that CCK is an endogenous hormone involved in the stimulation of satiety (9). However, systemic administration of CCK also has been reported to induce emesis in monkeys (30) and learned taste aversions in rats (7), and antiemetic drugs similarly reduce the CCK-induced inhibition of food intake in rats (17). It thus seems likely that gastric malaise accompanies the substantial decreases in food intake that occur when CCK is administered in relatively large doses, and this possibility makes uncertain the basis for the effects of CCK on feeding behavior.

Recent studies from our laboratories have shown that plasma levels of the neurohypophysial hormone oxytocin (OT) are increased in rats when LiCl and other nauseogenic agents are administered systemically in doses that could be used to produce learned taste aversions (29). Comparable effects also were observed when CCK was given in doses that reduced food intake (28). Much smaller increases in OT secretion were produced in rats by rapid consumption of a large meal (28) or by gastric distension (21). These results suggest that increased plasma levels of OT in rats reflect the activation of magnocellular oxytocinergic projections to the pituitary as well as the coactivation of other central pathways that are involved in the inhibition of feeding (29). To more fully characterize the relation between plasma OT and food intake, the first series of experiments measured the OT responses to LiCl and CCK in rats and associated them with the inhibition of food intake induced by these agents (experiment 1).

A second series of investigations determined the effects of LiCl, CuSO4, and CCK on gastric emptying. In this regard, observations that gastric emptying rates are reduced both by intestinal infusions of calories (13) and by systemic injection of CCK (6) are in agreement with the hypothesis that CCK is an endogenous satiety hormone. However, it remains to be determined whether gastric emptying also is reduced by treatments that appear to produce nausea. Thus we investigated the effects of LiCl, CuSO4, and CCK on gastric emptying in rats as a function of dose (experiment 2A) and time after administration (experiment 2B) and compared those results with one another and with the effects of these agents on food intake and OT secretion.

Methods

Subjects and Pretreatment Maintenance

Adult male rats of the Sprague-Dawley strain (Zivic-Miller, Allison Park, PA) were used. The animals were housed in open mesh stainless steel cages in a room that was illuminated each day from 8 A.M. to 8 P.M. Purina rodent chow and tap water were available continuously.

Two procedures were used for collection of blood samples in experiment 1. In one, rats were decapitated, and trunk blood was obtained. In the other, intracardiac catheters were implanted in rats weighing 275–325 g at the time of surgery. The catheters (Silastic, 0.063 cm ID) were positioned in the right atrium via the right jugular
vein of rats that were anesthetized with methoxyflurane. The catheters were filled with a solution of heparinized polyvinyl pyrrolidone to prevent blood from clotting within the tubing. At least 2 days were allowed for recovery from surgery.

Blood was collected in heparinized tubes (Vacutainer, Rutherford, NJ) and centrifuged at 4°C within 20 min (3,000 g for 3 min). Plasma was frozen at -20°C until radioimmunoassay for OT with the use of methods described previously (29).

Animals that were used to study gastric emptying in experiment 2 were equipped with stainless steel gastric cannulas (1.3 cm long, with a flange 1 cm in diameter at each end) as in earlier studies (15). Rats with gastric cannulas were housed individually in polypropylene cages (48 × 27 × 20 cm) and were allowed 6–7 days for recovery from surgery before testing.

Procedures

Experiment 1. In the first series of experiments, alterations in feeding behavior were examined after systemic injection of CCK or LiCl. Animals were given access to a balanced liquid diet (Bioserv, Frenchtown, NJ) twice each day, at 10 A.M. and 6 P.M., and they were allowed to consume 45 ml (which took ~60 min) and 65 ml (in 90 min), respectively. Intakes (±0.5 ml) were measured for the first 20 min in the morning session, and testing began when those values became stable, which usually occurred after 1 wk of training.

In experiment 1A, base-line intakes from the previous day were compared with the intakes of rats that were pretreated intraperitoneally with varying doses of CCK (1, 2.5, 5, 10, or 100 μg/kg) or LiCl (0.75, 1.25, 1.5, or 3.0 meq/kg). Food was presented to the animals 5 min after administration of CCK and 15 min after LiCl was given (n = 5–14 in each subgroup). In other animals given the same treatments, a blood sample was collected by decapitation 5 min after injection of CCK (n = 4–7 in each subgroup) and 15 min after LiCl (n = 4 in each subgroup). In rats with implanted catheters, a base-line blood sample was withdrawn at least 15 min before injection of each agent, and another blood sample was taken 5 min after CCK (n = 16) and 15 min after LiCl (n = 11). In the latter experiment, each rat received multiple doses of a single agent; the doses were administered in ascending order, and the same dose never was given more than once to a rat.

Experiment 1B examined the effects of CCK (10 μg/kg ip) and LiCl (1.5 meq/kg ip) on 20-min food intakes when the interval was varied between injection of the agent and presentation of food. The intervals used were 5, 20, and 60 min after injection of CCK and 15, 60, and 120 min after injection of LiCl (n = 5–7 in each subgroup). Food intakes are represented as a percent of the 20-min base-line intake from the previous day. A second group of identically treated rats was used for collection of trunk blood at the same time intervals (n = 5–10 in each subgroup).

Comparative experiments in rats that were treated with CuSO₄ before the feeding session were abandoned when these animals often failed to eat for several days thereafter, thus precluding repeated testing in the same animals.

Experiment 2. In the second series of experiments, we determined the rate at which a 10% dextrose solution emptied from the stomach. This rate was compared with values obtained from rats that were pretreated intraperitoneally with varying doses of CCK (2.5, 5, or 10 mg/kg), LiCl (0.75, 1.25, or 1.5 meq/kg), or CuSO₄ (2.5, 5, or 10 mg/kg), or with 75 mU/kg synthetic OT (215 IU/ml; Sigma Chemical, St. Louis, MO). The procedures used have been described in detail previously (15). Briefly, a 3-ml volume of 10% dextrose solution was administered intragastrically via the cannulas 5 min after injection of CCK, 15 min after LiCl and CuSO₄, and 2 min after OT were given. (Each rat received a single dose of the various agents.) The volume remaining in the stomach was withdrawn 10, 20, 30, or 40 min later (n = 5–11 at each time point) and was measured in calibrated tubes (~0.05 ml). Glucose concentration in the recovered volume was quantified by the glucose oxidase method with the use of a glucose analyzer (Beckman Instruments, Fullerton, CA).

The data are expressed as percent volume remaining in the stomach and were calculated as follows

\[
\text{% volume remaining} = \frac{\text{initial [glucose]} \times \text{volume recovered}}{\text{initial volume}}
\]

In experiment 2B, gastric emptying rates were measured after injection of CCK (10 μg/kg) or LiCl (1.5 meq/kg). The intervals between injection and administration of the glucose volume that were specified in experiment 1B (n = 5–11 in each subgroup) were used so that the temporal effects of the two agents on food intake, OT secretion, and gastric emptying could be compared.

RESULTS

Experiment 1. As expected from previous studies (28, 29), systemic injection of CCK and LiCl each decreased food intake and increased OT secretion in a dose-dependent fashion in rats (Table 1). These plasma OT concentrations and food intakes, measured in separate groups of animals, conformed closely with the results observed when both variables were measured in the same animals (Fig. 1). Highly significant negative correlations were found between plasma OT levels and food intake after administration of CCK (r = −0.87, P < 0.001) and LiCl (r = −0.91, P < 0.001). Thus high plasma levels of OT were associated with an inhibition of food intake in each case, whereas lower OT levels were observed when food intake was not as inhibited. The slopes of the two regression lines in Fig. 1, relating the stimulation of OT secretion and inhibition of feeding in response to CCK and LiCl, were not significantly different from one another; indeed, the responses to the two agents were similar that a single regression equation, with a statistically significant correlation coefficient (r = −0.84; P < 0.001), could be computed from the combined sets of data.
TABLE 1. Effect of cholecystokinin and LiCl on 20-min food intakes and plasma oxytocin levels

<table>
<thead>
<tr>
<th>Dose CCK, µg/kg</th>
<th>Food Intake, % basal</th>
<th>n</th>
<th>Plasma OT, µU/ml</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>87.3±5.2</td>
<td>6</td>
<td>3.4±0.6</td>
<td>4</td>
</tr>
<tr>
<td>2.5</td>
<td>51.8±4.0*</td>
<td>9</td>
<td>6.1±0.6*</td>
<td>4</td>
</tr>
<tr>
<td>5.0</td>
<td>35.6±4.6*</td>
<td>12</td>
<td>8.9±1.8*</td>
<td>4</td>
</tr>
<tr>
<td>10.0</td>
<td>21.1±6.1*</td>
<td>8</td>
<td>15.5±4.0*</td>
<td>7</td>
</tr>
<tr>
<td>100.0</td>
<td>1.6±1.0*</td>
<td>8</td>
<td>27.8±3.8*</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LiCl meq/kg</th>
<th>Food Intake, % basal</th>
<th>n</th>
<th>Plasma OT, µU/ml</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>98.7±0.8</td>
<td>6</td>
<td>3.9±0.8</td>
<td>4</td>
</tr>
<tr>
<td>1.125</td>
<td>74.3±6.4*</td>
<td>14</td>
<td>8.9±1.8*</td>
<td>4</td>
</tr>
<tr>
<td>1.5</td>
<td>28.2±4.8*</td>
<td>5</td>
<td>12.4±1.6*</td>
<td>4</td>
</tr>
<tr>
<td>3.0</td>
<td>8.2±2.5*</td>
<td>6</td>
<td>21.6±0.5*</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE. n = no. of animals tested at each dose. Mean basal food intakes = 27.7 ± 3.1 ml/20 min, mean basal plasma OT = 2.2 ± 0.5 µU/ml. * P < 0.001, † P < 0.01 relative to basal values.

The time courses of the OT responses to CCK and LiCl are shown in Fig. 2, A and B. Plasma OT levels peaked at 5 min after treatment with 10 µg/kg CCK (P < 0.001 in comparison to basal values) but were not significantly elevated by 20 or 60 min posttreatment. The duration of the OT response also was brief after injection of 1.5 meq/kg LiCl; plasma OT levels peaked at 15 min (P < 0.001) but were not significantly elevated by 1 or 2 h posttreatment.

Lengthening the interval between the injection of CCK or LiCl and access to food also decreased the inhibition of food intake produced by these agents. Thus 20-min food intakes were significantly reduced 5 min after CCK and 15 min after LiCl (P < 0.01 for both treatments), and a significant effect still was apparent 20 min after CCK and 60 min after LiCl (P < 0.05 in both cases). However, the 20-min food intakes were comparable to baseline values by 60 min after CCK and 120 min after LiCl. Thus the inhibition of food intake induced by these agents was more long-lasting than the increase in OT secretion, as is evident in Fig. 2, A and B.

FIG. 2. Plasma oxytocin (OT) levels and food intakes of rats given 10 µg/kg cholecystokinin (CCK; A) or 1.5 meq/kg LiCl (B) as a function of time. Values shown are means ± SE for 5–10 rats.

Experiment 2. The main finding of these experiments was that CCK, LiCl, and CuSO₄ each inhibited gastric emptying (Fig. 3), whereas OT administered systemically had no significant effect (data not shown).

Dose-dependent effects of CCK, LiCl, and CuSO₄ on gastric emptying are suggested by the significant overall difference of the slopes when the various doses were compared (all, P < 0.001). The largest doses of CCK (10 µg/kg), LiCl (1.5 meq/kg), and CuSO₄ (10 mg/kg) each resulted in significantly larger volumes remaining in the stomach at 20 min than those remaining either in non-injected controls (all, P < 0.001) or in the animals given the lowest doses of each agent (all, P < 0.01). However, the rate of gastric emptying was inhibited but not halted even at the highest doses used, as indicated by the slopes in Fig. 3, A, B, and C, all of which were significantly different from zero (P < 0.05).

As with food intake, the inhibition of gastric emptying by CCK (10 µg/kg) and LiCl (1.5 meq/kg) depended on the interval between injection of agent and intragastric administration of the glucose load (P < 0.001 for both cases). Figure 4, A and B, shows that both agents produced the most marked inhibition of gastric emptying when it was measured relatively soon after injection. This effect became smaller as the interval between injection and measurement of gastric emptying was lengthened.

A comparison of the potency of the effects of CCK and LiCl on gastric emptying and food intake indicated that both responses were inhibited similarly by each dose of the two agents (Fig. 5, A and B). Moreover, a comparison of the time courses of these effects revealed that the inhibition of gastric emptying produced by CCK and
LICl paralleled the inhibition of food intake (Fig. 6, A and B). These results are summarized in the linear function relating the effects of CCK and LiCl on food intake with those on gastric emptying, after the administration of each agent in various doses and at various intervals between injection and measurement of these effects (Fig. 7; $r = 0.91, P < 0.001$).

**DISCUSSION**

It is well known that the peptide hormone CCK and the nauseogenic agent LiCl both are effective in reducing the food intake of rats in a dose-dependent manner. Recent results from our laboratories indicate that a dose-related stimulation of OT release also is observed after the administration of either agent (28, 29). The present experiments confirm those findings and demonstrate parallel inhibitory effects of the two agents on gastric emptying. The close relation between these three responses to CCK and LiCl further suggests that both agents stimulate common central mechanisms whereby the behavioral, neuroendocrine, and autonomic functions are integrated (26).

CCK is thought to stimulate receptors located on afferent fibers in the gastric vagus (31) and to enhance sensory input from the stomach to the nucleus of the solitary tract in the brain stem (19, 20); accordingly, gastric vagotomy has been reported to abolish the CCK-induced stimulation of OT secretion and inhibition of
food intake and gastric emptying (16, 25, 28). In contrast, LiCl is thought to act centrally on chemoreceptors located in the area postrema (22) and not via peripheral afferents; accordingly, abdominal vagotomy does not interfere with the acquisition of a LiCl-induced learned aversion (14). Despite these differences in the receptor mechanisms and afferent pathways by which CCK and LiCl are detected, however, the results summarized in Figs. 1 and 7 strongly suggest that both agents share a final common efferent pathway in affecting food intake, OT secretion, and gastric emptying.

Although the effects of CCK and LiCl on OT release were highly correlated with their effects on food intake, experiment 1B indicated that food intake was inhibited for much longer periods of time than neurohypophyseal OT secretion was stimulated. In contrast, experiment 2B indicated that the inhibitory effects of CCK and LiCl on gastric emptying followed a time course that was strikingly similar to the inhibition of food intake. Moreover, gastric emptying also was inhibited by administration of CuSO4. In light of these effects of LiCl and CuSO4 on gastric function, it should be clear that the marked reduction of gastric emptying produced by CCK does not unambiguously support a role for this peptide hormone in satiety. In this regard, recent experiments have found that there are comparable inhibitory effects of CCK and LiCl on gastric motility in rats (8, 24), and X-irradiation also has been shown to decrease gastric emptying in rats, in parallel with the development of conditioned taste aversions (10). On the other hand, it must be recognized that the parallel effects of CCK and LiCl on food intake, OT secretion, and gastric emptying do not necessarily imply that the inhibition of feeding was accompanied by the same sensations after each treatment.

The magnocellular oxytocinergic neurons in the supraoptic and paraventricular nuclei (PVN) of the hypothalamus, which terminate in the posterior pituitary, almost certainly mediate the elevation in plasma OT levels seen after administration of CCK and LiCl. Because the inhibition of food intake and gastric emptying were obtained with the same treatments that stimulated pituitary OT secretion, it is reasonable to consider the possibility that circulating OT might mediate either or both of those effects. However, peripherally administered OT does not inhibit either feeding (29) or gastric emptying in rats (experiment 2A). Although the potential functional significance of the peripherally released OT is not yet clear, it would be adaptive for OT to act on vascular smooth muscle in the mesenteric arteries, as does vasopressin (1). Such an action would complement the inhibition of food intake and gastric emptying by reducing blood flow to the intestines and thereby limiting the absorption of ingested toxins. An alternative possibility is that the pituitary OT secretion is of no functional consequence and simply occurs when nonspecific stress and nociceptive pathways are activated by LiCl and CCK (11). Although it is likely that at least some component of the pituitary OT secretion is nonspecific (29), our data would suggest that activation of magnocellular oxytocinergic pathways is tightly linked to other pathways affecting both gastric motility and food intake.

In considering other pathways that could account for the observed inverse correlation between pituitary OT secretion and gastric motility, it is of interest that parvocellular oxytocinergic neurons are known to project from PVN to the dorsal motor nucleus of the vagus (27). Recently, Rogers and Hermann (23) reported that microinjection of OT into this brain stem region mimicked the inhibitory effects of PVN stimulation on gastric function, whereas administration of an OT antagonist produced an abrupt increase in gastric motility. These findings therefore suggest that activation of the PVN can stimulate vagal inhibitory neurons and thereby reduce gastric motility and that these effects may be mediated in part by parvocellular oxytocinergic neurons. Thus the coordinated stimulation of OT secretion and inhibition of gastric emptying produced by LiCl and CCK may be a result of coactivation of magnocellular and
parvocellular OT-containing neurons in the PVN (26). Although only a few percent of PVN efferents project to both pituitary and brain stem, there is anatomic and electrophysiologic evidence for communication between magnocellular and parvocellular neurons in PVN, perhaps via axon collaterals (27, 32), that could allow both systems to be activated simultaneously. Alternatively, similar afferent inputs into magnocellular and parvocellular neurons might allow simultaneous activation of both systems, with the consequent effects on OT secretion and gastric motility that have been described. Because PVN lesions, but not hypothalamic lesions, are known to increase feeding in rats (12), and PVN lesions eliminate the inhibitory effect of CCK on food intake (5), it seems likely that pathways projecting from parvocellular neurons also mediate the inhibition of food intake after systemic injection of CCK or LiCl. It remains to be determined whether the inhibition of feeding is mediated by central oxytocinergic projections or by altogether different afferent projections from the PVN or brain stem, which act in concert with this system.

The authors are grateful to Dr. Edda Thielis for her assistance with the statistical analyses of these data. This research was supported by National Institute of Mental Health Grant MH-25140.

Portions of this work were submitted by M. J. McCann in partial fulfillment of the requirements for the PhD degree.

Portions of this report were presented in preliminary form at the Society for Neuroscience meetings in Washington, DC (November 1986) and in New Orleans, LA (November 1987).

Present address of M. J. McCann and address for reprint requests:
Dept. of Physiology, Ohio State Univ. College of Medicine, 333 W. 10th Ave., Columbus, OH 43210.

Received 25 January 1988; accepted in final form 21 September 1988.

REFERENCES


