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Differential Changes in Central Monoaminergic Metabolism During First and Multiple Sodium Depletions in Rats

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FRANKMANN, S. P., L. BRODER, J. H. DOKKO AND G. P. SMITH. *Differential changes in central monoaminergic metabolism during first and multiple sodium depletions in rats.* PHARMACOL BIOCHEM BEHAV **47**(3) 617–624, 1994.—To investigate the role of central monoamines in the behavior of sodium appetite, serotonergic and dopaminergic metabolism in regions of the forebrain and dorsal hindbrain were measured in sodium-depleted rats. Male Sprague–Dawley rats were sodium depleted by injection of the diuretic-natriuretic drug, Lasix (furosemide, 10 mg), and maintained on a sodium-deficient diet overnight. Rats were tested at a first sodium depletion or after multiple sodium depletions. There were three test conditions: 1) mock depletion consisted of vehicle injection and overnight sodium-deficient diet; 2) sodium depletion alone consisted of Lasix injection and overnight sodium-deficient diet; and 3) intake of NaCl following sodium depletion consisted of 9-min access to 0.3 M NaCl after Lasix injection and overnight sodium-deficient diet. Dopamine (DA), DOPAC, HVA, 5-HT, and 5-HIAA were measured by HPLC in the olfactory tubercle, amygdala-pyriform lobe, n. accumbens, hypothalamus, caudate nucleus, and the dorsal hindbrain. Ingestion of 0.3 M NaCl for 9 min after the first sodium depletion increased 5-HIAA/5-HT ratio significantly in the n. accumbens and amygdala, and increased DOPAC/DA ratio in the n. accumbens and olfactory tubercle compared to the mock treatment. In contrast to the results after the first sodium depletion, rats that ingested 0.3 M NaCl after four or more sodium depletions had no changes in 5-HT and DA metabolism in the forebrain, but they did have a significantly larger 5-HIAA/5-HT ratio in the dorsal hindbrain than mock-treated rats. These results suggest that release of DA and 5-HT in the limbic forebrain regions may be important for the behavioral and neuroendocrine responses to the initial sodium depletion, but not to subsequent depletions. Alternatively, the changes in monoamine metabolism after the first sodium depletion may be due to the stress of that sodium depletion.

Sodium appetite Salt hunger Dopamine Serotonin Stress

WHEN rats are depleted of sodium, they increase their intake of sodium chloride (NaCl) (12). This appetite for NaCl appears to involve the central effects of angiotensin and aldosterone (11), and possibly some central inhibitory system mediated in part by oxytocin (15). After multiple depletions of sodium, intake of NaCl increases further (12). The central mechanisms that underlie the enhanced intake of NaCl after multiple depletions are not yet understood.

Despite the current interest in the role of central monoamines in feeding and drinking behavior, relatively little work has been done to analyze their role in sodium appetite. Using in vivo microdialysis, Chang et al. (2) measured an increased release of dopamine (DA) in the nucleus accumbens (n. accumbens) when sodium-depleted rats drank 2% NaCl after several sodium depletions. Other DA terminal fields were not investigated. More recently, Gentili et al. (7) demonstrated that the serotonergic antagonists, ketanserin and ritanserin, decreased the intake of NaCl elicited by administration of the mineralocorticoid, deoxycorticosterone acetate. Ketanserin also decreased intake of NaCl in rats that had been acutely depleted of sodium three times by administration of the natriuretic-diuretic drug, furosemide. Because the increased intake of NaCl after furosemide depends on a mineralocorticoid, aldosterone, Gentili et al. (7) suggested that central or peripheral serotonin (5-HT) receptors (5-HT_{1C} or 5-HT_2) are involved in the stimulation of NaCl intake by mineralocorticoids.

Because sodium depletion increases the acceptance of and

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preference for concentrated NaCl solutions, the experiments of Cooper and his colleagues (4,5) may also be relevant. They studied the intake of several concentrations of NaCl by rats after 22 h of water deprivation, a condition in which rats drink water, hypotonic or isotonic NaCl avidly, but drink little or no hypertonic NaCl. Administration of a 5-HT_{1A} agonist, 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) or gepirone, increased intake of hypertonic NaCl (1.8% and 2.7%), but did not increase intake of isotonic or hypotonic (0.45%) NaCl or water (4,5). In related experiments, Gilbert and Cooper showed that administration of SCH 23390, a dopaminergic D_1 antagonist, increased the preference for 1% NaCl and eliminated the aversion for 2.5% NaCl in comparison to water (8). The complementary result was also obtained—administration of SKF 38393, a dopaminergic D_1 agonist, decreased preference for 1% and 2.5% NaCl relative to water. These results suggest that decreased DA activity at D_1 receptors increases the preference for hypertonic NaCl solutions.

To investigate the problem further, we measured serotonergic and dopaminergic metabolism in regions of the forebrain and in the dorsal hindbrain of rats with an appetite for sodium produced by administration of furosemide at a first episode of sodium depletion and following several episodes of sodium depletion. A portion of these data has appeared in preliminary form (6).

METHOD

General

The subjects were male, Sprague–Dawley rats (Taconic Farms, Germantown, NY) weighing 250–500 g. They were individually housed in hanging stainless steel cages in a temperature-controlled room with a 12 L : 12 D cycle. Purina Chow and water were freely available unless otherwise noted.

Sodium Depletion

The rats were sodium depleted by injection of the diuretic-natriuretic drug, Lasix (furosemide), and removal of ambient sodium. Each rat received two injections of Lasix (5 mg/0.5 ml, SC) or the vehicle (0.15 M NaCl). The second injection was given 2 h after the first injection. At the time of the first injection, Purina pellets were replaced with a pelleted, sodium-deficient diet (BioServe AIN-76A). Overnight body weight and food and water intakes were measured. Rats were tested with one of two histories of sodium depletion: no previous episodes of sodium depletion or multiple previous episodes of sodium depletion (four to seven times).

Depletion Conditions

Forty-eight first depletion rats (n = 16/condition) and 36 multiple depletion rats (n = 12/condition) were assigned to one of three conditions: 1) mock depletion (M) consisted of vehicle injections and access to the sodium-deficient diet and water until tested; 2) sodium depletion alone (D) consisted of the two Lasix injections and overnight maintenance on sodium-deficient diet and water; and 3) intake of NaCl following sodium depletion (N) consisted of 9-min access to 0.3 M NaCl after two Lasix injections and overnight maintenance on sodium-deficient diet and water. For each rat in the N condition, the NaCl was available for 9 min from the time of the first lick as observed by the experimenter. Thus, the N group was the full experimental group, the D group provided a control for sodium depletion without ingestion of 0.3 M NaCl, and the M group provided a control for the nonspecific effects of two SC injections and the sodium-deficient diet.

Regional Brain Dissection

After decapitation with a guillotine, the brain of a rat was carefully removed from the skull and transferred to a chilled operating stage where regional dissections were performed with the aid of magnification. The regions obtained were olfactory tubercle, amygdala-pyriform lobe, n. accumbens, hypothalamus, caudate nucleus, and the dorsal hindbrain.

The brain was turned over so that it rested on its dorsal cortical surface. The olfactory tubercles were pinched off the base of the brain with fine microdissection forceps.

The caudate and n. accumbens were dissected from an ~2-mm slice of brain, the rear surface of which was in the frontal plane just anterior to the optic chiasm.

The hypothalamic tissue block was approached by removing the optic nerves and optic chiasm, and the meningeal tissue. Then an incision was made with a fine scissors along the medial edge of both optic tracts. This incision was extended posteromedially along the caudal edge of the mammillary bodies. The anterior face of the hypothalamic block was made by a frontal cut at the level of the optic chiasm. The dorsal boundary of the hypothalamic block was made by a horizontal incision at the dorsal edge of the fornix.

The brain was next placed on its lateral surface so that the pyriform lobe was accessible. The anterior cut of this tissue block was made in the frontal plane where the lateral olfactory tract enters the ventral surface of the brain at the level of the optic chiasm. The posterior cut was made in the frontal plane of the posterior edge of the mammillary body. Both the anterior and posterior cuts extended from the rhinal sulcus down to the lateral edge of the optic tract. A cut was made along the lateral edge of the optic tract to connect the anterior and posterior cuts. Then the block of pyriform lobe containing the amygdaloid nuclei, the entorhinal cortex, and the primary olfactory cortex was separated from the brain by dissecting along the rhinal sulcus and peeling the lobe out and down. This tissue is referred to as amydala throughout this paper. This dissection was done in a way that excluded the ventral portion of the hippocampus. Only one block of the pyriform lobe was removed for neurochemical analysis from each rat because preliminary experiments failed to measure any difference in monoamine metabolism between the right and left pyriform lobes.

Frontal sections of dorsal hindbrain were made at the level of the obex and ~2 mm anterior. Then the lower half of this section was dissected away and discarded. The sample tissue included the area postrema, nucleus of the solitary tract, dorsal motor nucleus of the vagus nerve, and other structures as defined in Figures 39–43 in Paxinos and Watson (10).

Sample Preparation

The tissue samples were wrapped in aluminum foil, weighed, and then frozen in dry ice. A homogenization solution, prepared on the day of the dissection, consisted of 0.1 N perchloric acid (Fisher), 0.008 M L-cysteine free base (Sigma), 1.0 mM EDTA (Fisher), and 0.025 ng/μl DHBA (Sigma), the internal standard. Homogenization solution (300–400 μl) was added to each tissue sample contained in a 4-ml polypropylene culture vial.

The tissue samples were homogenized by sonification with a Branson Sonifier 250 (Branson Ultrasonics Corp., Danbury, CT) and the homogenates were centrifuged for 20 min at

13,000 rpm and 4°C in a Dupont Sorvall RC-5 superspeed refrigerated centrifuge (Dupont Medical Instruments, Newtown, CT). Aliquots of the supernatants (from 300-400 μl) were placed into limited-volume inserts that were inserted into WISP autosampler vials (Sun Brokers, Wilmington, NC). The capped vials were stored at −80°C until HPLC analysis. The pellets from the centrifugation were saved and frozen at −80°C until a protein assay (1) was performed (Bio-Rad Laboratories, Rockville Centre, NY).

HPLC Neurochemical Measurements

The HPLC-ECD system consisted of a Waters model 510 pump (Waters, Milford, MA), a Waters WISP 710b autoinjector, and a BAS LC-4B amperometric electrochemical detector with a LC-22A temperature controller (Bioanalytical Systems, West Lafayette, IN). A glass carbon electrode (MF-1000) was utilized with an oxidative potential set at +750 mV against a Ag/AgCl reference electrode (RE-4). Full scale sensitivity was set from 2–5 nA with an active filter of 0.03 Hz.

The LC-17A flowcell was housed in a BAS CC-4 cell compartment with a BAS cell preheater module (EW-4055) maintaining the temperature of the incoming mobile phase at a constant 36°C. The analytical column was a Micro-Bondapak C18 10-μm cartridge installed in an 8 × 100 mm radial compression module (Waters, Milford, MA). Prior to the column, each system was fitted with a Waters Guard-Pak precolumn module containing a Micro-Bondapak C18 precolumn cartridge.

The mobile phase consisted of 0.06 M citric acid monohydrate, 0.04 M sodium phosphate monobasic, 1.0 mM EDTA, 0.55 mM 1-octanesulfonic acid sodium salt (Eastman Kodak, Rochester, NY), and 15% methanol by volume (Baker Analyzed, HPLC grade, J. T. Baker Co., Phillipsburg, NJ) with pH adjusted to 3.2 with 10 N sodium hydroxide. HPLC grade water was obtained from Baxter Healthcare Corp., McGraw Park, IL.

All values of monoamines and metabolites were determined by peak-height analysis by a Waters 730 data module and are reported as ng per mg protein. Dopamine metabolism was estimated by the ratios of dihydroxyphenylacetic acid to DA (DOPAC/DA) and homovanillic acid to DA (HVA/DA). Serotonin metabolism was estimated by the ratio of 5-hydroxyindoleacetic acid to 5-HT (5-HIAA/5-HT).

Statistical Analysis

The results of the first-depletion and multi-depletion groups were analyzed separately. Because there were no significant differences correlations between number of sodium depletions and changes in brain neurochemistry measurements, results of the multi-depleted rats were pooled. The neurochemical values for each region were analyzed by one-way ANOVA with experimental treatment (M, D, and N) as the factor. When a significant difference was found, a post hoc comparison of the treatments was made using Duncan's Multiple Range Test.

RESULTS

First Sodium Depletion

Ingestion of 0.3 M NaCl for 9 min (7.8 ± 0.7 ml, mean ± SE) after the first sodium depletion increased the 5-HIAA/5-HT ratio significantly in the n. accumbens and in the amygdala compared to mock treatment (Table 1). The significant increase of 5-HIAA/5-HT was due to a decrease of 5-HT and a small increase in 5-HIAA that were not statistically different from those of mock-treated rats (Table 1).

The 5-HIAA/5-HT ratio in the n. accumbens of rats that were sodium depleted, but not allowed to ingest 0.3 M NaCl (D), was also significantly larger than the ratio of mock-treated rats (Table 1). Note that sodium depletion alone increased the 5-HIAA/5-HT in n. accumbens as much as sodium depletion plus ingestion of 0.3 M NaCl (compare D and N, Table 1). Thus, the increase of 5-HT metabolism in the n. accumbens is primarily correlated with sodium depletion rather than with ingestion of 0.3 M NaCl.

Ingestion of 0.3 M NaCl after the first sodium depletion also increased the DOPAC/DA ratio significantly in the n. accumbens and olfactory tubercle compared to mock treatment, but not in comparison to rats that were sodium depleted but not permitted to ingest 0.3 M NaCl (Table 2). The significant increase of DOPAC/DA was the result of a decrease of DA that was not statistically significant combined with a smaller decrease of DOPAC (Table 2). Note that in contrast to the 5-HIAA/5-HT, the increase of DA metabolism in the n. accumbens was primarily correlated with ingestion of 0.3 M NaCl and not with sodium depletion alone.

There was no significant change of HVA/DA in any brain region sampled. The small number of n. accumbens samples (due to the lack of detectable concentrations of HVA in most samples) makes the measurement in this area uncertain.

Both sodium-depleted groups of rats (N and D) lost significantly more weight than mock-treated rats (Table 3). This was probably due to the natriuresis and diuresis produced by furosemide, although the overnight food intakes of these two groups was also significantly less.

Multiple Sodium Depletions

Rats that underwent multiple sodium depletions had different and less widespread changes in central 5-HT and DA metabolism than was observed in rats after the first sodium depletion. The only significant change in 5-HIAA/5-HT occurred in the dorsal hindbrain, where the ratio was significantly larger in rats that drank 0.3 M NaCl (8.3 ± 0.6 ml) after sodium depletion than in mock-treated rats (Table 4). Note that the 5-HIAA/5-HT ratio of the group allowed to ingest NaCl was not significantly larger than that of rats that were sodium depleted but not permitted to drink 0.3 M NaCl (Table 4).

No significant changes of DOPAC/DA or of HVA/DA occurred in any of the brain regions (Table 5). The significant increases of HVA in n. accumbens of D rats and in olfactory tubercle of the two groups of sodium-depleted rats (D, N) were correlated with smaller, nonsignificant increases of DA in these rats so that HVA/DA did not change significantly.

As in the first experiment, both groups of sodium-depleted rats lost significantly more weight and ate significantly less food than the mock-treated rats (Table 3). There were no significant correlations between number of sodium depletions and changes in brain neurochemistry measurements.

DISCUSSION

Ingestion of 0.3 M NaCl after the first depletion of sodium by furosemide was associated with increased metabolism of 5-HT (5-HIAA/5-HT) in n. accumbens and amygdala, and with increased metabolism of DA (DOPAC/DA) in n. accumbens and olfactory tubercle (Tables 1 and 2). The significant increase of 5-HIAA/5-HT in n. accumbens was due to the state of sodium depletion alone because it increased as much

TABLE 1

SEROTONIN METABOLISM AFTER THE FIRST SODIUM DEPLETION

Region	Treatment	5-HT	5-HIAA	5-HIAA/5-HT
N. accumbens	M	1.56 ± 0.25 (14)	1.16 ± 0.17 (14)	0.76 ± 0.03 (14)
	D	1.16 ± 0.13 (13)	1.01 ± 0.12 (13)	0.88 ± 0.03* (13)
	N	1.47 ± 0.21 (15)	1.28 ± 0.17 (15)	0.91 ± 0.05* (15)
Amygdala-piriform cortex	M	0.33 ± 0.05 (16)	0.25 ± 0.04 (16)	0.77 ± 0.03 (16)
	D	0.29 ± 0.04 (16)	0.25 ± 0.04 (16)	0.84 ± 0.04 (16)
	N	0.27 ± 0.03 (16)	0.26 ± 0.03 (16)	0.94 ± 0.04* (16)
Olfactory tubercle	M	1.67 ± 0.18 (16)	0.80 ± 0.08 (16)	0.49 ± 0.02 (16)
	D	1.85 ± 42 (16)	0.87 ± 0.15 (16)	0.50 ± 0.02 (16)
	N	1.46 ± 0.17 (16)	0.72 ± 0.09 (16)	0.50 ± 0.02 (16)
Caudate	M	0.24 ± 0.04 (15)	0.29 ± 0.04 (15)	1.35 ± 0.13 (15)
	D	0.26 ± 0.03 (15)	0.36 ± 0.04 (15)	1.45 ± 0.09 (15)
	N	0.23 ± 0.03 (15)	0.32 ± 0.04 (15)	1.56 ± 0.14 (15)
Hypothalamus	M	0.37 ± 0.05 (16)	0.36 ± 0.04 (16)	0.96 ± 0.04 (16)
	D	0.36 ± 0.04 (16)	0.37 ± 0.04 (16)	1.04 ± 0.05 (16)
	N	0.34 ± 0.05 (16)	0.35 ± 0.04 (16)	1.07 ± 0.05 (16)
Dorsal hindbrain	M	0.89 ± 0.11 (16)	0.70 ± 0.10 (16)	0.81 ± 0.04 (16)
	D	0.75 ± 0.09 (16)	0.58 ± 0.06 (16)	0.83 ± 0.04 (16)
	N	0.85 ± 0.08 (16)	0.72 ± 0.07 (16)	0.84 ± 0.03 (16)

Data are mean ± SEM for 5-HT, 5-HIAA (ng/mg protein), and the ratio of 5-HIAA/5-HT following mock sodium depletion (M), sodium depletion without NaCl ingestion (D), and sodium depletion and NaCl ingestion for 9 min (N). The number in parentheses is the number of brians from which the mean value was calculated.

*$p < 0.05$ compared to M by Duncan's Multiple Range Test.

in rats depleted of sodium as in rats depleted of sodium and allowed 9 min of NaCl intake (Table 1). Although ingestion of 0.3 M NaCl was necessary to produce the other significant increases of 5-HIAA/5-HT and DOPAC/DA, it should be noted that none of the ratios measured after sodium depletion and 0.3 NaCl ingestion were significantly larger than after sodium depletion alone (compare N and D, Tables 1 and 2).

Increased DA and 5-HT metabolism is often correlated with increased release of DA and 5-HT, respectively. Thus, the increased DOPAC/DA we measured in n. accumbens is consistent with the preliminary report of Chang et al. (2) of increased release of DA in the n. accumbens during sodium repletion. However, in the report of Chang et al., the rats had been depleted of sodium several times prior to the measurement of DA release in the n. accumbens. In our experiment, significant changes were detected in first- but not multiple-depleted rats.

In contrast to the results after the first sodium depletion, rats that ingested 0.3 M NaCl after four or more sodium depletions had no changes in 5-HT and DA metabolism in the forebrain, but they did have a significantly larger 5-HIAA/5-HT in the dorsal hindbrain than mock-treated rats (compare N and M, Table 3). These results suggest that the release of DA and 5-HT in the limbic forebrain regions may be important for the behavioral and neuroendocrine responses to the initial sodium depletion, but not to subsequent depletions. Alternatively, the changes of monoamine metabolism observed after the first sodium depletion may be due to the stress of that sodium depletion. It is also possible that there are small increases of DA or 5-HT metabolism after multiple depletions of sodium, but we did not detect them because of the dissection and neurochemical techniques that we used.

The increased 5-HIAA/5-HT observed in the dorsal hindbrain of rats that ingested 0.3 M NaCl after multiple sodium

TABLE 2

DOPAMINE METABOLISM AFTER THE FIRST SODIUM METABOLISM

Region	Treatment	DA	DOPAC	DOPAC/DA	HVA	HVA/DA
N. accumbens	M	6.18 ± 1.33 (14)	1.44 ± 0.23 (14)	0.25 ± 0.02 (14)	0.52 ± 0.06 (4)	0.09 ± 0.01 (4)
	D	4.10 ± 1.00 (12)	1.05 ± 0.25 (12)	0.28 ± 0.02 (12)	0.54 ± 0.14 (4)	0.10 ± 0.02 (4)
	N	4.35 ± 0.86 (15)	1.21 ± 0.22 (15)	0.33 ± 0.03* (15)	0.45 ± 0.11 (2)	0.12 ± 0.01 (2)
Amygdala-piriform cortex	M	0.40 ± 0.11 (16)	0.09 ± 0.02 (16)	0.25 ± 0.02 (16)	0.04 ± 0.01 (5)	0.15 ± 0.01 (5)
	D	0.34 ± 0.07 (16)	0.08 ± 0.02 (16)	0.24 ± 0.01 (16)	0.05 ± 0.01 (5)	0.19 ± 0.02 (5)
	N	0.39 ± 0.08 (16)	0.10 ± 0.02 (16)	0.27 ± 0.02 (16)	0.05 ± 0.01 (7)	0.18 ± 0.01 (7)
Olfactory tubercle	M	13.24 ± 1.73 (16)	2.16 ± 0.25 (16)	0.17 ± 0.01 (16)	0.61 ± 0.05 (12)	0.05 ± 0.00 (12)
	D	14.87 ± 4.06 (16)	2.51 ± 0.56 (16)	0.18 ± 0.01 (16)	0.63 ± 0.07 (14)	0.06 ± 0.00 (14)
	N	10.83 ± 1.28 (16)	2.13 ± 0.26 (16)	0.20 ± 0.01* (16)	0.69 ± 0.08 (16)	0.07 ± 0.00 (16)
Caudate	M	3.39 ± 0.57 (15)	0.72 ± 0.10 (15)	0.24 ± 0.03 (15)	0.35 ± 0.03 (15)	0.12 ± 0.01 (15)
	D	4.75 ± 0.79 (15)	1.03 ± 0.19 (15)	0.22 ± 0.02 (15)	0.55 ± 0.09 (15)	0.12 ± 0.01 (15)
	N	4.02 ± 0.73 (15)	1.02 ± 0.17 (15)	0.28 ± 0.03 (15)	0.52 ± 0.09 (15)	0.15 ± 0.01 (15)
Hypothalamus	M	0.22 ± 0.03 (16)	0.06 ± 0.01 (16)	0.28 ± 0.02 (16)	–	–
	D	0.20 ± 0.03 (16)	0.06 ± 0.01 (16)	0.29 ± 0.01 (16)	–	–
	N	0.19 ± 0.03 (16)	0.05 ± 0.01 (16)	0.29 ± 0.02 (16)	–	–
Dorsal hindbrain	M	0.22 ± 0.06 (16)	0.15 ± 0.09 (11)	0.53 ± 0.08 (11)	–	–
	D	0.15 ± 0.03 (15)	0.07 ± 0.01 (13)	0.46 ± 0.07 (13)	–	–
	N	0.17 ± 0.02 (16)	0.10 ± 0.02 (13)	0.51 ± 0.08 (13)	–	–

Data are mean ± SEM for DA, DOPAC, HVA (ng/mg protein), and the ratios of DOPAC/DA and HVA/DA following mock sodium depletion (M), sodium depletion without NaCl ingestion (D), and sodium depletion and NaCl ingestion for 9 min (N). The number in parentheses is the number of brains from which the mean value was calculated.

$p < 0.05$ compared to M by Duncan's Multiple Range Test.

TABLE 3

BODY WEIGHT, OVERNIGHT BODY WEIGHT CHANGE, AND FOOD INTAKE

Depletion Cond.	Treatment	Body Weight (g)	Body Weight Change (g)	Food Intake (g)
First	Mock	275.0 ± 12.8	−3.7 ± 1.0	26.4 ± 1.3
	Depletion	278.4 ± 14.9	−15.3 ± 1.5*	20.6 ± 1.9*
	Depletion with NaCl	270.1 ± 9.6	−13.9 ± 1.1*	21.6 ± 1.6*
Multiple	Mock	514.5 ± 24.7	−7.7 ± 3.1	29.4 ± 1.9
	Depletion	507.0 ± 24.5	−26.1 ± 3.0*	21.3 ± 2.7*
	Depletion with NaCl	509.9 ± 22.4	−20.8 ± 1.3*	20.8 ± 1.3*

Data are mean ± SEM. For each depletion condition *$p < 0.05$ v. Mock.

TABLE 4
SEROTONIN METABOLISM AFTER MULTIPLE SODIUM DEPLETIONS

Region	Treatment	5-HT	5-HIAA	5-HIAA/5-HT
N. accumbens	M	2.56 ± 0.40 (11)	1.93 ± 0.26 (11)	0.78 ± 0.05 (11)
	D	2.59 ± 0.45 (10)	2.04 ± 0.28 (10)	0.83 ± 0.07 (10)
	N	2.33 ± 0.48 (11)	1.83 ± 0.39 (11)	0.83 ± 0.07 (11)
Amygdala-piriform cortex	M	0.60 ± 0.05 (12)	0.44 ± 0.04 (12)	0.75 ± 0.05 (12)
	L	0.65 ± 0.08 (12)	0.47 ± 0.04 (12)	0.76 ± 0.05 (12)
	N	0.69 ± 0.08 (12)	0.47 ± 0.05 (12)	0.69 ± 0.02 (12)
Olfactory tubercle	M	2.37 ± 0.25 (12)	0.90 ± 0.05 (12)	0.40 ± 0.03 (12)
	D	2.60 ± 0.28 (12)	1.02 ± 0.07 (12)	0.41 ± 0.03 (12)
	N	2.69 ± 0.42 (12)	0.99 ± 0.09 (12)	0.40 ± 0.03 (12)
Caudate	M	0.43 ± 0.04 (12)	0.52 ± 0.03 (12)	1.28 ± 0.09 (12)
	D	0.38 ± 0.03 (12)	0.48 ± 0.04 (12)	1.32 ± 0.07 (12)
	N	0.37 ± 0.03 (12)	0.49 ± 0.03 (12)	1.39 ± 0.08 (12)
Hypothalamus	M	0.58 ± 0.09 (10)	0.45 ± 0.05 (10)	0.86 ± 0.07 (10)
	D	0.66 ± 0.05 (10)	0.54 ± 0.07 (10)	0.82 ± 0.06 (10)
	N	0.66 ± 0.05 (12)	0.55 ± 0.04 (12)	0.84 ± 0.04 (12)
Dorsal hindbrain	M	1.18 ± 0.08 (12)	0.75 ± 0.06 (12)	0.63 ± 0.02 (12)
	D	1.19 ± 0.10 (12)	0.82 ± 0.07 (12)	0.69 ± 0.03 (12)
	N	1.03 ± 0.08 (12)	0.76 ± 0.07 (12)	0.75 ± 0.04* (12)

Data are mean ± SEM for 5-HT, 5-HIAA (ng/mg protein), and the ratio of 5-HIAA/5-HT following mock sodium depletion (M), sodium depletion without NaCl ingestion (D), and sodium depletion and NaCl ingestion for 9 min (N). The number in parentheses is the number of brains from which the mean value was calculated.

*$p < 0.05$ compared to M by Duncan's Multiple Range Test.

depletions suggests the dorsal hindbrain as a site where ketanserin decreased NaCl intake after the third sodium depletion in the experiments of Gentili et al. (7). Given that the area postrema and the nucleus of the solitary tract have been implicated in the behavior of salt appetite (3,9), the current data suggest serotonin as a neurotransmitter that mediates the behavior. This suggestion requires testing.

Our neurochemical results with 0.3 M NaCl intake after multiple depletions of sodium can also be compared to NaCl intake after multiple water deprivations, the paradigm used by Cooper and his colleagues (4,5) for their pharmacological analysis of serotonergic and dopaminergic effects on the intake of hypertonic salt solutions (see the Introduction). Their results predict that increased intake of 0.3 M NaCl would be correlated with decreased DA and 5-HT metabolism (and release) in at least some of the forebrain regions we sampled, but we did not observe this. It should be noted, however, that the measurement of metabolism by ratio of metabolite to monoamine is less sensitive for measuring decreased metabolism than for measuring increased metabolism.

The pattern of changes in DA and 5-HT metabolism observed in these experiments is different from those we observed in previous experiments with sham feeding of sucrose or corn oil after 17 h of food deprivation or sham drinking of water after 17 h of water deprivation (13,14,16). In these experiments, the same neurochemical measurements were made in the same regions (except that the dorsal hindbrain was not sampled) after 9 min of ingesting 2.5%, 10%, or 40% sucrose, 100% corn oil, or water. Because rats were adapted to the sham drinking until intakes stabilized, these experi-

TABLE 5

DOPAMINE METABOLISM AFTER MULTIPLE SODIUM DEPLETIONS

Region	Treatment	DA	DOPAC	DOPAC/DA	HVA	HVA/DA
N. accumbens	M	12.00 ± 2.18 (10)	2.90 ± 0.30 (10)	0.31 ± 0.06 (10)	0.89 ± 0.13 (6)	0.07 ± 0.00 (6)
	D	14.40 ± 2.46 (10)	3.43 ± 0.36 (10)	0.36 ± 0.10 (10)	1.36 ± 0.14*† (5)	0.07 ± 0.00 (5)
	N	9.43 ± 1.60 (10)	2.58 ± 0.36 (10)	0.32 ± 0.05 (10)	0.90 ± 0.12 (7)	0.08 ± 0.01 (7)
Amygdala-piriform cortex	M	0.73 ± 0.16 (11)	0.16 ± 0.02 (11)	0.24 ± 0.02 (11)	0.09 ± 0.02 (8)	0.11 ± 0.01 (8)
	D	0.80 ± 0.09 (12)	0.17 ± 0.02 (12)	0.21 ± 0.01 (12)	0.10 ± 0.01 (8)	0.10 ± 0.01 (8)
	N	0.74 ± 0.11 (12)	0.14 ± 0.02 (12)	0.19 ± 0.02 (12)	0.09 ± 0.02 (9)	0.11 ± 0.01 (9)
Olfactory tubercle	M	13.88 ± 1.65 (12)	2.41 ± 0.27 (12)	0.18 ± 0.00 (12)	0.47 ± 0.05 (8)	0.04 ± 0.00 (8)
	D	16.16 ± 1.75 (12)	2.71 ± 0.25 (12)	0.17 ± 0.01 (12)	0.71 ± 0.06* (10)	0.05 ± 0.00 (10)
	N	16.72 ± 2.77 (12)	3.15 ± 0.61 (12)	0.18 ± 0.00 (12)	0.74 ± 0.08* (10)	0.05 ± 0.00 (10)
Caudate	M	9.83 ± 0.74 (12)	1.88 ± 0.11 (12)	0.20 ± 0.01 (12)	0.76 ± 0.05 (12)	0.08 ± 0.01 (12)
	D	9.18 ± 1.26 (12)	1.71 ± 0.16 (12)	0.20 ± 0.02 (12)	0.66 ± 0.05 (12)	0.08 ± 0.01 (12)
	N	9.37 ± 0.92 (12)	1.90 ± 0.12 (12)	0.22 ± 0.01 (12)	0.80 ± 0.05 (12)	0.09 ± 0.01 (12)
Hypothalamus	M	0.27 ± 0.02 (10)	0.08 ± 0.01 (10)	0.31 ± 0.03 (10)	0.03 ± 0.01 (3)	0.07 ± 0.01 (3)
	D	0.28 ± 0.02 (10)	0.09 ± 0.01 (10)	0.31 ± 0.02 (10)	0.03 ± 0.00 (3)	0.10 ± 0.02 (3)
	N	0.28 ± 0.03 (12)	0.09 ± 0.01 (12)	0.32 ± 0.03 (12)	0.03 ± 0.01 (2)	0.11 ± 0.03 (2)
Dorsal hindbrain	M	0.15 ± 0.01 (12)	0.06 ± 0.01 (8)	0.35 ± 0.03 (8)	–	–
	D	0.16 ± 0.01† (12)	0.06 ± 0.01 (8)	0.34 ± 0.03 (8)	–	–
	N	0.12 ± 0.01 (11)	0.05 ± 0.00 (6)	0.39 ± 0.06 (6)	–	–

Data are mean ± SEM for DA, DOPAC, HVA (ng/mg protein) and the ratios of DOPAC/DA and HVA/DA following mock sodium depletion (M), sodium depletion without NaCl ingestion (D), and sodium depletion and NaCl ingestion for 9 min (N). The number in parentheses is the number of brains from which the mean value was calculated.

*$p < 0.05$ compared to M, †$p < 0.05$ compared to N by Duncan's Multiple Range Test.

ments are most comparable to the multiple sodium depletion experiment reported here.

Sham intake of 10% or 40% sucrose, but not 5% sucrose, for 9 min increased DOPAC/DA in the hypothalamus (14), and sham drinking of water increased DOPAC/DA in the olfactory tubercle (13). There were no significant changes of DOPAC/DA in other regions. Sham feeding of 100% corn oil did not produce any significant changes of DOPAC/DA (16). Furthermore, no significant changes of 5-HIAA/5-HT occurred in any brain region in any of the experiments. The different patterns of neurochemical changes are apparently due to the interaction of deprivation state and orosensory effects of the substance ingested.

The sodium depletion experiments reported here were conducted under conditions when postingestive signals were allowed following the ingestion of NaCl. In contrast, in the experiments involving sucrose, water, and corn oil intake, postingestive signals were minimized because the ingested fluids drained out of an open gastric cannula. This may also have contributed to the different pattern of neurochemical changes.

Thus, there are two major results of these experiments. First, when rats drank 0.3 M NaCl after a first depletion of sodium, DA and 5-HT metabolism increased significantly in n. accumbens, amygdala, and olfactory tubercle. Second, when rats drank 0.3 M NaCl after multiple depletions of sodium, 5-HT metabolism increased in the dorsal hindbrain, but no significant changes of DA or 5-HT metabolism were measured in the forebrain.

Because none of these neurochemical changes occurred in

similar experiments we performed with sucrose, corn oil, or water, they appear to be characteristic of the interaction of NaCl intake and first or multiple sodium depletions. These results suggest that the release of DA and 5-HT in the limbic forebrain regions may be important for the behavioral and neuroendocrine responses to the initial sodium depletion, but not to subsequent depletions. Alternatively, the changes of limbic forebrain monoamine metabolism observed after the first sodium depletion may be due to the greater stress of that sodium depletion.

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