Opioids affect acquisition of LiCl-induced conditioned taste aversion: involvement of OT and VP systems

PAWEL K. OLSZEWSKI,1,2 QIUYING SHI,1,2 CHARLES J. BILLINGTON,1,2 AND ALLEN S. LEVINE1,2,3
1Minnesota Obesity Center, Research Service Veterans Affairs Medical Center, Minneapolis 55417; and Departments of 2Medicine and 3Psychiatry, University of Minnesota, Minneapolis, Minnesota 55455

Received 15 February 2000; accepted in final form 22 May 2000

Olszewski, Pawel K., Qiuying Shi, Charles J. Billington, and Allen S. Levine. Opioids affect acquisition of LiCl-induced conditioned taste aversion: involvement of OT and VP systems. Am J Physiol Regulatory Integrative Comp Physiol 279: R1504–R1511, 2000.—Aversive properties of lithium chloride (LiCl) are mediated via pathways comprising neurons of the nucleus of the solitary tract (NTS) and oxytocin (OT) and vasopressin (VP) cells in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. Because opioids act on brain regions that mediate effects of LiCl, we evaluated whether administration of opioids shortly before LiCl in rats influences 1) development of conditioned taste aversion (CTA) and 2) activation of NTS neurons and OT/VP cells. Neuronal activation was assessed by applying c-Fos immunohistochemical staining. Three opioids were used: morphine (MOR), a μ-agonist, butorphanol tartrate (BT), a mixed μ/κ-agonist, and nociceptin/orphanin FQ (N/OFQ), which binds to an ORL1 receptor. BT and N/OFQ completely blocked acquisition of CTA. MOR alleviated but did not eliminate the aversive effects. Each of the opioids decreased LiCl-induced activation of NTS neurons as well as OT and VP cells in the PVN and SON. We conclude that opioids antagonize aversive properties of LiCl, presumably by suppressing activation of pathways that encompass OT and VP cells and NTS neurons.

nociceptin/orphanin FQ; morphine; butorphanol tartrate; c-Fos; lithium chloride

CONDITIONED TASTE AVersions (CTA) are well-described phenomena, which develop when a novel taste is associated with a short-term unpleasant gastrointestinal sensation (12). Various chemical agents, such as cholecystokinin (CCK) or copper sulfate, have induced aversive effects when paired with novel flavors (7, 26); lithium chloride (LiCl) has been found to be particularly effective in generating CTA (27). LiCl administration also has caused a significant reduction in food intake, gastric motility, and gastric emptying (10, 24).

Peripheral injection of LiCl results in the onset of complex neural and endocrine mechanisms that underlie the development of anorexic and aversive responses. It has been shown to produce pronounced c-Fos immunoreactivity in several brain regions engaged in the regulation of ingestive behavior, including the nucleus of the solitary tract (NTS), central nucleus of amygdala (CeA), and the paraventricular nucleus of the hypothalamus (PVN) (29). In addition, dose-dependent neurohypophyseal secretion of oxytocin (OT) and vasopressin (VP) has been observed following the intraperitoneal administration of LiCl. Although there is still no agreement on the involvement of VP in mediating LiCl-induced effects, such a role for OT has been strongly substantiated (42). It is noteworthy that OT and VP have been recently proposed as satiety mediators (1, 19). Immunohistochemical studies revealed that hypothalamic supraoptic nuclei (SON) and PVN encompass the major populations of OT and VP cells (14). These morphologically diverse neuronal populations have been found to send projections to various brain areas, ranging from the autonomic centers in the brain stem to limbic structures and neocortex. Numerous terminals of OT and VP neurosecretory axons originating from the PVN and SON are present in the neurohypophysis (36, 39, 40). Both PVN and SON, along with their OT and VP neurons, receive a powerful input of visceral information via NTS-derived ascending pathways (2).

OT and VP reportedly interact with other neuropeptides in the regulation of neurobehavioral paradigms. A growing body of evidence based on anatomical, physiological, and behavioral studies indicates the importance of functional relations between endogenous opioids and OT/VP systems. Investigators have shown certain opioid-OT/VP interactions in analgesia and reinforcement (17, 18, 41); many opioid peptides and their agonists inhibit processes/events that occur upon the release of OT (33). Moreover, it has been shown that enkephalins and dynorphins colocalize with OT and VP, respectively, and influence each other’s release (33, 35). In addition, various subtypes of opioid recep-
titors have been detected in the SON and PVN, and it has been suggested that opioid peptides affect cellular activation of the two hypothalamic nuclei either directly via receptors present on cell bodies or indirectly by influencing presynaptic events (5, 6, 8, 16, 32).

Few studies have attempted to characterize the nature of opioid influence on the acquisition of LiCl-induced CTA. Of particular interest are results published by Blanquart et al. (3), who showed that some opioid agonists reduce taste aversion in rats. Flanagan et al. (10) found that naloxone potentiates the effects of CCK and LiCl on OT secretion, gastric motility, and feeding. On the basis of these observations, we hypothesize that opioid peptides affect the development of CTA by suppressing signals mediated via pathways that include OT and VP neurons localized in the PVN and SON. To test this hypothesis we addressed the following questions: 1) does administration of opioid peptides shortly before LiCl injection affect the acquisition of CTA? and 2) does administration of opioids prior to LiCl affect activation of NTS neurons as well as OT and/or VP cells in the PVN and SON?

Three opioid peptides known to increase consummatory behavior were used in the study: nociceptin/orphanin FQ (N/OFQ), an ORL1 agonist (4, 31), morphine (MOR), a μ-agonist (13), and butorphanol tartrate (BT), which binds to κ- and μ-receptors (22). A standard two-bottle test was used to determine acquisition of CTA to saccharin solution. Activation of neurons (in the PVN, SON, and NTS) as well as their biochemical characterizations (in the PVN and SON) were assessed by applying immunohistochemical techniques that included c-Fos staining as the marker of neuronal activation.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (Charles Rivers Laboratories, Portage, MI) weighing ~350 g at the beginning of the experiment were used in the studies. Animals were housed individually in wire-mesh cages with a 12:12-h light-dark schedule (lights on at 0700) in a temperature-controlled room (21°C).

Taste Aversion Experiments

Rats were accustomed to having access to water for 1 h (1300–1400) per day for 4 days (with such a water availability regimen, each animal started drinking immediately after a bottle was replaced and drank at least 10 ml within 15 min). On the 5th day, rats were given a novel 0.1% saccharin solution instead of water. At the moment of saccharin presentation they were injected with doses of MOR, N/OFQ, or BT known to increase consummatory behavior (see specifications below). Isotonic saline served as the control vehicle. Fifteen minutes later animals received an intraperitoneal injection of LiCl (5 meq/kg body wt; isotonic solution) or saline. During the next 2 days, rats were presented only with water. On the 8th day, a standard two-bottle preference test was used to assess acquisition of a CTA to the saccharin solution (9, 42).

As the opioid peptides used in our study are also known to stimulate feeding, food (Teklad laboratory chow pellets) was removed from cages for the period of time when animals had access to water or saccharin.

Control groups that underwent unconditioned stimulation (treatment not paired with the presentation of the saccharin solution) were also included.

Six animals per group were used in the taste aversion experiments.

Experiment 1: Morphine-LiCl interactions. To eliminate the sedative effect of MOR, animals were pretreated for 3 days with the dose of MOR that was to be used during the actual experiment. MOR (Wyeth Laboratories, Philadelphia, PA) was injected subcutaneously (4 mg/kg body wt).

Experiment 2: Nociceptin-LiCl interactions. Rats used in this experiment were equipped with an indwelling stainless steel cannula (20 gauge) in the right lateral ventricle. The cannula was positioned according to the atlas of Paxinos and Watson (31): 1.0 mm lateral to the midline, 1.5 mm caudal to bregma, and 3.5 mm below the surface of the skull (30). Dental cement was used to secure the cannula to two screws inserted in the skull. Implantations were performed under Nembutal anesthesia (50 mg/kg body wt ip). Ten days postoperative recovery were allowed before the experiment began.

Measurement of water intake following the administration of 100 ng of angiotensin II (Sigma Diagnostics, St. Louis, MO) provided the verification of cannula placement: those rats that drank less than 6 ml of water within 30 min after the injection of angiotensin were excluded from the study.

N/OFQ (Phoenix Pharmaceuticals, Belmont, CA) was injected intracerebroventricularly (icv) at a dose of 10 nmol in a volume of 5 μl saline.

Experiment 3: Butorphanol-LiCl interactions. BT (Bristol-Myers Squibb, Princeton, NJ) was administered sc at a dose of 4 mg/kg body wt.

Data analysis. The amount of ingested saccharin solution, expressed in milliliters and as the percentage of total fluid intake, was assessed during a two-bottle test per animal. These two parameters were used to determine the acquisition of CTA. The results were averaged per experimental group and analyzed using ANOVA followed by the Fisher least significant difference test (significant when P < 0.05) separately for experiments 1, 2, and 3.

Neurohistochemical Studies

Prior to the experiment animals had free access to food and water. On the experimental day rats (n = 5/group) underwent the same pharmacological treatment as described above in groups tested for taste aversion: administration of one of the opioid peptides or vehicle was followed by the injection of LiCl or saline 15 min later. Doses, the injection route of particular agents (i.e., ip, icv, or sc), surgical procedures, as well as MOR pretreatment schedule (in applicable groups) remained unchanged. To prevent c-Fos induction due to feeding or drinking, food and water were immediately removed from the cages of injected rats. All injections were performed between 1300 and 1400. An hour after the second (i.e., LiCl or saline) injection, animals were deeply anesthetized with Nembutal (100 mg/kg body wt ip) and perfused rapidly through the aorta with 75 ml of saline followed by 500 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and postfixed overnight in the same fixative at 4°C. The perfusion schedule and interpretation of results were based on data suggesting that maximum
c-Fos immunoreactivity can be observed ~60–90 min after the actual onset of neuronal activation (43).

Sectioning and immunohistochemistry. 40-mm-thick coronal Vibratome sections were cut through the regions of the hypothalamic PVN and SON and the NTS. They were processed as free-floating sections for standard single (NTS) or double (PVN, SON) immunostaining.

Hypothalamic and brain stem sections were pretreated for 10 min in 3% H2O2 in 10% methanol [diluted in Tris-buffered saline (TBS), pH 7.4–7.6] and routinely incubated for 36 h at 4°C in the primary goat anti-Fos antibody (diluted 1:9,000; Santa Cruz Biotechnology). Subsequently sections were incubated for 1 h at room temperature in the secondary rabbit-antigoat antibody (Vector Laboratories, Burlingame, CA). Following a 1-h incubation (room temperature) in the avidin-biotin complex, peroxidase in the tissue was visualized with 0.05% dianinobenzidine (DAB), 0.01 H2O2, and 0.3% nickel sulfate (time of reaction, 12 min). The vehicle for all incubations in antibodies was a mixture of 0.25% gelatin and 0.5% Triton X-100 in TBS. Intermediate rinsing steps were done in TBS alone.

Following the completion of c-Fos staining, PVN- and SON-containing sections were further processed to visualize OT or VP. The general procedure was similar to that in the staining for the first antigen; however, rabbit anti-OT (1:10,000; Chemicon, Temecula, CA) and rabbit anti-VP (1:6,000; supplied by Dr. Ruud M. Buijs from the Netherlands Institute of Brain Research, Amsterdam, The Netherlands) were used as primary antibodies; thus goat anti-rabbit (specifications as above) was used as the secondary antibody. Nickel sulfate was omitted from the DAB solution to obtain the brown instead of black staining.

Sections were mounted on gelatin-coated slides, air-dried, dehydrated in alcohols, soaked in Americlear (Baxter Diagnostics), and embedded in Entellan (Merck).

Data analysis. Activation of OT and VP neurons was studied by the analysis of c-Fos expression in the immunohistochemically characterized cells in the PVN and SON. Twelve sections per region that contained neurons expressing VP or OT (6 sections/peptide) were used for the analysis in each animal. The following estimates per section and then, by adding up the numbers, per region were assessed: 1) the total number of OT and VP neurons and 2) the total number of Fos-immunoreactive (Fos-IR) nuclear profiles colocalizing with these peptides. The percentage of Fos-positive OT and VP cells was calculated for the PVN and SON per animal. The percentages were averaged over the particular region and peptide for each experimental group.

The most pronounced increase of c-Fos expression in the NTS following LiCl injection has been observed in the portion of this region adjacent to the area postrema (29), sections encompassing this part of the NTS were used in the analysis. The number of Fos-IR nuclear profiles in the NTS was counted on three to four sections per animal. Images provided by Dage-MTI DC triple CCD camera attached to a Nikon Eclipse 400 microscope were analyzed using Scion Image software. Densities of Fos-positive nuclei (per 1 mm2) were averaged per animal and then per experimental group.

Statistical analysis of all data was performed using ANOVA followed by the Fisher least significance test. Values were considered significantly different when \( P < 0.05 \). Results were presented as means ± SE.

RESULTS

Taste Aversion Experiments

Administration of LiCl following the ingestion of 0.1% saccharin by rats significantly affected their later preference for the saccharin solution compared with controls. LiCl-treated animals drank on average less than 2 ml of 0.1% saccharin, whereas saline-injected rats ingested ~9 ml of the solution. BT and N/OFQ completely blocked the acquisition of CTA: animals that received one of these opioids shortly before the administration of LiCl did not significantly decrease intake of saccharin in a two-bottle choice test. Administration of BT or N/OFQ alone did not alter saccharin consumption compared with controls. MOR significantly reduced the development of CTA, however, rats that received MOR or LiCl and MOR drank on average less saccharin solution than control animals (Table 1). Unconditioned stimulation did not produce taste aversion (data not shown). Total fluid intake in a two-bottle test did not differ between groups.

Table 1. Saccharin solution intakes assessed during a two-bottle test in animals that were treated with LiCl alone or one of the opioid peptides followed by LiCl

<table>
<thead>
<tr>
<th>Saccharin Intake</th>
<th>LiCl</th>
<th>Opioid/LiCl</th>
<th>Opioid</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milliliters</td>
<td>1.69±0.70a</td>
<td>8.73±1.76†</td>
<td>7.81±2.44†</td>
<td>9.10±2.19†</td>
</tr>
<tr>
<td>% of Total fluid intake</td>
<td>7.84±1.13a</td>
<td>47.05±8.81†</td>
<td>41.18±13.05†</td>
<td>63.72±15.33†</td>
</tr>
</tbody>
</table>

| Butorphanol      | 1.40±0.34a | 8.78±1.79† | 8.01±1.50† | 9.22±1.75† |
| % of Total fluid intake | 6.58±1.54a | 62.00±11.73† | 50.49±9.45† | 67.69±12.80† |

| Nociceptin       | 1.81±0.30a | 6.74±1.01† | 6.09±1.33† | 8.99±1.12‡ |
| % of Total fluid intake | 8.18±0.05a | 40.43±6.08† | 38.92±8.49‡ | 59.28±7.38‡ |

Values are means ± SE. LiCl, lithium chloride. Superscripts in a line that differ from each other indicate significant differences (\( P < 0.05 \)).
Neurohistochemical Studies

Injection of LiCl-induced c-Fos expression in ~50% of VP and OT neurons in the PVN. Fos-positive OT and VP cell bodies were found throughout the whole PVN, and their presence was not limited to any particular portion(s) of the nucleus. Administration of BT, N/OFQ, or MOR prior to LiCl injection caused a significant decrease in the percentage of activated OT and VP neurons, relative to control (Table 2; Figs. 1 and 2).

Upon intraperitoneal injection of LiCl, the majority of OT and VP neurons in the SON displayed c-Fos immunoreactivity. Administration of opioids prior to LiCl significantly reduced Fos expression in these SON neurons; however, the percentage of activated OT and VP cells in this nucleus was higher than in control animals. When each of the opioid peptides was injected alone, it did not produce any change in the activation of OT and VP neurons in the PVN or SON compared with controls (Table 3; Figs. 1 and 2).

LiCl administration resulted in significant increase of NTS cells displaying c-Fos compared with the control level of immunoreactivity. Injection of opioids shortly before LiCl significantly reduced c-Fos expression in the NTS, but density of Fos-positive nuclear profiles was still higher than in the NTS of control animals. Elevated numbers of Fos-IR nuclei were also detected in the NTS of rats that received only opioids

<table>
<thead>
<tr>
<th>% of Fos-Positive PVN Neurons</th>
<th>LiCl</th>
<th>Opioid/LiCl</th>
<th>Opioid</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Butorphanol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>52.7 ± 4.2 a</td>
<td>3.0 ± 2.0 †</td>
<td>5.8 ± 2.7 †</td>
<td>6.9 ± 4.1 †</td>
</tr>
<tr>
<td>OT</td>
<td>47.1 ± 7.7 a</td>
<td>4.8 ± 1.6 †</td>
<td>8.1 ± 3.0 †</td>
<td>7.6 ± 2.8 †</td>
</tr>
<tr>
<td><strong>Nociceptin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>53.1 ± 4.3 a</td>
<td>5.6 ± 2.1 †</td>
<td>2.6 ± 1.1 †</td>
<td>4.4 ± 1.9 †</td>
</tr>
<tr>
<td>OT</td>
<td>46.6 ± 7.3 a</td>
<td>6.7 ± 0.9 †</td>
<td>3.0 ± 1.6 †</td>
<td>8.2 ± 3.6 †</td>
</tr>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>50.3 ± 6.2 a</td>
<td>6.4 ± 1.6 †</td>
<td>5.2 ± 2.3 †</td>
<td>3.6 ± 1.5 †</td>
</tr>
<tr>
<td>OT</td>
<td>51.2 ± 8.1 a</td>
<td>8.0 ± 3.1 †</td>
<td>8.6 ± 4.2 †</td>
<td>6.1 ± 3.4 †</td>
</tr>
</tbody>
</table>

Values are means ± SE. VP, vasopressin; OT, oxytocin; PVN, paraventricular nucleus. Superscripts in a line which differ from each other indicate significant differences (P < 0.05).

Table 2. Percentages of vasopressin and oxytocin PVN neurons colocalizing with c-Fos in animals that were treated with LiCl alone or one of the opioid peptides followed by LiCl

<table>
<thead>
<tr>
<th>% of Fos-Positive SON Neurons</th>
<th>LiCl</th>
<th>Opioid/LiCl</th>
<th>Opioid</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Butorphanol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>65.7 ± 2.9 a</td>
<td>18.2 ± 9.0 †</td>
<td>4.0 ± 1.3 †</td>
<td>6.1 ± 1.8 ‡</td>
</tr>
<tr>
<td>OT</td>
<td>63.2 ± 7.1 a</td>
<td>18.0 ± 8.0 †</td>
<td>6.5 ± 2.4 †</td>
<td>5.4 ± 3.2 ‡</td>
</tr>
<tr>
<td><strong>Nociceptin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>61.7 ± 4.9 a</td>
<td>20.0 ± 6.0 †</td>
<td>3.9 ± 2.9 †</td>
<td>9.2 ± 3.3 ‡</td>
</tr>
<tr>
<td>OT</td>
<td>65.0 ± 3.9 a</td>
<td>16.2 ± 3.3 †</td>
<td>2.7 ± 1.6 †</td>
<td>4.4 ± 3.8 ‡</td>
</tr>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>60.5 ± 7.0 a</td>
<td>20.8 ± 4.1 †</td>
<td>6.3 ± 2.1 †</td>
<td>8.1 ± 4.8 ‡</td>
</tr>
<tr>
<td>OT</td>
<td>51.2 ± 8.1 a</td>
<td>8.0 ± 3.1 †</td>
<td>3.8 ± 0.9 †</td>
<td>2.4 ± 1.2 ‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. SON, supraoptic nucleus. Superscripts in a line which differ from each other indicate significant differences (P < 0.05).

Table 3. Percentages of vasopressin and oxytocin SON neurons colocalizing with c-Fos in animals that were treated with LiCl alone, one of the opioid peptides followed by LiCl, or saline

Fig. 1. Photomicrographs depicting coronal sections through the paraventricular nucleus (PVN) (A and B) and supraoptic nucleus (SON) (C and D) of rats injected with lithium chloride (LiCl) (left) or butorphanol tartrate (BT) and LiCl (right). Sections were double-stained for c-Fos and vasopressin (VP). Open arrows, Fos-positive VP neurons; thin arrows, VP neurons devoid of Fos. Scale bar = 0.05 mm.
compared with saline-injected animals. However, c-Fos expression in the NTS of opioid-treated animals was significantly lower than in rats that were given LiCl (Table 4; Fig. 3).

**DISCUSSION**

The endogenous opioid system has been implicated in the regulation of numerous physiological processes and behaviors. Of particular interest are data indicating that opioids regulate consummatory behavior, presumably by providing the “rewarding” properties of an ingestant (21). Therefore, the possible influence of opioid peptides on the development of CTAs has been suggested. Attempts to characterize the modulatory role of opioid peptides on the acquisition of CTA have led to contradictory results: both anti-aversive and aversive properties have been attributed to these substances (3, 11, 15).

The results of the present study clearly demonstrate that MOR, BT, and N/OFQ affect acquisition of LiCl-induced CTA in rats.

The µ-agonist, MOR, alleviated but did not eliminate the aversive effects of LiCl. Interestingly, MOR administered alone also induced mild taste aversion, unlike the other peptides used in our experiment. In 1987, Blancquaert and colleagues (3) conducted a study with a design similar to ours and found that MOR at a dose of 3 mg/kg body wt had anti-aversive effects against CTA induced by apomorphine, but it did not antagonize LiCl-dependent taste aversion. They did not, however, observe the development of CTA in rats that received only MOR, although high variability in sucrose solution consumption was notable in the group of MOR-treated animals (3). It is noteworthy that other authors have suggested aversive properties of MOR (3, 20, 42). It is likely that differences in methodology between studies (e.g., doses, route, and time of MOR administration) contributed to profound discrepancies in the experimental results. The fact that rats used in our study were accustomed to receiving MOR seems to be of a crucial importance, as MOR causes strong sedative effect in MOR-naive animals. Thus, in rats unaccustomed to MOR injections, sedation can possibly become an “unpleasant sensation” contributing to the development of CTA. On the basis of the results of the current experiment, we conclude that MOR antagonizes the aversive effects of LiCl but also produces mild taste aversion by itself.

To our knowledge, this is the first study to investigate the influence of BT and N/OFQ on the acquisition of CTA. N/OFQ, an ORL1 agonist (4), and BT, which acts via µ- and κ-receptors (22), completely blocked the development of CTA.

### Table 4. Densities of c-Fos immunoreactive nuclear profiles in the NTS of animals that underwent treatment with LiCl, opioids, opioids followed by LiCl, or saline

<table>
<thead>
<tr>
<th>Density</th>
<th>LiCl</th>
<th>Opioid/LiCl</th>
<th>Opioid</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Butorphanol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>152.7 ± 10.3*</td>
<td>101.4 ± 12.2†</td>
<td>111.2 ± 7.6†</td>
<td>52.7 ± 5.9‡</td>
<td></td>
</tr>
<tr>
<td><strong>Nociceptin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>148.6 ± 11.1*</td>
<td>73.1 ± 12.0†</td>
<td>102.1 ± 11.7†</td>
<td>49.4 ± 8.2‡</td>
<td></td>
</tr>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>152.7 ± 10.3*</td>
<td>87.1 ± 6.5†</td>
<td>96.6 ± 9.3†</td>
<td>66.0 ± 4.6‡</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. NTS, nucleus of the solitary tract. Superscripts in a line which differ from each other indicate significant differences (P < 0.05).
BT, a morphinan congener, is known to stimulate consummatory behavior more effectively than most other opioid agonists, including MOR and N/OFQ (22, 31). In the current study we demonstrated that anti-aversive effects of BT were stronger than those of MOR. In addition, administration of BT alone did not induce CTA. Such a powerful influence of BT may be attributed to the fact that this peptide binds to both κ- and μ-receptors, contrary to MOR, which is only a μ-agonist. As the stimulation of κ-receptors alone has not been found to antagonize the aversive properties of LiCl (3), and the stimulation of μ-receptors did not fully block the development of taste aversion, complete prevention of CTA by a mixed μ/κ-agonist BT indicates the importance of μ- and κ-system interactions in the regulation of aversive responses. Additional studies are needed to further address this issue.

N/OFQ is a novel peptide that exerts its action via the ORL1 receptor system (28). It does not, however, activate μ-, κ-, or δ-receptors, nor does ORL1 bind μ-, κ-, or δ-ligands with high affinity (34). Therefore, the N/OFQ/ORL1 system has been proposed to be separate from, but possibly interacting and/or overlapping with, the three remaining opioid systems (28, 31). Recently, N/OFQ has been found to stimulate consummatory behavior similarly to other opioids (31). Our data show that N/OFQ is a potent anti-aversive factor that powerfully antagonizes the effects of LiCl. Thus the N/OFQ/ORL1 system appears to be involved in the modulation of aversive responsiveness in rats.

In the current series of experiments, we have attempted to elucidate the nature of opioid influence on the acquisition of LiCl-induced CTA by characterizing some neural bases of opioid/LiCl interactions.

The complexity of the heterogeneous opioid system in the mammalian central nervous system, i.e., vast distribution of μ-, κ-, δ-, and ORL1 receptors, as well as opioid-containing fibers and neurons (23, 25, 28), suggests that opioids exert their action via intricate neuroendocrine mechanisms. A growing body of evidence indicates the importance of functional relations between opioids and OT/VP systems. The μ-, κ-, and ORL1 receptors are present in the SON (25, 28, 37, 38), and μ- and ORL1 are in the PVN (25, 28, 44). Injections of opioids of all types into the PVN generate a variety of physiological and behavioral responses (21). It has also been demonstrated that opioid agonists decrease, whereas antagonists potentiate, OT and VP secretion under various experimental conditions (33). The κ- and μ-agonists inhibit activation of OT-containing neurons both directly, causing hyperpolarization of these cells, and indirectly, through presynaptic actions (5, 6, 16, 32). VP neurons are relatively less sensitive to inhibition by μ-agonists, but their activation can be readily suppressed by κ-agonists (33). Orphanin has been shown to inhibit both OT and VP cells (8). In addition, the release of dynorphins and enkephalins from magnocellular dendrites has been detected (33, 35).

LiCl-induced activation of OT and VP neurons in the SON and PVN, which is in agreement with the outcome of studies by Verbalis et al. (43), who in 1986 showed that injection of LiCl at a dose similar to that used in our experiments results in the release of both OT and VP. Administration of MOR, BT, or N/OFQ prior to LiCl dramatically reduced activation of these cells. Significantly lower c-Fos expression was also observed in the NTS of animals treated with opioids and LiCl compared with rats that received only LiCl injection. It is noteworthy that OT and VP are thought to be involved in the mediation of the aversive effect of LiCl (42). However, their direct role in the acquisition of LiCl-dependent CTA is still not completely understood. The activation of cells containing these peptides probably reflects activity in a final common pathway for LiCl-induced responses. The NTS, as the major visceral sensory relay cell group, is an important ele-
ment of this pathway (29). Importantly, NTS contains all types of opioid receptors (23, 25, 28).

It should be noted that the suppression by opioids of LiCl-induced c-Fos immunoreactivity appears to be greater in the hypothalamic nuclei than in the brain stem, suggesting that the reduction in afferent activation of the PVN and SON from the NTS may only be a part of the reason for the decreased activation of OT and VP neurons. Considering the routes of opioid administration (icv or sc), the action of these peptides was not restricted to a particular site. Therefore, the observed changes in activation of OT and VP neurons may also be attributed to the effects of opioids on local hypothalamic receptors and/or on extrahypothalamic receptors present in the areas that send projections to the hypothalamus.

In general, the results of our study provide additional evidence to support the hypothesis that opioid peptides alleviate aversive properties of LiCl by influencing neural networks that encompass OT and VP cells in the PVN and SON as well as NTS neurons.

Perspectives

Treatments that influence consummatory behavior have become a useful tool with which to elucidate neural mechanisms involved in the regulation of feeding. LiCl, besides its aversive properties, is known to induce a powerful anorexic effect (24). OT and VP have been recently proposed as satiety factors (1, 19); thus it is likely that the inhibitory influence of LiCl on food intake is mediated via pathways that comprise OT and VP neurons. Importantly, opioids play a crucial role in the regulation of consummatory behavior. Considerable evidence indicates that the endogenous opioid system is primarily responsible for the maintenance rather than the initiation of feeding, presumably by affecting the “rewarding” properties of an ingestant (21). In our study, we showed that opioids alleviate aversive effects of LiCl and suppress LiCl-induced activation of OT and VP cells in the PVN and SON. Therefore, our results appear to be valuable also in relation to the involvement of opioid peptides in the control of feeding. We hypothesize that the endogenous opioid system influences food intake by suppressing activity of satiety-mediating pathway(s) that encompass OT and VP neurons.

We are grateful to Dr. Ruud M. Buijs (Netherlands Institute for Brain Research, Amsterdam, The Netherlands) for the generous gift of the rabbit VP antibody.

This work was supported by the Department of Veterans Affairs, by National Institute on Drug Abuse Grant DA-03999, and by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-42698 and P30-DK-50456.

REFERENCES

24. McCann MJ, Verbalis JG, and Stricker EM. LiCl and CCK inhibit gastric emptying and feeding and stimulate OT secretion.


