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Hypothalamic Levels and Utilization of Noradrenaline and 5-Hydroxytryptamine in the Sodium-Depleted Rat

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MUNARO, N. AND E. CHIARAVIGLIO. *Hypothalamic levels and utilization of noradrenaline and 5-hydroxytryptamine in the sodium-depleted rat.* PHARMAC. BIOCHEM. BEHAV. 15(1) 1–5, 1981.—The present report investigated the time course of hypothalamic noradrenaline (NA) and 5-hydroxytryptamine (5-HT) metabolism in male rats with acute sodium depletion by intraperitoneal dialysis (IPD). The hypothalamic level and utilization of NA start to rise significantly 12 to 24 hrs after IPD. In contrast, 24 hrs after IPD, sodium-depleted rats allowed to drink sodium in a half hour drinking test significantly decreased NA steady state and utilization. Electrochemical stimulation of the pre-limbic cortex 5 hrs after IPD significantly increased the utlization but not the concentration of NA, whereas sodium replenishment decreased both of them. The metabolism of hypothalamic 5-HT was not affected 12–24 hrs after sodium depletion or by electrochemical stimulation, while both the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) increased significantly in sodium-depleted rats allowed to drink sodium. These results suggest that sodium deficiency promotes an increased hypothalamic NA utilization while failing to affect 5-HT metabolism.

Noradrenaline Sodium depletion 5-Hydroxytryptamine Sodium load

IT is a well known fact that brain catecholamines (CA) and 5-hydroxytryptamine (5-HT) play an important role in the regulation of consummatory behavior. It has been reported that the central injection of noradrenaline (NA) evoked eating [1,10] and eating and drinking in rats [12]. Moreover, changes in NA and dopamine (DA) levels [2, 14, 20] and modifications of 5-HT turnover [11,17] in specific areas of rat brain have been reported in relation to food intake. The more effective sites responsive to CA injection and in which changes in the levels of monoamines have been seen, were mainly located in the hypothalamus [8,13].

Studying specific sodium appetite in rats Chiaraviglio and Taleisnik [4] have demonstrated that, NA applied into the 3rd ventricle evoked sodium intake in non-deprived rats, whereas, sodium intake induced by acute depletion of body sodium by peritoneal dialysis (p.d.) was inhibited or abolished by alpha adrenergic blockers, noradrenergic stores depletors [4] or CA synthesis inhibitors [5]. All these facts suggest that specific sodium appetite is mediated by a catecholaminergic system. In addition electrochemical stimulation of the medial surface of the frontal cortex at the prelimbic area, induces a significant sodium intake by shortening the latency of appearance of sodium appetite [7].

The aim of the present experiment was to investigate the hypothalamic utilization of NA and 5-HT in rats with sodium deficiency. The effect of electrochemical stimulation of the limbic cortex on the utlization of these neurotransmitters was also studied in sodium depleted rats.

METHOD

Animals

The experiments were performed in male rats weighing 200–400 g housed in a temperature (22°C) and light controlled room (light on from 6 a.m. to 8 p.m.).

Tissue Sampling

The rats were killed by decapitation at 9 a.m. and the brain was quickly removed. The preoptic area and anterior hypothalamus were dissected by a frontal section placed at the rostral border of the optic chiasma and a caudal section at the level of the supraoptic commissure. Lateral sections were made at hypothalamic fissures. This block of tissue, 2 mm thick, weighing 10–12 mg included the periventricular area, preoptic area, paraventricular nucleus and the lateral hypothalamic nucleus.

Sample Analysis

The concentration of noradrenaline (NA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were simultaneously measured by a spectrophotofluorometric assay [3,16]. Following homogenization of the tissue in acid butanol, the monoamines were extracted with solvents and then purified through activated alumina and Dowex 50-WX4. The metabolite, 5-HIAA, was extracted from the organic phase with $NaCo_3H$. The fluorophores of NA, 5-HT

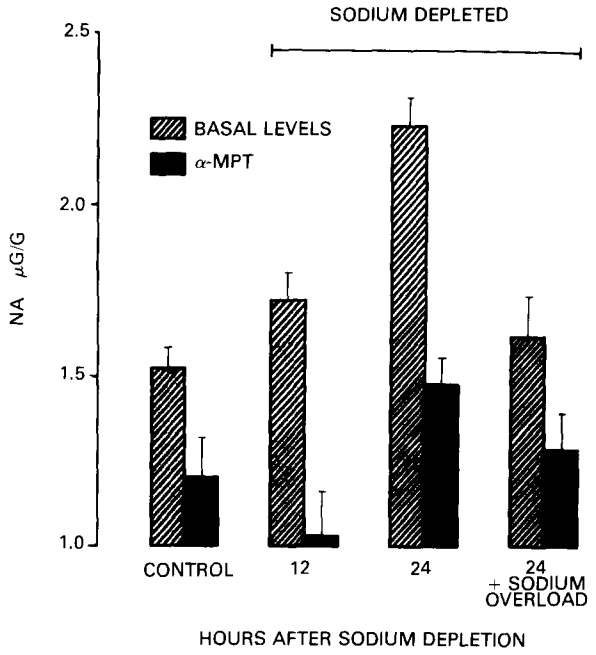

FIG. 1. NA concentration in the hypothalamus of sodium depleted rats before and after α-MPT treatment. Abbreviations: C=control. Ns=not significant. Control NA basal values for rats 12 and 24 hrs after sodium depletion has been grouped in one bar. The same after α-MPT treatment. Each bar represents the mean of six determinations ± SE of mean. Analysis of variance: Time effect $F(1,40)=17.0$, $p<0.001$; Sodium effect, $F(1,40)=27.5$, $p<0.001$; α-MPT effect, $F(1,40)=74.5$, $p<0.001$. Interaction F=Ns. Duncan's test: For NA basal levels—C vs 12 hrs, $p<0.05$; C vs 24 hrs, $p<0.01$; 12 hrs vs 24 hrs, $p<0.01$; 24 hrs vs 24 hrs+Na load, $p<0.01$. For levels after α-MPT—C vs 24 hrs, $p<0.05$; 12 hrs vs 24 hrs, $p<0.01$.

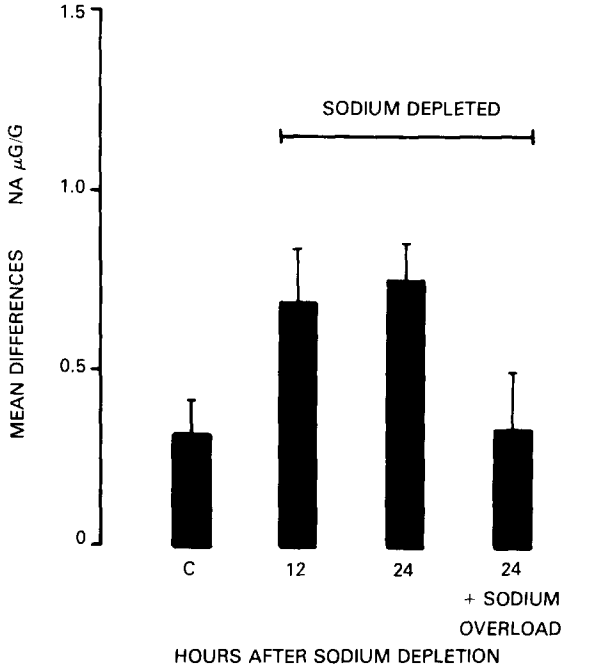

FIG. 2. Mean differences for NA levels before and after α-MPT treatment. Groups of rats as in Fig. 1. Abbreviations: C=control. Ns=not significant. C vs 12 hrs, $p<0.05$; 12 hrs vs 24 hrs, Ns; 24 hrs vs 24 hrs+Na load, $p<0.01$.

and 5-HIAA were obtained through oxidation with I_2 for NA, or condensation with o-phthaldialdehyde (OPT) for 5-HT and 5-HIAA. The hypothalamic utilization of NA [19] was measured by injecting the rats with α-methyl p-tyrosine (α-MPT) to inhibit the synthesis of catecholamines, and the tissue was assayed for NA. Each sample value was obtained from one animal.

Procedure

Several experimental groups were tested:

1. Control rats. A group of animals were sham dialyzed and individually caged, without food and with access only to distilled water.

2. Sodium-depleted rats. Depletion of body sodium was performed by peritoneal dialysis. The technique [6] consisted of a peritoneal injection of a 5% glucose solution, in a volume equivalent to 10% of rat body weight. This volume was removed 1 hr later by inserting an 18-gauge needle into the peritoneal cavity. The dialyzed rats were caged individually without food and with access only to distilled water until they were killed 5, 12 or 24 hrs after the dialysis was performed.

3. Sodium depleted-stimulated rats. A group of sodium-depleted rats were electrochemically stimulated (Anodic d.c. 100 μA-30 sec) in the medial surface of the frontal lobe cortex by means of a permanently implanted stainless steel electrode. The rats were killed 5 hrs after the dialysis and the stimulation was performed 30 min to 1 hr before decapitation.

4. Sodium-depleted-repleted rats. The 24 hrs sodium-depleted group and the 5 hrs sodium-depleted-stimulated group were given 8 and 3 ml respectively of sodium chloride solution (1.8%) in order to replete the sodium lost, 30 min before decapitation.

5. Rats from all groups. Six animals from groups 1, 2, 3 and 4 were injected with α-MPT (30 mg/kg) in order to estimate changes in the utilization of NA. The difference between basal levels of NA and those measured three hours after the administration of the drug was taken as an index of the utilization of the amine.

Statistical Analysis

The appropriate analysis of variance was performed on the NA, 5-HT and 5-HIAA data. Post-hoc comparisons between means were made by Duncan's test. To determine the significance of the utilization of NA between different groups, the difference *t*-test was applied. The level of significance chosen was $p<0.05$.

RESULTS

Effect of Sodium Depletion on the Levels and Utilization of Hypothalamic NA

Twelve hours after sodium depletion basal concentration of NA in the hypothalamic fraction was significantly higher than in control rats ($p<0.05$). A greater enhancement of NA basal levels was measured 24 hrs after sodium depletion ($p<0.01$). This effect was reversed by sodium load, which

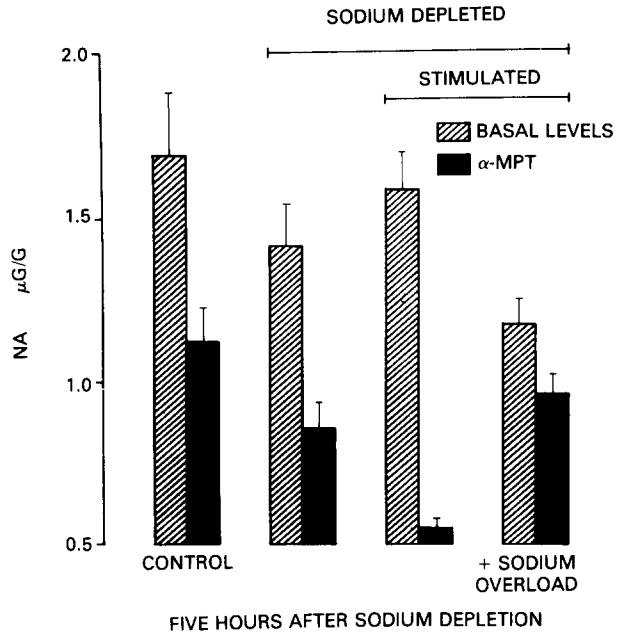

FIG. 3. NA concentration in the hypothalamus of sodium depelted stimulated rats before and after α-MPT treatment. Abbreviations: C=control. St=stimulated. Each bar represents the mean of six determinations±s.e. of mean. Analysis of variance: α-MPT effect, $F(1,40)=86.4$, $p<0.001$; Stimulation-sodium effect, $F(3,40)=6.4$, $p<0.001$; Interaction, $F(3,40)=6.8$, $p<0.001$. Duncan's test: For NA basal levels—C vs 5 hrs+St+Na load, $p<0.01$; 5 hrs vs 5 hrs+St+Na load, $p<0.01$; 5 hrs+St vs 5 hrs+St+Na load, $p<0.01$. For levels after α-MPT: C vs 5 hrs+St, $p<0.01$; 5 hrs vs 5 hrs+St, $p<0.05$; 5 hrs+St vs 5 hrs+St+Na load, $p<0.01$.

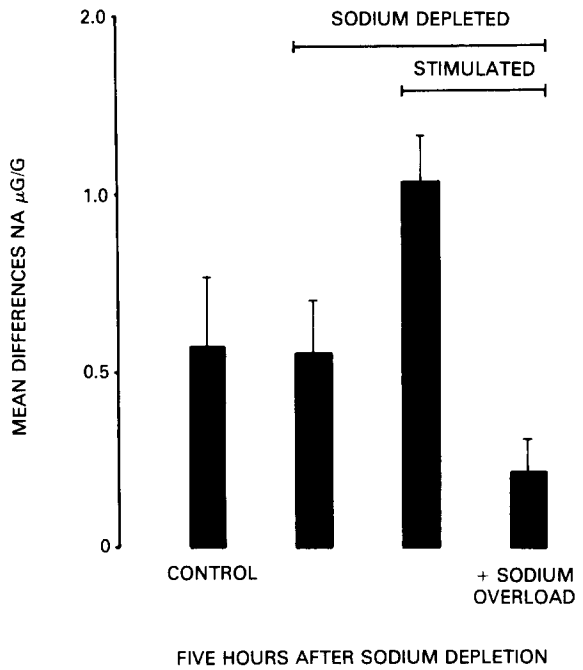

FIG. 4. Mean differences for NA levels before and after α-MPT treatments. Groups of rats and abbreviations as in Fig. 3. C vs 5 hrs, Ns; 5 hrs vs 5 hrs+St, $p<0.01$; 5 hrs+St vs 5 hrs+St+Na load, $p<0.01$.

decreased significantly the level of NA ($p<0.01$) (Fig. 1). Three hours after α-MPT treatment the levels of NA decreased significantly in all the groups studied ($p<0.05$) (Fig. 1).

Figure 2 shows that the degree of NA depletion induced by α-MPT was higher in the groups killed 12 and 24 hrs after sodium depletion than in control rats ($p<0.05$). In spite of the fact that 24 hrs after sodium depletion NA basal levels were higher than those measured 12 hrs later (Fig. 1), α-MPT administration resulted in no difference in NA utilization between the two groups. In contrast, α-MPT treatment in sodium loaded animals provoked a significant ($p<0.01$) decrease in NA depletion, indicating a diminished NA utilization.

Effect of Electrical Stimulation of the Limbic Cortex and Sodium Load on the Levels and Utilization of Hypothalamic NA in Sodium-Depleted Rats

Five hours after sodium depletion basal levels of NA in the hypothalamic fraction did not differ significantly from controls and those measured 30 min after electrical stimulation (Fig. 3). Sodium load on the contrary, provoked a significant decrease in NA basal levels when compared with that of depleted stimulated ($p<0.01$) or non-depleted rats ($p<0.01$), but not when compared with sodium-depleted animals. α-MPT treatment produced 3 hrs later a significant decrease in NA concentration in all the groups ($p<0.05$) (Fig. 3).

After inhibition of CA synthesis by α-MPT (Fig. 4) the depletion of NA was similar in controls and sodium-depleted rats, suggesting no modifications in the utilization of the amine. However, in sodium-depleted rats electrical stimulation, which did not produce changes in NA basal levels, induced a marked increase in NA depletion after α-MPT treatment, ($p<0.01$) indicating an augmented utilization of the amine. Sodium load to depleted stimulated rats significantly retarded ($p<0.01$) the depletion of NA by α-MPT, reflecting a lower utilization of NA.

Effect of Sodium Depletion on the Levels of Hypothalamic 5-HT and 5-HIAA

Table 1 shows that sodium depletion did not affect the levels of 5-HT since they remained without changes 12 and 24 hrs after IPD. On the contrary, sodium load in rats 24 hrs after sodium depletion significantly increased the levels of 5-HT. 5-HIAA content in the same hypothalamic fraction also was unaffected by sodium depletion, although a small non-significant decrease was observed. Sodium load resulted in a significant increase in the content of 5-HIAA.

Effect of Electrical Stimulation and Sodium Overload on the Levels of Hypothalamic 5-HT and 5-HIAA

The level of 5-HT in the hypothalamic fraction 5 hrs after sodium depletion showed no substantial changes occurring when compared with that of the control group. Depleted rats electrically stimulated 30 min before killing showed a significant increase in the content of 5-HT which was enhanced by sodium load. Neither sodium depletion nor electrical stimulation affected the levels of 5-HIAA, but a significant increase was observed after sodium load in depleted-stimulated rats (Table 2).

TABLE 1

EFFECT OF SODIUM DEPLETION ON HYPOTHALAMIC 5-HT AND 5-HIAA CONCENTRATION (μg/g)

Treatment	5-HT	5-HIAA
Control	1.68 ± 0.12†	1.13 ± 0.14#
After depletion		
12 hrs	1.93 ± 0.15	0.95 ± 0.18§
24 hrs	1.79 ± 0.02†	0.84 ± 0.12§
24 hrs + Na load	2.23 ± 0.05*	1.65 ± 0.04‡

Means ± S.E. (n=6) are presented.

Duncan's test: For values of 5-HT: *vs † $p<0.01$. For values of 5-HIAA: ‡vs § $p<0.01$; ‡vs # $p<0.05$.

TABLE 2

EFFECT OF ELECTRICAL STIMULATION AND SODIUM LOAD ON HYPOTHALAMIC 5-HT AND 5-HIAA CONCENTRATION (μg/g)

Treatment	5-HT	5-HIAA
Control	1.53 ± 0.25†	0.86 ± 0.07†
After depletion		
5 hrs	1.34 ± 0.13§	0.98 ± 0.10†
5 hrs + stimulation	2.01 ± 0.09‡	1.15 ± 0.10†
5 hrs + stimulation + Na load	3.14 ± 0.12*	1.98 ± 0.18*

Mean ± S.E. (n=6) are presented.

Duncan's test: For values of 5-HT: *vs †, ‡, § $p<0.01$; ‡vs § $p<0.05$. For values of 5-HIAA: *vs † $p<0.01$.

DISCUSSION

The above results show that in the rat an activation of the hypothalamic noradrenergic system takes place after sodium depletion. This activation takes time to become evident since in both non-depleted rats or in rats 5 hrs after sodium depletion NA basal levels and utilization are similar, while 12 hrs after IPD higher NA basal levels are accompanied by an enhanced amine utilization. Although 24 hrs after sodium depletion an augmented level of NA is found, the utilization does not increase, pointing to the fact that NA synthesis is higher than its utilization.

Ferreyra and Chiaraviglio [9] have demonstrated that the onset of sodium appetite appears 10–14 hrs after acute sodium depletion. The time that sodium appetite needs to become evident is in agreement with the time course of noradrenergic utilization observed in this work, and seems to indicate that a temporal relationship exists between the onset of sodium appetite and the metabolism of hypothalamic NA. A possible explanation for the observed effect of sodium depletion on the utilization of NA could be derived from the fact that *in vitro* experiments show that the lack of sodium enhances the efflux of NA from hypothalamic synaptosomes [18]. However, the increase in NA utilization is observed when natremia has returned to normal levels [9] and the specific sodium appetite appears. Alternatively, brain tissue could still have been sodium-depleted at the time that NA utilization was enhanced, but the fact that 5 hrs after sodium depletion the utilization of NA was similar to that of non-depleted rats leads to the suggestion that sodium deficiency may initiate a process which culminates with the changes observed in NA metabolism. Furthermore 5 hrs after sodium depletion electrochemical stimulation of the limbic cortex, which shortened the time of appearance of specific sodium appetite [7], increased hypothalamic utilization of NA, while sodium replenishment in these animals or in rats 24 hrs after sodium depletion significantly decreased hypothalamic utilization of NA, indicating a rather specific dependence on the presence of Na^+.

In relation to the metabolism of 5-HT, following sodium depletion hypothalamic levels of 5-HT did not change significantly, and although the levels of 5-HIAA slightly decreased, the differences were not significant. Possibly if several distinct hypothalamic nuclei were assayed a variation in serotonergic metabolism could be demonstrated [11]. Electrochemical stimulation increased the levels of 5-HT, whereas no changes were found in its metabolite, suggesting an increased synthesis of the amine [15]. On the contrary, sodium load promoted a significantly larger formation of 5-HT and 5-HIAA indicating that more 5-HT was being metabolized. These results showed that although the lack of sodium did not alter the metabolism of 5-HT, sodium load, on the contrary, induced a marked increase in the rate of synthesis and release of 5-HT. Such effects would explain the rise in concentration of 5-HIAA.

From these results it appears that acute sodium depletion triggers a series of events resulting in an increase in NA metabolism together with the development of specific sodium appetite. The fact that the metabolism of 5-HT was not modified by sodium lack, does not rule out the possibility that by using more sensitive methods a change in 5-HT metabolism could be detected.

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