Interaction of serotonin and cholecystokinin in the lateral parabrachial nucleus to control sodium intake

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The lateral parabrachial nucleus (LPBN) is an important central site involved in the control of water and NaCl intake in rats (2, 17–21, 23, 24). This structure in the rat lies dorsolateral to the superior cerebellar peduncle in the pons and is reciprocally connected with several forebrain areas such as the paraventricular nucleus of the hypothalamus, central nucleus of amygdala, median preoptic nucleus (1, 7, 10, 11, 13), and more caudal regions such as the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS) (9, 14, 22). Recent studies have shown that bilateral LPBN injections of methysergide, a serotoninergic receptor antagonist, markedly increase NaCl intake induced by ANG II administered either intracerebroventricularly or into the subfornical organ (2, 21). Methysergide injected bilaterally into the LPBN also increased NaCl intake induced by combined treatment with the diuretic furosemide (Furo) and the angiotensin converting enzyme inhibitor captopril (Cap) injected subcutaneously (21). The injection of 2,5-dimetoxy-4-iodoamphetamine hydrobromide [DOI, a serotonergic 5-hydroxytryptamine (5-HT)2A/2C-receptor agonist] into the LPBN reduced Furo + Cap-induced NaCl intake (21). Other studies have shown that methysergide injected into the LPBN increases water and hypertonic NaCl intake induced by 24 h of water deprivation or by injection of Furo followed by 24 h of sodium-deficient diet (18). Not only 5-HT, but also CCK action in the LPBN is involved in the inhibition of water and NaCl intake. Bilateral injections of proglumide, a CCK-receptor antagonist, into the LPBN increased ANG II and Furo + CAP-induced 0.3 M NaCl intake (20).

Serotonin and CCK inhibit food intake (3–5, 8, 15, 29, 30). The reduction of food intake induced by intraperitoneal injection of CCK-8 was antagonized by intraperitoneal injection of metergoline (a 5-HT-receptor antagonist) (8). Devazepide, a type A CCK-receptor antagonist, injected subcutaneously, significantly antagonized the reduction in meal size induced by intraperitoneal injection of fenfluramine, an indirect 5-HT agonist, showing a reciprocal interaction between 5-HT and CCK (8). To explain the effects of antagonists and agonists of these two neurotransmitters on food intake, Cooper and Dourish (3) proposed a model of interdependence and cooperation between 5-HT and CCK. The authors suggested that, in response to food ingestion, both endogenous 5-HT and CCK are released. The cooperativity assumption is that elevated 5-HT release and action tend to increase CCK release and action and vice versa. The interdependence assumption is that both 5-HT and CCK action at their respective receptors is necessary for the normal development of satiety. Pharmacological interventions that block either 5-HT or CCK receptors reduce the satiety-inducing effects of

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either transmitter. In the present study, we investigated whether the model proposed by Cooper and Dourish to control food intake could also be applied to 5-HT and CCK in the LPBN to inhibit water and sodium intake.

MATERIALS AND METHODS

Animals. Male Holtzman rats weighing 280–300 g were used. The animals were housed in individual stainless steel cages with free access to normal sodium diet (Purina Rat Chow), water, and 1.8% NaCl solution. Temperature was maintained at 23 ± 2°C, and humidity was maintained at 55 ± 10% on a 12:12 light-dark cycle with light onset at 7:30 AM.

Cerebral cannulas. Rats were anesthetized with trichloroethylene (200 mg/kg of body wt) and placed in a Kopf stereotaxic instrument. The skull was leveled between bregma and lambda. Stainless steel 23-gauge cannulas were implanted bilaterally into the LPBN using the following coordinates: 9.5 mm caudal to bregma, 2.2 mm lateral to the midline, and 4.1 mm below the dura mater. The tips of the cannulas were positioned at a point 2 mm above each LPBN. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. A 30-gauge metal obturator filled the cannulas between tests. When surgery was complete, the rats were allowed to recover 6 days before drug injections into the LPBN.

Injections into the LPBN. Injections into the LPBN were made using 10-μl Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. A 30-gauge metal obturator filled the cannulas between tests. When surgery was complete, the rats were allowed to recover 6 days before drug injections into the LPBN.

Drugs. Furo (Sigma Chem., St Louis, MO) was administered subcutaneously at 10 mg/kg of body weight. Cap (Sigma Chemical, St Louis, MO) was administered subcutaneously at 5 mg/kg of body weight. Methysergide maleate, DOI, proglumide sodium, and CCK-8 sulfated [cholecystokinin fragment 26–33, [Tyr β]amide] were purchased from Research Biochemicals Internationals (Natick, MA). Methysergide (0.5 and 4 μg/0.2 μl) was dissolved in propylene glycol and water 2:1 (vehicle), DOI (5 μg/0.2 μl) and proglumide (20 and 50 μg/0.2 μl) were dissolved in saline. CCK-8 (1 μg/0.2 μl) was dissolved in distilled water.

Water and NaCl intake. Rats were tested in their home cages. Water and 1.8% NaCl were provided from burettes with 0.1-ml divisions that were fitted with metal drinking spouts. Water and 1.8% NaCl intake were induced by treatment with subcutaneous Furo (10 mg/kg of body wt) + Cap (5 mg/kg of body wt) as described previously (6, 21). Cumulative water and 1.8% NaCl intakes were measured at 30, 60, 90, and 120 min starting 1 h after Furo + Cap treatment when water and NaCl were available for animals.

Rats received Furo + Cap treatments and were returned to their home cages in the absence of water and 1.8% NaCl solution. Forty minutes later, the animals received the first bilateral injections into the LPBN (first treatment) followed 10 min later by the second bilateral injections into the LPBN (second treatment) according to the combinations of treatments presented in Table 1. Water and 1.8% NaCl were available to the animals 10 min after the second LPBN treatment.

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Results

Histological analysis. Similar to the results seen in previous reports (2, 18–21), the LPBN injection sites were centered in the central lateral and dorsal lateral portions of the LPBN [see Fulwiler and Saper (7) for definitions of LPBN subnuclei]. Figure 1 shows typical LPBN injection sites. Injections reaching the ventral lateral and external lateral portions as well as the Kölliker-Fuse nucleus were observed in some rats, and the results from these rats were included in the analysis.

From a total of 72 rats used in this study, 53 had histologically confirmed LPBN bilateral injections as described above.

Effects of bilateral injections of methysergide + proglumide into the LPBN on Furo + Cap-induced 1.8% NaCl and water intake. With the higher doses of the drugs tested, bilateral injections of only methysergide

### Table 1. Summary of the combinations of treatments into the LPBN

<table>
<thead>
<tr>
<th>First Treatment</th>
<th>Second Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>saline</td>
</tr>
<tr>
<td>Methysergide (0.5 μg/0.2 μl)</td>
<td>saline</td>
</tr>
<tr>
<td>Vehicle</td>
<td>proglumide (20 μg/0.2 μl)</td>
</tr>
<tr>
<td>Methysergide (0.5 μg/0.2 μl)</td>
<td>proglumide (20 μg/0.2 μl)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>saline</td>
</tr>
<tr>
<td>Methysergide (4 μg/0.2 μl)</td>
<td>saline</td>
</tr>
<tr>
<td>Vehicle</td>
<td>proglumide (50 μg/0.2 μl)</td>
</tr>
<tr>
<td>Methysergide (4 μg/0.2 μl)</td>
<td>proglumide (50 μg/0.2 μl)</td>
</tr>
<tr>
<td>Saline</td>
<td>saline</td>
</tr>
<tr>
<td>Proglumide (50 μg/0.2 μl)</td>
<td>DOI (5 μg/0.2 μl)</td>
</tr>
<tr>
<td>Saline</td>
<td>DOI (5 μg/0.2 μl)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>water</td>
</tr>
<tr>
<td>Methysergide (4 μg/0.2 μl)</td>
<td>water</td>
</tr>
<tr>
<td>Vehicle</td>
<td>CCK-8 (1 μg/0.2 μl)</td>
</tr>
<tr>
<td>Methysergide (4 μg/0.2 μl)</td>
<td>CCK-8 (1 μg/0.2 μl)</td>
</tr>
</tbody>
</table>

LPBN, lateral parabrachial nucleus; DOI, 2,5-dimetoxy-4-iodoamphetamine hydrobromide.
Fig. 1. Photomicrograph of transverse section of a rat brain showing injection sites, as indicated by the arrows, in the lateral parabrachial nucleus (LPBN).

(4 μg/0.2 μl each site) or of only proglumide (50 μg/0.2 μl each site) into the LPBN produced similar increases of 1.8% NaCl intake (14.5 ± 4.1 and 14.2 ± 3.2 ml/2 h, respectively, vs. control: 2.9 ± 0.9 ml/2 h), [Fig. 2A; F(3,36) = 3.07; P < 0.05]. The combination of both antagonists injected into the LPBN resulted in no additional change in 1.8% NaCl intake (16.3 ± 3.2 ml/2 h) compared with the effect of each antagonist alone delivered to the LPBN (Fig. 2A). The increase in water intake was similar for all treatments (methysergide: 17.7 ± 2.3 ml/2 h; proglumide: 17.4 ± 2.3 ml/2 h; and methysergide + proglumide: 20.4 ± 3.4 ml/2 h vs. control: 10.3 ± 1.1 ml/2 h; Fig. 2B). An ANOVA showed significant interaction between treatments and time for water intake [F(9,108) = 2.56; P < 0.05].

With the low doses of the drugs, bilateral injections of only methysergide (0.5 μg/0.2 μl) or only proglumide (20 μg/0.2 μl) into the LPBN produced similar increases of 1.8% NaCl intake (13.2 ± 2.7 and 11.1 ± 2.1 ml/2 h, respectively, vs. control: 2.8 ± 0.8 ml/2 h), (Fig. 3A). The combined bilateral injections of methysergide (0.5 μg/0.2 μl) and proglumide (20 μg/0.2 μl) into the LPBN produced greater increases in 1.8% NaCl intake (20.6 ± 2.3 ml/2 h) compared with the effect of each antagonist alone [Fig. 3A; F(3,36) = 10.29; P < 0.01]. As in the effects observed with the high doses, the low doses of the antagonists produced similar increases of water intake in all treatments (methysergide: 17.7 ± 3.2 ml/2 h; proglumide: 19.4 ± 1.9 ml/2 h; and methysergide + proglumide: 23.0 ± 3.1 ml/2 h vs. control: 12.1 ± 1.7 ml/2 h; Fig. 3B). An ANOVA showed a significant interaction between treatments and time for water intake [F(9,108) = 3.00; P < 0.01].

Effects of bilateral injections of proglumide + DOI into the LPBN on Furo + Cap-induced 1.8% NaCl and water intake. Bilateral injections of DOI (5 μg/0.2 μl each site) into the LPBN decreased 1.8% NaCl intake [1.8 ± 0.4 vs. control: 4.9 ± 0.9 ml/2 h; Fig. 4A; F(3,76) = 14.03; P < 0.01]. Proglumide (50 μg/0.2 μl each site) injected bilaterally into the LPBN significantly increased 1.8% NaCl intake (15.4 ± 2.8 ml/2 h). With the combination of bilateral injections of proglumide and DOI into the LPBN, the 1.8% NaCl intake was not different from the control treatment (5.9 ± 1.3 vs. control: 4.9 ± 0.9 ml/2 h; Fig. 4A).

Water intake significantly decreased with bilateral injections of DOI (5 μg/0.2 μl) into the LPBN [6.4 ± 0.8 vs. control: 11.7 ± 0.9 ml/2 h; Fig. 4B; F(3,76) = 11.38; P < 0.01]. Proglumide increased water intake (17.3 ± 2.2 ml/2 h). With the combination of proglumide and DOI injected into the LPBN, water intake was not different from control (9.6 ± 1.4 vs. saline: 11.7 ± 0.9 ml/2 h; Fig. 4B).

Effects of bilateral injections of methysergide + CCK-8 into the LPBN on Furo + Cap-induced 1.8% NaCl and water intake. Bilateral injections of only methysergide (4 μg/0.2 μl, each site) into the LPBN increased 1.8% NaCl intake (16.3 ± 3.7 vs. control: 3.0 ± 0.9 ml/2 h; Fig. 5A). CCK-8 (1 μg/0.2 μl, each site) for water intake [Furo] 1.8% NaCl and water intake. Bilateral injections of only methysergide (4 μg/0.2 μl, each site) into the LPBN increased 1.8% NaCl intake (15.4 ± 2.8 ml/2 h). With the combination of proglumide and DOI into the LPBN, the 1.8% NaCl intake was not different from the control treatment (5.9 ± 1.3 vs. control: 4.9 ± 0.9 ml/2 h; Fig. 4A).

With the low doses of the drugs, bilateral injections of only methysergide (0.5 μg/0.2 μl) or only proglumide (20 μg/0.2 μl) into the LPBN produced similar increases of 1.8% NaCl intake (13.2 ± 2.7 and 11.1 ± 2.1 ml/2 h, respectively, vs. control: 2.8 ± 0.8 ml/2 h), (Fig. 3A). The combined bilateral injections of methysergide (0.5 μg/0.2 μl) and proglumide (20 μg/0.2 μl) into the LPBN produced greater increases in 1.8% NaCl intake (20.6 ± 2.3 ml/2 h) compared with the effect of each antagonist alone [Fig. 3A; F(3,36) = 10.29; P < 0.01]. As in the effects observed with the high doses, the low doses of the antagonists produced similar increases of water intake in all treatments (methysergide: 17.7 ± 3.2 ml/2 h; proglumide: 19.4 ± 1.9 ml/2 h; and methysergide + proglumide: 23.0 ± 3.1 ml/2 h vs. control: 12.1 ± 1.7 ml/2 h; Fig. 3B). An ANOVA showed a significant interaction between treatments and time for water intake [F(9,108) = 3.00; P < 0.01].

Effects of bilateral injections of proglumide + DOI into the LPBN on Furo + Cap-induced 1.8% NaCl and water intake. Bilateral injections of DOI (5 μg/0.2 μl each site) into the LPBN decreased 1.8% NaCl intake [1.8 ± 0.4 vs. control: 4.9 ± 0.9 ml/2 h; Fig. 4A; F(3,76) = 14.03; P < 0.01]. Proglumide (50 μg/0.2 μl each site) injected bilaterally into the LPBN significantly increased 1.8% NaCl intake (15.4 ± 2.8 ml/2 h). With the combination of bilateral injections of proglumide and DOI into the LPBN, the 1.8% NaCl intake was not different from the control treatment (5.9 ± 1.3 vs. control: 4.9 ± 0.9 ml/2 h; Fig. 4A).

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Effects of bilateral injections of methysergide + CCK-8 into the LPBN on Furo + Cap-induced 1.8% NaCl and water intake. Bilateral injections of only methysergide (4 μg/0.2 μl, each site) into the LPBN increased 1.8% NaCl intake (16.3 ± 3.7 vs. control: 3.0 ± 0.9 ml/2 h; Fig. 5A). CCK-8 (1 μg/0.2 μl, each site)
alone injected bilaterally into the LPBN produced no change in 1.8% NaCl intake (3.7 ± 1.3 ml/2 h). During the first 30 min of the test with the combination of bilateral LPBN injections of methysergide and CCK-8, 1.8% NaCl intake (6.7 ± 2.7 ml/30 min) was reduced compared with NaCl intake with methysergide injected alone into the LPBN \[ F(3,32) = 8.02; P < 0.01; \text{Fig. 5A} \].

Injections of only CCK-8 into the LPBN did not alter the water intake (10.5 ± 1.7 vs. control: 10.1 ± 1.2 ml/2 h; Fig. 5B). Water intake increased with bilateral injections of only methysergide and with the association of methysergide and CCK-8 into the LPBN \[ 18.3 ± 2.4 \text{ and } 20.1 ± 3.4 \text{ ml/2 h; } F(3,32) = 4.91; P < 0.01 \].

Specificity of injections into the LPBN to produce the reported results. The specificity of the LPBN as the site of injections that produce the effects of the drugs was confirmed by results from rats in which the injections did not reach one or both LPBN sites. Table 2 shows the total ingestion of water and 1.8% NaCl (120 min of intake) produced by the different treatments in rats in which the injections did not reach one or both LPBN sites. ANOVA showed no significant differences on cumulative water and NaCl intake in these rats with any of the treatments.

DISCUSSION

The effects of the combined administration of serotonergic and CCKergic agonists and antagonists into the LPBN on Furo + Cap-induced NaCl intake suggest the presence of important interactions between the two neurotransmitters in the control of sodium intake. On the other hand, the results showed no clear interaction between 5-HT and CCK in the control of Furo + Cap-induced water intake. In most of the experiments, the rats ingested significant amounts of 1.8% NaCl that can, in turn, induce significant water intake due to the increase in body fluid osmolality. Consequently, the water intake recorded in the protocols was likely to have been confounded by large intakes of hypertonic NaCl. Therefore, it is not appropriate to consider pos-
possible interactions between 5-HT and CCK to control water intake in the protocol used in the present study.

The present results show an interaction between inhibitory serotonergic and cholecystokinin mechanisms in the LPBN on the control of sodium chloride intake (sodium appetite) that seems to work like the model of cooperativity and interdependence between 5-HT and CCK proposed by Cooper and Dourish to explain the inhibitory control of feeding by these two neurotransmitters (3). Cooper and Dourish proposed that elevated 5-HT release and action tend to increase CCK release and action and vice versa. The interdependence assumption is that both 5-HT and CCK action at their respective receptors is necessary for the normal development of satiety. Pharmacological interventions that block either 5-HT or CCK receptors reduce the satiety-inducing effects of either transmitter. One assumption of this idea is that elevated 5-HT and CCK activity is necessary for satiety to be fully expressed.

The combination of methysergide and proglumide administered in high doses into the LPBN produced an increase of 1.8% NaCl and water intake that was equivalent to the increase produced by the injections of either antagonist alone. With low doses, bilateral LPBN injections of only methysergide or only proglumide produced comparable increases in 1.8% NaCl intake. The combined treatment with low doses of both antagonists administered into the LPBN produced a significantly greater increase in sodium intake. Considering the effects of bilateral LPBN injections of methysergide and proglumide in light of the model of cooperativity and interdependence, the treatment with a high dose of methysergide not only prevents the action of 5-HT, but also may reduce CCK release, therefore nearly abolishing the action of both inhibitory mechanisms within the LPBN. Similarly, proglumide treatment prevents the action of CCK but also may reduce 5-HT release, producing the same effects as methysergide. Thus the effects of one or both antagonists in high doses injected in the LPBN are the same; that is, with high doses, combined injections of both antagonists did not produce a greater 1.8% NaCl intake, because the blockade of only one receptor system seems to be sufficient to abolish the action of both systems. Injections of low doses of each antagonist, methysergide or proglumide, produce only a partial impairment in each mechanism. The combination of both receptor antagonists given in low doses into the LPBN can produce a more intense blockade of each mechanism compared with single injections of each antagonist, thereby producing an increased sodium intake.

Bilateral injections of the serotonergic receptor agonist DOI alone into the LPBN decreased water and 1.8% NaCl intake. With the combination of bilateral LPBN injections of proglumide and DOI, water and 1.8% NaCl intake returned to control level. This suggests that the association of proglumide and methysergide treatment prevents the action of CCK but also may reduce 5-HT release, producing the same effects as methysergide. Thus the effects of one or both antagonists in high doses injected in the LPBN are the same; that is, with high doses, combined injections of both antagonists did not produce a greater 1.8% NaCl intake, because the blockade of only one receptor system seems to be sufficient to abolish the action of both systems. Injections of low doses of each antagonist, methysergide or proglumide, produce only a partial impairment in each mechanism. The combination of both receptor antagonists given in low doses into the LPBN can produce a more intense blockade of each mechanism compared with single injections of each antagonist, thereby producing an increased sodium intake.

Table 2. Water and 1.8% NaCl intake induced by combined subcutaneous treatment with Furo + Cap in rats in which 1 or both injections did not reach LPBN sites

<table>
<thead>
<tr>
<th>LPBN Treatments</th>
<th>1.8% NaCl Intake (ml/120 min)</th>
<th>Water Intake (ml/120 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh + sal</td>
<td>6</td>
<td>2.5 ± 1.1</td>
</tr>
<tr>
<td>Methy (4 μg) + sal</td>
<td>6</td>
<td>4.0 ± 2.0</td>
</tr>
<tr>
<td>Veh + prog (50 μg)</td>
<td>6</td>
<td>4.1 ± 1.5</td>
</tr>
<tr>
<td>Methy (4 μg) + prog (50 μg)</td>
<td>6</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>Veh + sal</td>
<td>5</td>
<td>6.2 ± 1.5</td>
</tr>
<tr>
<td>Methy (0.5 μg) + sal</td>
<td>5</td>
<td>8.8 ± 3.8</td>
</tr>
<tr>
<td>Veh + prog (20 μg)</td>
<td>5</td>
<td>8.6 ± 1.6</td>
</tr>
<tr>
<td>Methy (0.5 μg) + prog (20 μg)</td>
<td>5</td>
<td>8.2 ± 1.3</td>
</tr>
<tr>
<td>Sal + sal</td>
<td>5</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Sal + DOI (5 μg)</td>
<td>5</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>Prog (50 μg) + sal</td>
<td>5</td>
<td>3.7 ± 1.3</td>
</tr>
<tr>
<td>Prog (50 μg) + DOI (5 μg)</td>
<td>5</td>
<td>4.7 ± 2.0</td>
</tr>
<tr>
<td>Veh + water</td>
<td>4</td>
<td>5.2 ± 1.8</td>
</tr>
<tr>
<td>Veh + CCK-8</td>
<td>4</td>
<td>5.1 ± 3.3</td>
</tr>
<tr>
<td>Methy (4 μg) + water</td>
<td>4</td>
<td>4.9 ± 2.4</td>
</tr>
<tr>
<td>Methy (4 μg) + CCK-8 (1 μg)</td>
<td>4</td>
<td>5.8 ± 2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats; veh, vehicle; sal, saline; methy, methysergide; prog, proglumide; CCK-8, cholecystokinin fragment 26–33. Furo, furosemide; Cap, captopril.
DOI abolished the inhibition of sodium intake produced by activation of the serotonergic inhibitory mechanism with DOI as well as the increase of sodium intake produced by the deactivation of cholecystokininergic mechanism with proglumide. According to the model of cooperativity and interdependence between 5-HT and CCK, the activation of the serotonergic inhibitory mechanism of the LPBN with DOI may also stimulate local CCK release, resulting in a reduction of NaCl intake as a consequence of the action of DOI and CCK on their respective receptors. Proglumide treatment prevents CCK action on its receptors and may reduce the inhibitory effect of DOI, which is also dependent on CCK action. Why would the proglumide effect disappear when proglumide is associated with DOI? Proglumide blocking CCK receptors prevents the direct inhibitory action of CCK and also the stimulant effect of CCK on 5-HT release. The result may be due also to a reduced endogenous 5-HT release and almost a complete blockade of both inhibitory mechanisms within the LPBN. However, DOI injections activating serotonergic receptors can partially restore the inhibition, impairing the normal effect of proglumide. The effect of DOI is partial, because without CCK participation, the effect of serotonergic receptor activation is not complete.

Bilateral injections of CCK-8 alone into the LPBN did not change either water or 1.8% NaCl intake. Compared with the intake with bilateral injections of methysergide alone, the combination of CCK-8 and methysergide injected into the LPBN reduced 1.8% NaCl intake during the first 30 min of testing. The lack of an effect of CCK-8 when administered alone into the LPBN on NaCl intake is consistent with previous results showing that bilateral injections of CCK-8 into the LPBN did not reduce Furo + Cap-induced 1.8% NaCl intake (20). To explain the lack of an effect of CCK-8 injected into the LPBN on NaCl intake, Menani and Johnson (20) proposed that the CCK normally present in the LPBN is enough to interact with 5-HT and sufficient to exert a complete inhibitory effect on sodium intake. Thereby, exogenous CCK-8 would not increase the inhibition of sodium intake. According to the model of cooperativity and interdependence between 5-HT and CCK, the blockade of 5-HT receptors impairs CCK release. Therefore, only methysergide treatment can increase 1.8% NaCl intake by indirectly reducing CCK release. Replacing CCK by injecting exogenous CCK-8 may restore part of the inhibition, so it is possible to observe a partial reduction of 1.8% NaCl intake in association with methysergide and CCK-8 into the LPBN.

The LPBN is a major site that receives ascending projections from AP/mNTS and sends efferent projections to areas of the forebrain involved in the control of fluid and electrolyte balance such as hypothalamic areas and amygdala (1, 7, 9–11, 13, 14). The AP/mNTS is an important area of the hindbrain that receives afferent projections from volume receptors (arterial baroreceptors, cardiopulmonary receptors), gustatory receptors, and other visceral receptors that can influence water and NaCl intake (12, 22, 27, 28). Central or peripheral administration of the atrial natriuretic peptide (ANP) has also been shown to reduce water and sodium intake (16). Because the AP lacks a blood-brain barrier, plasma ANP released by atrial distension may have activated this area, and this information about blood-borne levels of ANP is then carried to the LPBN. Neuropeptides such as 5-HT and CCK have been identified in the projections from AP/mNTS to the LPBN (14, 25, 26). Thus the LPBN can play a key role in the central circuitry that controls water and NaCl intake. Figure 6 is representing the interdependence and cooperativity between 5-HT and CCK in the LPBN to control NaCl intake. The proposed model suggests that the release and the action of 5-HT and CCK in the LPBN are essential for complete inhibition of NaCl intake.

**Perspectives**

The demonstration of an interaction between inhibitory serotonergic and CCKergic mechanisms in the LPBN to control NaCl intake represents an important advance in our understanding of the central mechanisms involved in the control of fluid and electrolyte balance. Future studies should deal with the origin and nature of the stimuli that induce serotonin and CCK release in the LPBN and whether 5-HT and CCK interact in the LPBN to control water intake.

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