FUNCTION OF ADH AND THIRST MECHANISMS IN THE FACE OF CONSUMING SALTY WATER IN MALE WISTAR RAT

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Background: Seawater comprises 99% of water resources, and because a decrease in raining, the degree of water saltiness increased in some geographical areas. Access to fresh potable water has been limited, hence people and animal in such habitats are compelled to use salty water. It is important for us to know the function of antidiuretic hormone (ADH) and thirst mechanisms in the face of consuming salty water. **Methods**: One hundred and forty male Wistar rats, weighing 300±20 g were allocated randomly to seven groups. Test groups consumed 1, 3, 5, 7 and 9% salt concentrations as drinkable water, another test group used distilled water, and control group consumed potable water. At the onset of the experiment and after 120 hours, blood samples were drawn to determine serum sodium level and plasma osmolality. This study repeated with 0.5, 1, 1.2, 1.4, 1.6, 1.8 and 2 percent salt concentrations. **Results**: Serum sodium level and plasma osmolarity have a direct relation with salt concentration in distilled water. This is evident that, when the rate of salt concentration in distilled water exceeds 1.4 %, serum sodium level and plasma osmolarity increase significantly. **Conclusion**: ADH and thirst could control serum sodium level and plasma osmolarity in a limited wide of salt and water intake. ADH and thirst mechanisms could not control serum sodium level and plasma osmolarity, when the rate of salt concentration in distilled water exceeded 1.4 %.

Key words: ADH, Concentration, Osmolarity, Salt, Thirst

INTRODUCTION

Water is fundamental to existence as total body water is tightly regulated within ±0.2% of body weight each day. Sodium is the major extracellular cation in the body fluid and is therefore one of the most important osmotically active solutes. Sodium is regulated within narrow limits (137–141 mOsm/L). Like human, physiologic serum sodium concentration in the rat ranges from 135–146 mOsm/L. The serum sodium concentration and thus serum osmolality are closely controlled by water homeostasis, which is mediated by thirst, arginine vasopressin, and the kidneys. Thirst and ADH act coherently and parallel to each other to accurately control plasma osmolality and sodium concentration. Osmoregulation is normally so efficient that mean plasma osmolality is maintained within a range of 1 to 2 percent (usually between 280 and 290 mOsm/L), despite wide variations in sodium and water intake. The stimulation of thirst, which promotes fluid intake, and the actions of ADH to stimulate water retention, are synergistic in returning plasma osmolality to normal. As seawater comprises 99% of water resources and because a decrease in raining, the degree of water saltiness increased, thus free access to fresh potable water in some geographical areas is limited. People and animal in such habitats are compelled to use salty water. It is very important to know the action of thirst and ADH mechanisms in the face of consuming salty water. The function ADH and thirst mechanisms in the face of consuming salt concentrations in rat on regulation of serum sodium level and plasma osmolarity were studied.

MATERIAL AND METHODS

We received permission for this study from the animal experimentation committee at Zanjan University of Medical Sciences, Zanjan, adopting principles of laboratory animal care (National Institutes of Health publication No. 86-23, revised 1985). Animals were housed five per cage under a standard 12-h light/dark cycle, food was available always for all groups of animals, the temperature of the testing room was kept at 24 °C. Animals were purchased from Iranian Razi Institute, Ketamine hydrochloride was purchased from Sankyo Co. (Tokyo, Japan) and NaCl was purchased from Merck Co (Darmststdt, Germany). First evaluation of ADH and thirst mechanisms was done by adding 10, 30, 50, 70 and 90 gm of NaCl to one liter of distilled water, leading to preparations of 1, 3, 5, 7 and 9 percent salt concentrations. Resultant osmolarity was 170, 510, 850, 1190 and 1530 mOsm/L of Na and Cl each, respectively. One hundred and forty male Wistar rats, weighing 300±20 g were allocated randomly to seven groups. Throughout the experiment, different test groups consumed special salt concentration as the only source of drinking water, another test group used distilled water.
water as drinking water, and control group consumed potable water. All groups were fed with a diet containing 0.5% salt, and other living conditions were identical. At the beginning of the experiment and after 120 hours, blood samples were drawn to measure the serum sodium level and plasma osmolarity from the animal’s tail, under anesthesia condition. Ketamine hydrochloride at an anesthetic dosage of 125 mg/kg administered intra-peritoneally. 

Serum sodium level was measured by flame photometer, the model of flame photometer was Corning 480 (Tokyo, Japan). Plasma osmolarity was measured by clinical refractometer (Erma, Tokyo, Japan). Physical examinations were performed daily and weight changes were measured in animals and development of dehydration was evaluated. The rate of water and salt concentrations intake between different groups were evaluated as the main parameter for evaluation of thirst mechanism. Wet bed and body contamination with faeces were considered as indicators of polyuria. When severe dehydration developed in test groups and some animals died due to salt poisoning, we finished the experiment and all of the remaining animals were, gradually treated with tap water. Craniotomy was performed in the dead animals and intracranial bleeding was evaluated. The data were presented as mean±S.D. Statistical analysis about collected data was done by using different groups ANOVA test and p<0.05 was considered as significant changes. In the second experiment, exact evaluation was performed by using 0.5, 1, 1.2, 1.4, 1.6, 1.8 and 2 percent salt concentrations in distilled water. Na and Cl content of 0.5, 1, 1.2, 1.4, 1.6, 1.8 and 2 percent salt concentrations is about 85, 170, 205, 240, 275, 305 and 340 mOsm/L, respectively. Osmolarity of 0.5, 1, 1.2, 1.4, 1.6, 1.8 and 2 percent salt concentrations is about 170, 340, 410, 480, 550, 610 and 680 mOsm/L, respectively. One hundred and sixty male Wistar rats, weighing 300±20 g allocated randomly to eight groups and other conditions of this experiment were similar to the preceding experiment.

RESULTS

The rate of salt concentration intake in test groups consuming 1% and 3% NaCl concentration, showed an increase of 78% and 36%, respectively, as compare to control group. In contrast, test groups maintained on 5, 7 and 9 percent salt concentration showed a decrease of 52, 69 and 80 percent in salt concentration consumption, respectively.

The group maintained on distilled water showed 11% decrease in water intake. The rate of urine output in groups consuming 1% and 3% salt concentrations, were also considerably increased. Animal beds were always wet and animal bodies contaminated with faces. While, the rate of urine output in other groups were also considerably decreased and animal beds were always dry.

At the beginning of experiment, mean serum sodium levels in test groups (1, 3, 5, 7 and 9%) were 139.5±2.2, 140.5±2.3, 140.1±2.4, 138.9±2.7 and 140.5±2.3 mOsm/L respectively. In group maintained on distilled water (control group) it was 140.1±1.3 and 139.5±2.7 mOsm/L respectively and there was no significant difference in serum sodium levels between these groups.

One hundred and twenty hours after NaCl consumption, mean serum sodium levels in test group which consumed 1, 3, 5, 7 and 9 percent salt, were 147.5±2.2, 150.6±3.4, 162.8±2.7, 165.5±2.8, and 171.7±4.1 mOsm/L respectively. It was 136.3±2.6 mOsm/L in the group maintained on distilled water, while in control group it was 138.1±2.6 mOsm/L with p=0.06, p=0.008, p=0.004, p=0.001, p=0.0006 and p=0.15 respectively.

At the beginning of experiment, mean plasma osmolarity in test groups (1, 3, 5, 7 and 9%) was 285.8±3.6, 289.5±2.4, 288.6±2.8, 284.8±3.6, and 289.2±3.4 mOsm/L respectively. In the group maintained on distilled water it was 286.4±2.8 mOsm/L, and in control group it was 286.8±2.6 mOsm/L and was not different significantly.

One hundred and twenty hours after salt consumption, mean plasma osmolarity in test groups which consumed 1, 3, 5, 7 and 9 percent salt concentrations were 304.6±3.4, 312.6±2.8, 315.8±3.2, 328.8±3.4, and 350.8±4.8 mOsm/L respectively. In group maintained on distilled water it was 272.8±2.6 mOsm/L, and in control group it was 285.6±2.6 mOsm/L and p=0.07, p=0.004, p=0.002, p=0.0018, p=0.0004 and p=0.38, respectively.

Serum sodium level and plasma osmolarity had a direct relation with the amount of salt in distilled water. One percent salt concentration and distilled water did not increase serum sodium level and plasma osmolarity significantly. However 3, 5, 7 and 9 percent salt concentrations resulted in a significant increase in serum sodium level and plasma osmolarity. Table-1 compares the rate of salt concentrations intake in test groups versus the control group and shows mean serum sodium level and plasma osmolarity in different groups at the onset and 120 hours later. Distilled water and 1% salt concentration could be tolerated for a long period without significant complications, but higher salt concentrations could not be tolerated for a long period of time. Using 3% and higher salt concentrations was associated with continuous body water loss, weight loss, development of dehydration and hypernatremia. Concordant with these changes, different signs of hypernatremia include muscle tremour, lethargy, decreased sensitivity to stimuli, sensory and motor disturbances, and rotatory rhythmic movements appeared.
in the animals. Most of the affected animals died due to nervous problems as intracranial bleeding was observed after craniotomy on autopsy. Exact evaluations in the second experiment provided evidence that, when the salt concentration in distilled water exceeded 1.4%, plasma osmolarity and serum sodium level increased significantly.

### DISCUSSION

The present study produced several key findings as a result of consuming different salt concentrations as drinkable fluid which differentially affected the amount of salty water intake regulating ADH secretion and thirst mechanism. One percent and 3% of NaCl concentration accompanied by 78 and 36% percent rise in salt concentrations intake, and groups maintained on distilled water and 5, 7 and 9 percent salt concentrations showed decrease of 11, 52, 69 and 80 percent in water intake and salt concentrations consumption. The rate of urine output in groups which consumed 1% and 3% salt concentrations, were also considerably increased (ADH secretion was almost normal), while, the rate of urine output in other groups were also considerably decreased. (ADH secretion was high). The stimulation threshold for NaCl solution in human is 0.01 M. The rate of salt in 0.01 M solution is about 0.6 g/L. When the rate of salt in distilled water increased, neural protective mechanism in relation to salt consumption got activated. One percent salt concentration osmolarity is approximately 340 mOsm/L, 1% salt concentration did not provoke the neural protective mechanism strongly and thirst sensation did not suppress, so the animal greedily consumed 1% salt concentration. Three percent salt concentration osmolarity is about 1000 mOsm/L, as the maximal concentrating ability of the kidneys is also about 1200 mOsm/L, so 3% salt concentration as the only source of water supply for the rats is not quite beneficial. Through a stronger action of the neural protective mechanism and in spite of unsuppressed thirst sensation, animal showed a lower tendency to drink 3% salt concentration in comparison with 1% salt concentration group. As a result, the amount of 3% salt concentration intake was considerably decreased. Distilled water is devoid of electrolytes and its osmolarity is zero, so consuming distilled water as drinking water resulted in a greater drop in plasma osmolarity, lack of electrolytes in distilled water makes it a less desirable drinking water for the animals and some part of 11% decrease related to this phenomenon.

Thirst is important for maintaining body fluid homeostasis. Normally, when the serum sodium rises, thirst develops and ADH secretion is stimulated. Although ADH release may occur earlier, it is thirst that provides the ultimate protection against hypernatremia. Hypernatremia will not occur in individuals with an intact thirst mechanism and free access to water, due to a potent osmolar stimulus to drink. Hence, a significant disorder in thirst sensation lead to hypernatremia. Five, 7 and 9 percent salt concentrations osmolarity are about 1700, 2380 and 3060 mOsm respectively and could not be used as a water source to compensate for body fluid loss. Second, high salt concentrations provoked neural protective mechanism more powerful and the animal had no tendency to drink these salt concentrations. Five, 7 and 9% salt concentrations put a positive feedback loop into action and cause a progressive body water loss leading to enhanced osmolarity and thirst induction. If the neural protective mechanism did not counteract the thirst sensation, the animal would drink excessive amount of salt concentrations and became massively dehydrated and die in a short time. The animal’s refusal from drinking highly concentrated salt concentrations will keep it alive for some days and if it can get fresh water at this time it will escape death. Third, there was a direct relation between serum sodium level and plasma osmolarity with the amount of salt in distilled water. One percent salt concentration and distilled water did not create a significant changes in serum sodium level and plasma osmolarity, but, 3, 5, 7 and 9 percent salt

### Table-1: Percentage rate of salt concentrations intake in test groups as compare to water intake in control group showing mean serum sodium levels and plasma osmolarity at the onset of experiment and 120 hours later.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Salt concentration consumption as compare to water intake in control group</th>
<th>Mean serum sodium levels 120 hours after beginning the experiment (mEq/L)</th>
<th>p Value</th>
<th>Plasma osmolarity 120 hours after beginning the experiment (mOsm/L)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>100%</td>
<td>138.1±2.6</td>
<td>0.06</td>
<td>285.6±2.6</td>
<td>0.07</td>
</tr>
<tr>
<td>1% Nacl group</td>
<td>178%</td>
<td>147.5±2.2</td>
<td>0.008</td>
<td>312.6±2.8</td>
<td>0.004</td>
</tr>
<tr>
<td>3% Nacl group</td>
<td>136%</td>
<td>150.6±3.4</td>
<td>0.004</td>
<td>315.8±3.2</td>
<td>0.002</td>
</tr>
<tr>
<td>5% Nacl group</td>
<td>48%</td>
<td>162.8±2.7</td>
<td>0.001</td>
<td>328.8±3.4</td>
<td>0.0018</td>
</tr>
<tr>
<td>7% Nacl group</td>
<td>31%</td>
<td>165.5±2.8</td>
<td>0.0006</td>
<td>350.8±4.8</td>
<td>0.0004</td>
</tr>
<tr>
<td>9% Nacl group</td>
<td>20%</td>
<td>171.7±4.1</td>
<td>0.15</td>
<td>272.8±2.6</td>
<td>0.38</td>
</tr>
<tr>
<td>Distilled water group</td>
<td>89%</td>
<td>136.3±2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
concentrations resulted to a significant increase in serum sodium level and plasma osmolarity. Forth, ADH and thirst could control serum sodium level and plasma osmolarity when 1% salt concentration and distilled water used as drinking water, but when 3, 5, 7, and 9 percent salt concentrations were used as drinking water, ADH and thirst could not control serum sodium level and plasma osmolarity. As the kidney ability for maximal urine concentration in different species of animals are not identical and other parameter like age and weight could affect urine maximal concentration, so the result of our study could not expended to different species of animals completely. The results of our study are in agreement with other findings. Pervious research emphasized that, thirst and ADH mechanisms could control sodium concentration and plasma osmolarity in a limit wide of salt and water intake. Ingestion of seawater (approximately 350 to 500 Na mOsm/L) not only lead to hypernatremia but also lead to water loss from solute diuresis or osmotic diarrhea. 13 Shipwreck victims who drink seawater, for every litre of seawater drunk, 2 litre of urine volume would be required to get rid of solutes ingested, this would result in a net fluid loss of 1 litre for every litre of seawater drunk, explaining the rapid dehydration and hypernatremia that occurs in shipwreck. 6

CONCLUSION
Exact evaluation in the second experiment suggests that ADH and thirst mechanisms could control serum sodium level and plasma osmolarity until the salt concentrations in distilled water is lower than 1.4% and when the salt concentration in distilled water exceeds 1.4%, these systems could not control plasma osmolarity and serum sodium level, and serum sodium levels and plasma osmolarity increased significantly.

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REFERENCES

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