Sodium appetite and Fos activation in serotonergic neurons

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Fos-serotonin immunoreactivity; area postrema; raphe system

RECENTLY, there has been increased interest in identifying the neural network subserving sodium appetite. Forebrain structures such as the circumventricular organs (CVOs) of the lamina terminalis, the organum vasculosum of the lamina terminalis, and the subfornical organ have been identified as key targets processing angiotensin-, osmotic-, and sodium-related information involved in the control of sodium appetite. For that purpose, we analyzed the number of Fos-immunoreactive (Fos-ir) cells and double-labeled Fos-serotonin (5-HT)-ir neurons within different nuclei of the hindbrain raphe system and the area postrema (AP). Sodium depletion and sodium appetite were induced by peritoneal dialysis. Twenty-four hours after peritoneal dialysis, a 2% NaCl solution intake test was given to peritoneal dialyzed animals [PD-with access (PD-A) group] and to control dialyzed animals [CD-with access (CD-A) group]. Two additional groups of animals received either peritoneal dialysis or control dialysis but were not given access to the 2% NaCl [CD-no access (CD-NA) group or PD-no access (PD-NA) group]. The number of Fos-ir neurons within different nuclei of the raphe system was increased in spontaneous and induced sodium ingestion of CD-NA and PD-NA groups. The PD-NA group had significantly fewer double-labeled cells along the raphe system compared with the animals in near-normal sodium balance (CD-NA and CD-A) or in the process of restoring sodium balance by consuming NaCl (CD-A). The AP of the PD-A group showed a significant increase in the number of Fos-ir and Fos-5-HT-hir cells compared with the PD-NA and CD groups. Our results suggest that serotonergic pathways with cell bodies in the AP and the raphe system are involved in the control of sodium appetite.

Fos-serotonin immunoreactivity; area postrema; raphe system

The aim of the present work was to evaluate whether activity in hindbrain serotonergic neurons is changed during the presence of sodium appetite induced by peritoneal dialysis and during the process of satiety. For that purpose, we analyzed the number of Fos-immunoreactive (Fos-ir) cell nuclei within the different serotonergic cell groups in brain stem nuclei after sodium ingestion induced by peritoneal dialysis. In addition, we used 5-HT-immunohistochemical staining in combination with nuclear Fos-ir to provide a quantitative estimation of the serotonergic neurons that were activated. This approach was selected because Fos, the nuclear protein product of the immediately early gene c-fos, has been used as a marker of neural activation in response to a wide variety of stimuli. The analysis of the pattern of c-fos expression in the central nervous system preceding and after depletion-induced sodium ingestion potentially identifies the individual cellular components of functional neural networks ac-
comparing the presence of sodium appetite and then its satiety. Fos-ir has been applied in analysis of forebrain systems activated by the different procedures used to induce sodium appetite (12, 20, 30, 38, 43, 44).

MATERIALS AND METHODS

The experiments used 32 adult Wistar-derived male rats born and reared in the breeding colony at the Instituto Ferreyra. The animals weighing 250–300 g were housed singly in hanging wire cages for at least 1 wk before the experiments were begun and had free access to food, water, and 2% NaCl. Room lights were on for 14 h/day, and temperature was controlled at 23°C.

A two × two factorial experimental design with sodium-replete or sodium-depleted animals with either no access to 2% NaCl or access to 2% NaCl as the factors was used. Specifically, 18 animals received intraperitoneal dialysis and the remainder did not; then approximately half of each of these two groups was given access to 2% NaCl for 1 h, and the other half of each of the two groups was not. The groups were designated as control dialyzed with no access to 2% NaCl (CD-NA), peritoneal dialyzed with no access to 2% NaCl (PD-NA), control (sham) dialyzed with access to 2% NaCl (CD-A), and peritoneal dialyzed with access to 2% NaCl (PD-A).

Sodium appetite was stimulated by an acute sodium depletion induced by peritoneal dialysis. The technique, described previously (11), consisted of an intraperitoneal injection of a 5% glucose solution warmed to 37°C in a volume equivalent to 10% of rat body weight. After 1 h, the ascitic fluid was recovered by inserting a needle into the peritoneal cavity. The amount of Na⁺ withdrawn by this method in the dialyzed groups (i.e., PD-NA and PD-A) was 0.84 ± 0.02 meq/100 g body wt (mean ± SE, n = 18). In control dialyzed rats (CD-NA and CD-A), the needle was inserted into the peritoneal cavity, but no injection was given. The dialyzed and control dialyzed rats were caged individually without food and with only distilled water available. Twenty-four hours after control or peritoneal dialysis, access to 2% NaCl was given for the CD-A and PD-A groups and sodium intake was measured at 60 min. Behavioral data were analyzed by Student’s t-test. Ninety minutes after the termination of the intake test or a comparable period but with no access to 2% NaCl, the animals were perfused for immunohistochemical detection of Fos and 5-HT.

Immunohistochemistry. After the completion of the different experimental procedures, the rats were anesthetized with thiopentone (100 mg/kg ip) and perfused transcardially with ~200 ml of normal saline followed by 400 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.2. The brains were then removed, fixed overnight in the perfusion solution, and stored at 4°C in PB containing 30% sucrose. Two series of 40-μm coronal sections were cut using a freezing microtome. Immediately before immunostaining, sections were placed in a mixture of 10% H₂O₂-10% methanol until oxygen bubbles ceased appearing. They were then incubated in 10% normal horse serum (NHS) in PB for 1 h to block sites of nonspecific binding of serum products. Fos-ir was detected using a standard avidin-biotin peroxidase protocol. The free-floating sections were incubated overnight at room temperature in an antibody raised in rabbits against a synthetic 14-amino acid sequence corresponding to residues 4–17 of human Fos (Ab-5, batch no. 60950101; Oncogene Science, Manhasset, NY) diluted 1:20,000 in a solution of PB containing 2% NHS and 0.3% Triton X-100. After being washed in PB, the sections were incubated in biotin-labeled anti-rabbit immunoglobulin and avidin-biotin peroxidase complex (Vector, 1:200 dilution in 1% NHS-PB) for 1 h at room temperature. The peroxidase label was detected using diaminobenzidine hydrochloride (DAB; Sigma) intensified with 1% cobalt chloride and 1% nickel ammonium sulfate. This method produces a blue-black nuclear reaction product. One series of Fos-labeled sections was also processed for immunocytochemical localization of 5-HT. Sections were incubated for 72 h at 4°C in polyclonal rabbit anti-5-HT antibody (Biogenex, batch no. PU0680399, San Ramon, CA) diluted 1:250 in a solution of PB containing 2% NHS and 0.3% Triton X-100. After incubation, sections were rinsed and incubated in the appropriate biotinylated secondary anti-serum and avidin-biotin peroxidase complex (Vector, 1:200 dilution in 1% NHS-PB). Cytoplasmic 5-HT-ir was detected using intensified DAB to produce a brown reaction product. Finally, the free-floating sections were mounted on gelatinized slides, air-dried overnight, dehydrated, cleared in xylene, and placed under a coverslip with DePeX. Controls for the Fos-ir were conducted by placing sections in primary Fos antibody that had been preabsorbed with an excess of the Fos peptide or by processing sections without the primary antibody. No Fos-ir neurons were observed after either of these control procedures.

Cytarachitectural and quantitative analysis. Serotonergic hindbrain groups of cells including the different nuclei of the raphe system and the AP were microscopically examined using low-power magnification (×10), and on the basis of Fos staining observed to be associated with one or more of the sodium appetite-related treatments, the dorsal raphe nucleus (DR), median raphe nucleus (MrN), raphe magnus nucleus (RMg), raphe pallidus nucleus (RPa), and AP were chosen for quantification. The raphe obscurus nucleus (ROb) and the raphe pontis nucleus (RPn) did not show Fos-ir cells in any group of animals analyzed. The brain nuclei evidencing Fos-ir were identified and delimitated on the basis of the plates from the rat brain atlas of Paxinos and Watson (31). The number of Fos-ir nuclear profiles was counted in sections of the DR at three levels, i.e., anterior, medial, and posterior (corresponding to plates with a distance from bregma of −7.64, −8.30, and −8.80 mm, respectively); the MrN at two levels, i.e., anterior and medial (distance from bregma of corresponding plates: −7.64 and −8.30 mm, respectively); the RMg and the RPa in plate with a distance from bregma of −11.60 mm; and the AP in plate with a distance from bregma of −13.80 mm (31).

Fos-ir nuclei were quantified using a computerized system that included a Zeiss microscope equipped with a video camera attached to a contrast enhancement device. Video images were digitized and analyzed using Scion Image PC, based on the National Institutes of Health 1997 version. Fos-ir cells in each section were counted by setting a size range for cellular nuclei (in pixels) and threshold level for staining intensity. Representative sections in control and experimental groups were acquired at exactly the same level, with the aid of the Adobe Photoshop Image Analysis Program, version 5.5. The counting procedure was done in four or five animals from each condition, and it was repeated at least twice on each section analyzed to ensure that the numbers of profiles obtained were similar. The data, expressed as means ± SE, were analyzed by a two-way ANOVA. Factors were depletion vs. sham depletion and access to 2% NaCl vs. no access. Least significant difference (LSD) tests were used as followups to identify significant differences between groups.

Results were considered to be significantly different at \( P < 0.05 \). The data were not corrected for double counting or by using a stereological technique. However, because the objects we were counting (nuclei) did not change in size and because
section thickness did not vary between experimental and control groups, any systematic error should be identical for both groups. Hence, the results are meant to provide relative data on expression of Fos-ir but are not meant to be accurate estimates of absolute cell counts. Two series of sections were used in the quantitative analysis; in one series of sections, Fos-ir neurons were counted using the computerized image analysis previously described, and in the other series of sections, Fos- and 5-HT-labeled neurons were hand counted.

**Electrolyte assays.** Animals separate from those used in the immunohistochemical studies were divided into different treatment conditions (PD-A, PD-NA, CD-A, CD-NA) and bled to analyze plasma sodium and potassium concentrations. Blood samples were taken 90 min after the termination of the intake test in the PD-A and CD-A groups or after a comparable period in the PD-NA and CD-NA groups, which had no access to 2% NaCl. Trunk blood was collected into chilled tubes containing EDTA (final concentration 2 mg/ml blood) for centrifugation at 3,000 g for 10 min at 4°C. A small aliquot of the plasma was measured immediately for sodium and potassium concentration using a Hitachi 911 automatic analyzer. The data were subjected to a two-way ANOVA. Post hoc comparisons were made using LSD tests with significance levels set at P < 0.05.

**RESULTS**

**Sodium intake in animals studied for Fos-ir.** Cumulative volume of 2% NaCl drunk during the intake test (1 h) was 3.91 ± 0.5 and 0.72 ± 0.4 ml/100 g body wt in the PD-A (n = 5) and the CD-A (n = 4) groups, respectively (t = 5.29, P < 0.001).

**Sodium intake and plasma electrolyte determinations.** In animals studied to determine plasma electrolyte concentrations, the cumulative volumes of 2% NaCl drunk during the intake test (1 h) of the PD-A group (n = 13) was 3.43 ± 0.26 ml/100 g body wt and of the CD-A group (n = 11) was 1.01 ± 0.21 ml/100 g body wt (t = 7.24, P < 0.001).

In general, the treatments produced only small effects on plasma electrolytes. CD-NA and PD-NA groups had lower plasma sodium concentrations than CD-A and PD-A groups (F1,12 = 26.4; P < 0.0001; 2-way ANOVA, LSD test; Table 1). Potassium plasma levels did not differ significantly among any of the groups analyzed (F1,12 = 1.0; P = 0.3; 2-way ANOVA, LSD test; Table 1).

**Fos-ir in the raphe system.** Spontaneous and peritoneal dialysis-induced sodium ingestion produced a pattern of highly localized and intense Fos-ir cells in different nuclei of the raphe system. Within the DR, the Fos-ir cells were mainly located in the ventrolateral and dorsal subdivision (DRVL and DRD, respectively) at the anterior and medial levels (Figs. 1 and 2, respectively). The greater activation in the DR was observed at the medial level, where the number of Fos-ir cells of CD-A and PD-A rats was significantly increased when compared with these regions of the CD-NA and PD-NA groups (F1,14 = 28.86; P < 0.0001; 2-way ANOVA, LSD test; Figs. 2 and 3). In the MnR, RMg, and RPa, the groups displaying both “spontaneous” (CD-A) and induced (PD-A) sodium ingestion showed enhanced expression of c-fos above levels found in the groups of animals that did not have access to NaCl (MnR: F1,13 = 12.28; P < 0.001; RMg: F1,13 = 7.71; P < 0.05; RPa: F1,13 = 29.26; P < 0.0001; Fig. 3).

In all of these cases, an ANOVA revealed a significant difference in c-fos expression between animals with and without access to the 2% NaCl. At a more posterior level of the DR (DRp), the number of Fos-ir neurons was not significantly different among the groups (Fig. 3).

Fos expression in the CD-A and also in the PD-A groups showed a tendency to vary in relation to the volume of saline consumed by an individual animal. For example, in the CD-A group, the largest individual intake (1.4 ml NaCl/100 g body wt) was ingested early in the test period vs. later (case 1: Fos-ir 0.41 mmol/l, 2-way ANOVA, P = 0.04; case 2: Fos-ir 0.24 mmol/l, 2-way ANOVA, P = 0.001).

**Table 1. Plasma Na+ and K+ concentration in four groups of sodium-depleted and sham-depleted rats with and without access to the 2% NaCl intake test**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Na+, mmol/l</th>
<th>K+, mmol/l</th>
</tr>
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<tbody>
<tr>
<td>PD-A</td>
<td>13</td>
<td>148.8 ± 2.106</td>
<td>6.6 ± 0.41</td>
</tr>
<tr>
<td>PD-NA</td>
<td>8</td>
<td>132.2 ± 2.81</td>
<td>6.1 ± 0.24</td>
</tr>
<tr>
<td>CD-A</td>
<td>11</td>
<td>144.5 ± 3.17†</td>
<td>6.3 ± 0.34</td>
</tr>
<tr>
<td>CD-NA</td>
<td>9</td>
<td>134.4 ± 2.62</td>
<td>6.1 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. PD-A and PD-NA, peritoneal dialyzed rats with and without access to 2% NaCl, respectively; CD-A and CD-NA, control dialyzed rats with and without access to 2% NaCl, respectively. *Significantly different from CD-NA and PD-NA groups.

Double-immunolabeling in the raphe system. The number of double-labeled cells (Fos-5-HT) found in the different nuclei of the raphe system is shown in Fig. 4. Most of the serotonergic Fos-ir neurons in the DR were localized in the DRVL and DRD subdivisions. Photomicrographs (Fig. 5, A, B, and F) show double-immunolabeled neurons (arrowheads) in the DRVL.

There was a significantly lower number of Fos-5-HT cells along the DR and MnR in PD-NA compared with animals in normal sodium balance (CD-NA and CD-A) or in the process of reaching this homeostatic balance through ingesting 2% NaCl (i.e., PD-A group). An
ANOVA indicated that there was a significant difference in the number of Fos-5-HT-ir neurons along the DR and MnR related to both factors analyzed, i.e., depletion vs. sham depletion (DR: $F_{1,11} = 13.96, P < 0.01$; MnR: $F_{1,11} = 15.15, P < 0.01$) and access to 2% NaCl vs. no access (DR: $F_{1,11} = 11.39, P < 0.01$; MnR: $F_{1,11} = 51.84, P < 0.0001$). The percentage of Fos-5-HT cells in relation to the total number of 5-HT-ir neurons found in the sections analyzed is shown in Table 2, and it indicated that the PD-NA group had a lower percent-

Fig. 1. Photomicrographs of coronal sections of the anterior level of the dorsal raphe nucleus (DR) showing the Fos-immunoreactive (ir) neurons after different treatments: peritoneal dialysis with access to 2% NaCl (PD-A group; A), peritoneal dialysis with no access to 2% NaCl (PD-NA group; B), control dialysis with access to 2% NaCl (CD-A group; C), and control dialysis with no access to 2% NaCl (CD-NA group; D). Bars, 100 μm. DRD, DRV, and DRVL, dorsal, ventral, and ventrolateral subdivisions of the DR, respectively.

Fig. 2. Photomicrographs of coronal sections of the medial level of the DR showing the Fos-ir neurons after different treatments: PD-A (A), PD-NA (B), CD-A (C), and CD-NA (D). Bars, 100 μm. See Fig. 1 for group descriptions.
age of Fos-5-HT cells along the DR and MnR compared with the other three groups of animals.

Spontaneous and induced sodium ingestion of the CD-A and PD-A animals, respectively, showed significant increases in the number of Fos-5-HT cells in the RMg and the RPa groups above the levels found in the groups of animals that did not have access to NaCl. In both nuclei, an ANOVA revealed a significant difference in the number of Fos-5-HT-ir cells that was related to the sodium intake test (RMg: $F_{1,10} = 33.65, P < 0.001$; RPa: $F_{1,9} = 42.89, P < 0.0001$).

**Fos-ir and double-immunolabeling in the AP.** For the AP, only the PD-A group showed a significant increase in the number of Fos-ir and Fos-5-HT-ir cells compared with the other three groups of animals (Figs. 3 and 4). The photomicrographs of Fig. 5, C, D, and G, show double-immunolabeled neurons (arrowhead) in the AP. An ANOVA for the number of Fos-ir cells indicated that there was a significant interaction between both factors analyzed, i.e., depletion vs. sham depletion and access to 2% NaCl vs. no access ($F_{1,12} = 13.0; P < 0.01$). Furthermore, in the case of the number of Fos-5-HT-ir cells in the AP, the ANOVA also indicated that there was a significant interaction associated with both factors ($F_{1,12} = 8.74; P < 0.01$).

**DISCUSSION**

The results of our experiments indicate that the activity of cells in hindbrain serotonergic nuclei is related to body sodium status. Fos expression in many of these cells was decreased when the animals were sodium depleted and increased when the animals were either in near normal sodium balance (CD-NA and CD-A) or were in the process of restoring sodium ho-
meostasis by ingesting a 2% solution of NaCl (i.e., PD-A; Fig. 4; Table 2). The proportion of activated serotonergic cells in relation to all activated neurons and to all serotonergic cells in control and depleted animals before and after they drink sodium indicates that non-5-HT cells in these structures are also activated by the ingestion of sodium (Figs. 3 and 4; Table 2).

Previous work from our laboratory indicates that after sodium depletion by PD, plasma and cerebrospinal fluid sodium levels decrease rapidly, particularly during the first 4 h after PD. This reduction is followed by a period of slow recovery and reaches baseline levels by ~24 h. In addition, the dialyzed animals show a significant decrease in the blood volume immediately after PD that returns to control values 12 h later (5, Table 2).

Table 2. Number of total 5-HT-ir neurons and the percentage of double-immunolabeled cells in sections of three nuclei of the raphe system

<table>
<thead>
<tr>
<th>Group</th>
<th>Dorsal Raphe</th>
<th>Median Raphe</th>
<th>Raphe Pallidus</th>
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<tbody>
<tr>
<td></td>
<td>Anterior Level</td>
<td>Medial Level</td>
<td>Anterior Level</td>
</tr>
<tr>
<td></td>
<td>5-HT-ir neurons</td>
<td>% of Fos-5-HT cells</td>
<td>5-HT-ir neurons</td>
</tr>
<tr>
<td>PD-A</td>
<td>446.7 ± 61.72</td>
<td>5.99</td>
<td>312.0 ± 68.08</td>
</tr>
<tr>
<td>PD-NA</td>
<td>477.5 ± 47.79</td>
<td>1.84</td>
<td>377.2 ± 34.95</td>
</tr>
<tr>
<td>CD-A</td>
<td>488.5 ± 45.63</td>
<td>6.55</td>
<td>353.0 ± 60.81</td>
</tr>
<tr>
<td>CD-NA</td>
<td>506.2 ± 34.77</td>
<td>5.16</td>
<td>418.5 ± 29.98</td>
</tr>
</tbody>
</table>

Values are means ± SE. 5-HT, serotonin; ir, immunoreactive. See Table 1 for description of groups.

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In the present work, plasma sodium concentration of the PD-NA group reached the same level of its respective control group (CD-NA). Both the PD-NA and CD-NA groups had significantly lower plasma sodium concentrations compared with animals with access to NaCl. Therefore, the activity of the raphe serotonergic neurons does not appear to be directly correlated with changes in plasma sodium concentration because only the Fos-5-HT-ir cells of the PD-NA group were significantly decreased compared with the other three groups of animals (Fig. 4; Table 1). In other words, a change in plasma sodium levels is not a condition associated with increased activity in raphe 5-HT cells.

When 5-HT neuronal cell groups were first described in the brain stem, Dahlström and Fuxe (8) introduced a coding system to identify the major groupings of cell bodies. Following this system for nomenclature, the major clusters of 5-HT cells found in the rat brain stem were numbered first caudally and proceeding rostrally, as the B1–B9 cell groups. Although there are some exceptions, the B cell groups mostly overlap with major raphe nuclei. In addition to the major B1–B9 cell groups, there are other smaller groups located in other structures. The AP contains one of the smaller 5-HT groups (8, 13). The DR (B-6, B-7), MnR (B5, B8), and RPa (B-1; see Ref. 41 for discussion) studied in the present experiments are collectively referred to in the following discussion as the raphe system and the AP as one of the distinct nonraphe groups.

Animals with electrolytic lesions of the DR or depletion of 5-HT (from all serotonergic neurons) by central administration of p-chlorophenylalanine (ANP) release both under resting conditions and after volume expansion (33). Reis and colleagues (33) suggested that serotonergic neurons in the raphe system tonically inhibit ANP neurons. This pathway is proposed to control tonic ANP release and to inhibit water intake (33). Sodium intake was not evaluated in the experiments by Reis et al. (33), but because central ANP inhibits both water and sodium intake, it is possible that DR lesions might also reduce an inhibitory influence on sodium appetite. Our results are consistent with the idea that there is tonic inhibition of sodium appetite by serotonergic cells of the raphe system. Such an inhibitory influence would probably be reduced in the state of sodium deficiency and increased when the animals ingest NaCl and restore body sodium balance. This system is therefore likely to prevent excess NaCl and/or water consumption and reduce the possibility of excess extracellular volume expansion.

In the case of the AP, only peritoneal dialysis-induced sodium consumption (i.e., in the PD-A group) was associated with enhanced expression of c-fos (Fig. 3). The PD-A group that showed greater Fos activation in all cell types also showed a significantly greater number of double-immunolabeled cells for both 5-HT and Fos (Figs. 4 and 5). These data are consistent with previous studies showing that the increase in c-fos expression in the AP after stimulated sodium ingestion was correlated with the activation of local osmo-/sodium-sensitive cells or the arrival of synaptic signals conveying information about body fluid status (12). The AP may be influenced by afferent inputs from the NTS, which is a major relay site for taste and viscerosensory inputs from NaCl-associated gustatory input, hepatic and renal osmo- and/or sodium receptors, and cardiovascular baroreceptors (4, 25). Sodium depletion per se does not significantly elevate c-fos expression in the AP or the NTS (12, 15). The fact that Fos-ir was significantly increased in both nuclei after stimulated sodium ingestion (12, 15) may be the result of either orosensory stimulation produced by 2% NaCl, the postingestive consequences (e.g., stimulation of gastric, hepatopetal, or cardiopulmonary baroreceptors), or changes in systemic sodium plasma concentration. Further experiments are necessary to distinguish among these possibilities.

Our results are consistent with lesion and pharmacological evidence suggesting that there is an inhibitory hindbrain circuit controlling sodium intake (6, 7, 9, 16, 23, 34; see Ref. 18 for review). This proposed hindbrain network involves the AP/mNTS and LPBN. Increased NaCl consumption that is observed after lesions centering on the AP (7, 9) may be the result of damage of osmo- or sodium receptors or associated elements located in the AP/mNTS. AP neurons are responsive to hypertonic NaCl applied locally (1), and AP neurons also receive afferent signals from sodium receptors located in the hepatic portal vein (2, 25, 39). Thus the AP, which lacks a blood-brain barrier, may sense changes in blood or cerebrospinal fluid sodium concentration while also receiving neural viscerosensory information from the periphery. Both types of inputs may contribute to the modulation of sodium appetite. In this light, the present work might be interpreted as identifying serotonergic neurons located in the AP that are activated by sodium ingestion and that may release 5-HT into other brain regions such as the LPBN (18).

An early report by Lança and van der Kooy (19) describes a prominent serotonergic pathway from 5-HT cells in the AP and mNTS that projects to the LPBN (19). Recent pharmacological studies suggest that ascending serotonergic pathways play an important role in hydromineral homeostasis. Serotonergic inputs to the LPBN may normally exert an inhibitory action on sodium appetite. When drugs that modify serotonergic transmission are injected into the LPBN, sodium appetite can be enhanced or inhibited. For example, injection of methysergide, a nonselective 5-HT1/5-HT2 receptor antagonist, into the LPBN increases the intake of water and NaCl induced by intracerebroventricular ANG II and by various systemic treatments such as furosemide combined with captopril. On the other hand, bilateral injections of (±)2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI), a 5-HT2 receptor agonist, into the LPBN produce inhibition of sodium appetite (22, 23; see Ref. 18 for review).

Modulation of activity in the raphe nuclei and AP may be mediated, in part, through defined reciprocal connections with the NTS (36, 40), which receives gus-
tatory and viscerosensory inputs. Serotonergic pathways from the raphe system project not only to the LPBN (32, 40) but also to forebrain structures such as the paraventricular nucleus of the hypothalamus (35, 42), the central nucleus of the amygdala (21), the subfornical organ, and the organum vasculosum of the lamina terminalis (3). All of these rostral areas have been implicated in sodium appetite regulation (see Refs. 17 and 18 for reviews). Systemic dexfenfluramine, a drug that globally enhances serotonergic transmission, inhibited spontaneous and depletion-induced NaCl intake without affecting water intake, whereas the nonspecific 5-HT antagonist metergoline produced significant increases in these responses (34). These observations suggest that 5-HT may have a very general inhibitory effect on NaCl intake. This interpretation is further supported by previous results showing that sodium loading of rats 24 h after peritoneal dialysis-induced sodium depletion had significantly increased hypothalamic levels of 5-HT and 5-hydroxydoleacetic acid (26). Thus these results suggest that an increase in the synthesis and release of 5-HT may occur in peritoneal dialysis sodium-depleted rats once they drink NaCl. Thus, in light of the anatomic and functional evidence and the present results, it is reasonable to postulate the presence of serotonergic pathways with cell bodies in the raphe nuclei and in the AP that project to the LPBN as well as forebrain structures (paraventricular nucleus, central nucleus of the amygdala, CVOs) and that these act to exert both tonic and phasic inhibitory tone in the control of NaCl intake.

Perspectives

The present findings suggest that 5-HT neurons in nuclei of the raphe system and in the AP change their activity as a function of the status of body sodium and when animals are in the process of restoring body sodium homeostasis by ingesting salt. The results are consistent with the idea that under conditions of satiety, the raphe 5-HT system tonically acts to inhibit sodium intake and that the 5-HT neurons in the raphe system and in the AP as well are activated in the process of sodium ingestion to phasically increase inhibitory control and thereby limit excess NaCl intake.

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