Changes in Volemia and Natremia and Onset of Sodium Appetite in Sodium Depleted Rats

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FERREYRA, M. DEL C. AND E. CHIARAVIGLIO. Changes in volemia and natremia and onset of sodium appetite in sodium depleted rats. PHYSIOL. BEHAV. 19(2) 197-201, 1977. – Rats depleted of sodium by the IP injection of 10% b.w. of isotonic glucose for 30 min, developed a specific sodium appetite. The blood volume decreased 16% of the control value immediately after the treatment, returning to normal levels 2 to 4 hr later. The plasma sodium concentration showed a sudden and significant decrease, returning to control value 10-12 hr after the dialysis. In contrast, the sodium appetite became evident 10-12 hr after dialysis and reached the highest volume of ingestion at the sixteenth hr when both volemia and natremia had returned to normal. Since the appearance of the sodium appetite was not initiated during the state of hypovolemia and hyponatremia, the results show that the decrease in plasma volume and sodium concentration of the intravascular fluid that occurs after acute sodium depletion by IPD does not initiate an immediate onset of the sodium appetite.

Sodium depletion Peritoneal dialysis Natremia Volemia Onset of sodium appetite Urinary volume

THE VOLUNTARY sodium intake in animals, as well as in man, appears closely related to internal sodium regulation and probably shares common receptors and osmotic, endocrine and neural mechanisms [12].

Richter [10] observed in rats that one week after adrenalectomy a strong sodium appetite is developed in response to the uncontrolled loss of sodium. In normal rats maintained on sodium-free diet a week is also necessary to elicit an increase in sodium appetite [6]. Artificial methods to induce a rapid and strong sodium deficit have been used in different laboratories. Subcutaneous (SC) injection of Formalin [14], polyethylene glycol [13] or the use of diuretics [16] are the most frequently reported. All these methods induce a shift of entracellular fluid as well as NaCl. However, whereas thirst is provoked immediately after plasma volume deficit (as is the case in SC Formalin and polyethylene glycol), sodium appetite can not be elicited as rapidly as thirst, and manifests itself only several hours after an experimental maneuver to reduce body sodium. It seems that sodium appetite is subserved by a receptor mechanism less sensitive, or more slowly responsive, than the mechanism for thirst.

We have been studying the regulation of sodium appetite in rats, after depleting sodium by intraperitoneal dialysis (IPD) against isotonic glucose [2,3]. It seemed worthwhile to study the electrolyte changes that take place after IPD and the temporal relationship between these changes and the onset of sodium appetite.

METHOD

Animals

The experiments were carried out in male albino rats weighing 220–330 g at the beginning of the experiment. All the rats were naive to food, water or sodium deprivation. The rats were caged in groups of 10 with food and water ad lib up to the time they were used in the experimental procedure.

Sodium Depletion

Acute sodium depletion was performed by intraperitoneal dialysis in unanesthetized rats. The technique, which has been described [2], consisted in an intraperitoneal injection of a 5% glucose solution warmed to body temperature, in a volume equivalent to 10% of body weight. Thirty min later a similar volume was removed by inserting an 18 g needle into the peritoneal cavity. The sodium chloride removed was 0.843 ± 0.023 mEq/100 g body weight (Mean ± SE of 18 rats) [1]. In control rats no injection was given but the needle was inserted into the peritoneal cavity. Dialysed and control rats were caged

1 Supported by the Consejo Nacional de Investigaciones Científicas y Técnicas.
2 The R131 SA was generously supplied by the Laboratorio Central de Radioisótopos. Hospital Nacional de Clínicas, Córdoba.
3 Member of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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individually without food and with distilled water as the only drink.

Water and NaCl intake, blood volume, plasma sodium concentration, hematocrit, urinary volume and urinary sodium excretion, were monitored at the same selected period of time after sodium depletion, in order to determine the degree of correspondence between these physiological parameters.

Intake Test

After sodium depletion, 11 groups of 10 rats each were assigned for testing intakes of 1% NaCl solution and water. After recording body weight, experimental and control rats were given a two-bottle choice test at different time periods after dialysis. The volume drank was measured at the end of 30 min.

Plasma Sodium Concentration

Rats depleted of sodium as described above, were bled by cardiac puncture at different time intervals after peritoneal dialysis. The blood was centrifuged and the serum sodium concentration was determined by flame photometry. Drinking water was not available between dialysis and bleeding.

Blood Volume

In six groups of sodium-deprived rats the blood volume was measured at different time intervals after removing the peritoneal dialysate, by means of the radiiodide 131I-serum-albumin (RI[31SA]) according to Kuschnir et al. [9].

![FIG. 1. Sodium chloride (upper) and distilled water (lower) ingested after sodium depletion by IPD. The points represent ml/100 g body weight (mean ± SE) ingested for different group of rats in a two-bottle test during 30 min.](image)

### TABLE 1

BLOOD VOLUME, PLASMA SODIUM CONCENTRATION AND SODIUM INTAKES AFTER ACUTE SODIUM DEPLETION BY INTRAAPERITONEAL DIALYSIS AGAINST ISOTONIC GLUCOSE

<table>
<thead>
<tr>
<th>Time after IPD Hours</th>
<th>Blood volume (ml/100 g)</th>
<th>Plasma Na concentration (mEq/L)</th>
<th>1% NaCl intake (ml 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Na depleted</td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>7.45±0.14</td>
<td>6.64±0.38**</td>
<td>145.54±0.81</td>
</tr>
<tr>
<td>(5)</td>
<td>(9)</td>
<td>(7)</td>
<td>(5)</td>
</tr>
<tr>
<td>2</td>
<td>8.08±0.19</td>
<td>7.58±0.23</td>
<td>144.90±2.33</td>
</tr>
<tr>
<td>(7)</td>
<td>(7)</td>
<td>(10)</td>
<td>(10)</td>
</tr>
<tr>
<td>4</td>
<td>7.93±0.19</td>
<td>7.36±0.23</td>
<td>142.70±7.21</td>
</tr>
<tr>
<td>(7)</td>
<td>(7)</td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>149.73±2.95</td>
</tr>
<tr>
<td>(8)</td>
<td>(8)</td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td>8</td>
<td>7.67±0.18</td>
<td>7.20±0.24</td>
<td>149.03±2.90</td>
</tr>
<tr>
<td>(12)</td>
<td>(11)</td>
<td>(8)</td>
<td>(6)</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>145.08±2.20</td>
</tr>
<tr>
<td>(6)</td>
<td>(6)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>148.73±2.15</td>
</tr>
<tr>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>16</td>
<td>8.15±0.19</td>
<td>8.65±0.39</td>
<td>-</td>
</tr>
<tr>
<td>(7)</td>
<td>(7)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>24</td>
<td>8.58±0.17</td>
<td>8.50±0.19</td>
<td>142.11±0.95</td>
</tr>
<tr>
<td>(8)</td>
<td>(7)</td>
<td>(10)</td>
<td>(10)</td>
</tr>
</tbody>
</table>

Mean ± SE ( ) number of animals

*P<0.05  **P<0.01  ***P<0.001 Vs. control group.

Each data represents a separate group of rats.

1) 20 min. two - bottles test.
The rats were injected with 40 μl of RI131SA (containing 0.4 μ Ci) through a jugular vein, allowing 5–7 min for mixing. A blood sample was withdrawn from the opposite jugular vein with a heparinized syringe. Capillary microhematocrit tubes were filled with blood for hematocrit determination. The remaining blood was centrifuged and 0.4 ml of plasma was used for counting.

Urine Collection

After sodium depletion by IPD and before performing the intake test, seven groups of 10 rats each were transferred to special cages for urine collection. The amount of urine was measured at different times and aliquots were used for sodium determination by flame photometry.

RESULTS

Fluid Intake

In the first 8 hr after IPD, the volume of 1% NaCl solution ingested was low and similar in both the controls and the experimental groups, but afterwards a significant increase occurred in the sodium-depleted group. The highest intake was observed at the sixteenth hr after dialysis: 8.2 ± 0.6 ml/100 g body weight (Mean ± SE) thereafter, a plateau was obtained which lasted for at least 24 hr (Fig. 1). Compared with the control group (2.1 ± 0.3 ml/100 g body weight), the difference was statistically significant (t: 5.3, p<0.001). The amount of water ingested was negligible in the dialyzed group (Fig. 1).

Blood Volume

Compared with the control value (7.9 ± 0.08 ml/100 g body weight) the blood volume in the dialyzed group, showed a significant decrease immediately after IPD (6.6 ± 0.38 ml/100 g body weight), t = 4.75, p<0.001. The hypovolemia was a transient phenomenon reaching normal levels shortly after dialysis with a tendency to overcome the control value 16 hr later (Fig. 2).

Hematocrit

The hematocrit value for the control animals was 43.6 ± 0.18%. Immediately after dialysis it increased significantly (52.0 ± 0.32%) (p<0.001) returning to control value at the twelfth hr (Fig. 2). The changes in hematocrit showed a reciprocal relationship with the variations observed in the blood volume.

Plasma Sodium Concentration

Due to the sodium chloride removed by the IPD the plasma sodium concentration decreased immediately after dialysis (Fig. 2). The control value of 145.7 mEq/l dropped to 133.3 ± 1.1 (t = 4.9, p<0.001). The difference remained significant during the first six hours, returning to control level afterwards. A higher sodium intake was observed when the natremia reached the control level. Table I shows the changes in blood volume and plasma sodium concentration following IPD. The sodium intake began 10–14 hr after dialysis, at which time rats were neither hypovolemic nor hyponatremic.

Volume of Urine and Urinary Sodium Excretion

The effect of acute sodium depletion by IPD on urinary volume and urinary sodium excretion is shown in Table 2. The urinary volume excreted was significantly higher in the depleted group (p<0.001) as compared with the control. On the contrary, the sodium excretion was negligible in the depleted animal while control rats even excreting a small volume, the sodium excretion was higher.

DISCUSSION

Specific appetite for saline solution can be defined as a behavioral response elicited by a deficit of body sodium. However, the present experiment shows that, after an acute depletion of sodium, the appearance of sodium appetite takes from 10–14 hr, suggesting that the sensory system evoking sodium intake needs some time to evolve.

The fact that natremia returns to normal levels even though the rat had no access to sodium salt suggests that sodium could be removed from some unknown deposit [11] in order to compensate the natremia.
TABLE 2

URINARY VOLUME AND URINARY SODIUM EXCRETION AFTER ACUTE SODIUM DEPLETION BY INTRAPERITONEAL. DIALYSIS AGAINST ISOTONIC GLUCOSE

<table>
<thead>
<tr>
<th>Collection periods Hours after IPD</th>
<th>Urinary volume ml 100 g body w.</th>
<th>Urinary Na Excretion μEq 100 g b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Na depleted</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>0.07±0.03 (34)</td>
<td>0.09±0.03 (27)</td>
</tr>
<tr>
<td>4</td>
<td>0.18±0.04 (23)</td>
<td>1.75±0.17*** (24)</td>
</tr>
<tr>
<td>6</td>
<td>0.43±0.05 (22)</td>
<td>2.97±1.03*** (22)</td>
</tr>
<tr>
<td>8</td>
<td>0.56±0.05 (21)</td>
<td>3.61±0.24*** (20)</td>
</tr>
<tr>
<td>10</td>
<td>0.72±0.02* (15)</td>
<td>4.6±0.29*** (15)</td>
</tr>
<tr>
<td>12</td>
<td>0.98±0.15* (10)</td>
<td>4.38±0.45*** (9)</td>
</tr>
<tr>
<td>24</td>
<td>3.46±0.83 (10)</td>
<td>8.67±1.02*** (11)</td>
</tr>
</tbody>
</table>

Mean ± SE ( ) number of animals
*P<0.05  **P<0.01  ***P<0.001
Vs. control group. The data for each hour of collection represents a separate group of rats.

depleted reservoir the information may travel to the specific neural structure promoting sodium appetite. Our results are contradictory with the hypothesis that hyponatremia elicits sodium intake [15] but since nothing is yet known about receptors evoking sodium appetite, it may be that the hyponatremia is the trigger which activates the system.

In addition to the hyponatremia, a transient hypovolemia, with a simultaneous rise in the hematocrit, was seen after dialysis. Volemia was normalized before natremia, indicating that volume regulation may have a higher priority than osmoregulation [8].

With the present data we can not demonstrate that the acute removal of sodium and other solutes from the extracellular fluid, could drive water to the intracellular space, leading to hypovolemia and cellular expansion. We could assume that water is driven by ion movements to leave the cells and move to the extracellular space. Further experimental evidence will be necessary to elucidate this point.

The sodium appetite appeared and persisted when the plasma sodium concentration and the blood volume had returned to normal levels. Therefore, neither hypovolemia nor hyponatremia has a temporal relationship with the appearance of sodium appetite.

In our experiment volemia decreased 16% from the normal value immediately after depletion. This stimulus, which should have induced thirst [7,11], actually inhibited all drinking behavior. In fact, the water ingested during 24 hours was negligible and the ingestion of 1% NaCl started 8 hr after IPD. It seems that the hypovolemia associated with hyponatremia may induce a depressive state capable of inhibiting the thirst mechanism. Although little is currently known about this possibility, it may be that the sudden extrusion of ions produced by the experimental procedure could activate some inhibitory fibers connected with the thirst circuit. It has been observed in this laboratory that sodium depletion by intraperitoneal dialysis inhibits water and food intake in both thirsty and hungry rats (unpublished results).

The sodium-depleted rats lost more urinary water than the control group. The urinary excretion may result in a negative water balance, consequently the Na-depleted rats should became dehydrated.

The physiological and behavioral effects of the IPD as a method for depleting body sodium have not been studied in detail up to this time. The present observations are in disagreement with the idea that hyponatremia and hypovolemia can, by themselves, induce sodium appetite, because their changes after sodium depletion are not immediately related with the appearance of sodium intake. Indeed, they should act as a first trigger. Hypovolemia as well as
Hyponatremia and sodium depletion are known as stimuli for the release of renin from the kidney [5] and the renin-angiotensin system has been implicated in the regulation of sodium appetite [3,4]. Considering that the onset of the renin-angiotensin system occurs shortly after any physiological challenge, the implication of this system in the present experiment is questionable. On the other hand, it could be suggested that receptors related with the sodium reservoir [15] could be responsible for extracellular sodium level regulation, perhaps by an action of the mineralocorticoids on sodium transport.

The etiology of sodium appetite seems to be very complex and its elucidation deserves further work.

REFERENCES