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Responses Evoked by the Pharyngolaryngeal Application of Saline in Vasopressinergic Cells

Takao AKAISHI and **Shinji HOMMA**

Department of Physiology, Niigata University School of Medicine, Niigata, Japan

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Summary. **The roles of the pharynx and larynx in body water regulation were studied in the urethane-anesthetized (1.2 g/kg, b.wt.) rat by recording the electrical activities of supraoptic vasopressin (AVP) producing cells in the hypothalamus. The pharyngo/laryngeal application of 0.15 ml/kg b.wt. water caused a decrease in the electrical activity of the AVP producing cell, whereas 0.3 M saline caused an increase in the firing activity. Isotonic saline had no effect, nor did applications of 0.3 M saline to the dorsal tongue. Application of 0.05 mM amiloride, a gustatory sodium antagonist, caused a decrease in the firing activity. Hypertonic saline (0.3 M) containing 0.05 mM amiloride caused an insignificant change in their activity. Isotonic saline in amiloride had no effect. The pharyngeal application of 0.3 M choline chloride had also no effect on the firing activity of the AVP producing cells.**

These data suggest the following points: 1) afferents from oropharyngeal and laryngeal mucosa regulate the activity of AVP cells; 2) these afferents are influenced by the concentration of sodium chloride involved in the ingested solution; and 3) the gustatory reception of sodium is involved in this mechanism.

Key words—pharyngolarynx, saline, vasopressin, rat.

INTRODUCTION

Our recent studies[2,3] have revealed that humans taking 20 min to drink a very small volume (0.15 ml/kg b.wt.) of water or hypertonic (0.3 M) saline results in a slight hypotonic diuresis or hypertonic antidiuresis, respectively, whereas isotonic saline has no effect. Additionally, changes in urine volume were inversely associated with that in urine osmolality. Actually, there was a significant linear relationship between both changes in urine volume and osmotic pressure throughout the concentration range (from 0 to 0.3 M) of sodium chloride in the solutions ingested. These data strongly suggest the existence of neural afferent pathways from the oropharyngeal/laryngeal mucosa to the hypothalamic mechanism regulating vasopressin (AVP) release.

In the present study, we therefore recorded the single unit activity of the hypothalamic AVP producing cell before and after the pharyngeal application of water, 0.15 and 0.3 M saline, in the male rat. We were further examined whether or not a gustatory factor is involved in this mechanism by using amiloride, a gustatory antagonist for sodium.[6,16]

MATERIALS AND METHODS

Animals and operations

A total of 32 Wistar male rats (250-360 g) were used in the present series of experiments. They were housed in a controlled environment at 23±1°C (mean±SEM) and 50±5% humidity, with a 14:10 h light-dark cycle (light on at 7:00 a.m.). Just before the experiment, they were anesthetized with urethane (1.2 g/kg, b.wt.) and anesthesia was supplemented when necessary. Every attempt was made to maintain a level of anesthesia no deeper than the point where voluntary ocular movements were abolished. They underwent a tracheotomy to avoid any effects originating from the trachea mucosa. The esophagus was also ligated to eliminate the gastric factor.[7] A double barred vinyl tubing (1.0 mm outer diameter) was inserted into the oral cavity so that the tip of the tube reached the pharyngeal mucosa, and was fixed on to the lower lip by means of a thread. In some

Correspondence: Dr. Takao Akaishi, Department of Physiology, Niigata University School of Medicine, Asahimachi 1, Niigata 951, Japan.

animals (N=5), the tip of the vinyl tube was placed on the dorsal tongue to stimulate the gustatory mechanism on the tongue. For electrophysiological examinations, the rats were mounted in a stereotaxic instrument in the prone position. A bipolar stimulating electrode made from stainless wire (0.2 mm diameter) was implanted in the neurohypophysis to apply the electrical stimulation (0.5 ms duration, 0.8-1.0 Hz) for antidromic identification of the unit discharge activity of the supraoptic magnocellular neurosecretory cells in the supraoptic nucleus (SON). The antidromic identification of discharge activities of the SON neurosecretory cells was followed by conventional criteria proposed in the past.[8] Namely, antidromic action potentials had a discrete threshold, responded at a constant latency for the stimulus at a fixed rate and intensity, and followed paired-pulse stimuli at a short interval. Furthermore, responses induced by secondary stimuli were canceled by collision with spontaneous generated spikes. Those neurons that displayed a phasic firing pattern (see Fig. 1) in their spontaneous discharge activity were identified as vasopressinergic cells. The antidromically identified SON neurosecretory cells that showed a tonic firing pattern were classified as presumed oxytocin producing cells, and were not considered in the present study. Other electrophysiological techniques were as in our previous study.[1]

To examine the effects of the pharyngeal application of test solutions on blood pressure, the right femoral artery was also cannulated in an additional six animals. All aspects of the present experiments were conducted in conformity with the guiding principles for the care and use of animals approved by the council of the Japan Physiological Society (1995).

Chemicals used

The solution applied to the pharynx/larynx contained various concentrations of sodium chloride (0, 0.15 and 0.3 M). These solutions were made up of distilled water or 0.05 mM amiloride (Sigma). Additionally, 0.3 M choline chloride (Sigma) was also tested. Each stimulation was 0.15 ml/kg, b.wt. of a test solution applied within several sec. After each test, the oral cavity was rinsed with 0.15 M saline and wiped off with a cotton applicator. Application of the test solution was performed at intervals of more than an hour.

Data analysis

Both the single unit activity of the AVP cells and blood pressure were analyzed on- or off-line with a Macintosh IIsi computer with a MacLab package (Analog Digital Instruments). Spontaneous activity was subtracted from the evoked response to yield the net number of spikes for each 30 sec for 3 min before and after stimulation. The data on the effect of each test solution on the discharge activity were analyzed by ANOVA.

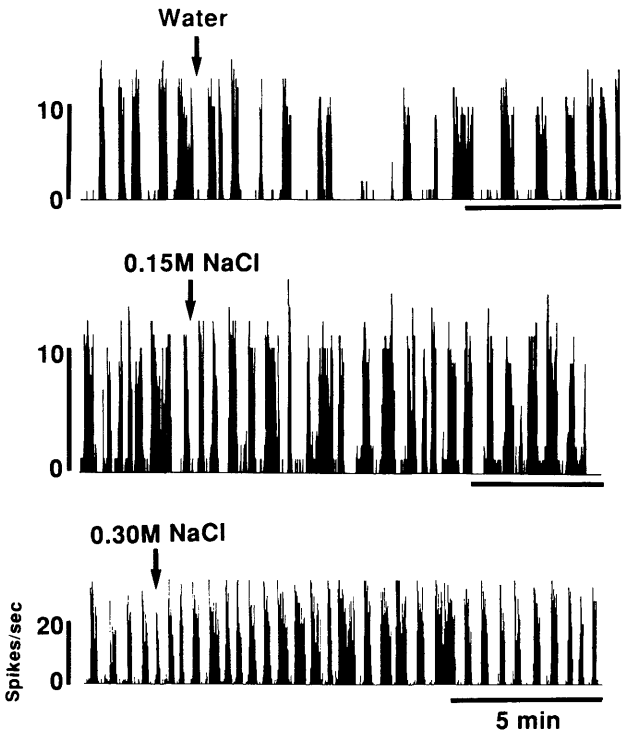

Fig. 1. Ratemeter recordings of the response of a representative AVP neuron in each group to water (0.15 ml/kg, b.wt., upper), 0.15 M saline (middle) and 0.3 M saline (lower) applied to the pharyngeal/laryngeal mucosa.

RESULTS

A total of eighty-four supraoptic AVP cells were identified in 26 animals and studied. The mean discharge rate of these AVP cells was 6.7±1.1 spikes/sec (mean±SEM).

Effects of water and saline

In the first step, the effects of the pharyngeal/laryngeal application of distilled water (N=11), 0.15 M (N=10) and 0.3 M sodium chloride (N=13) (0.15 ml/kg, b.wt.) on the spontaneous activity of AVP cells were studied (Fig. 1). The application of distilled water caused the firing activity to decrease (Fig. 2A). In 1.5-2 min after the application, the discharge

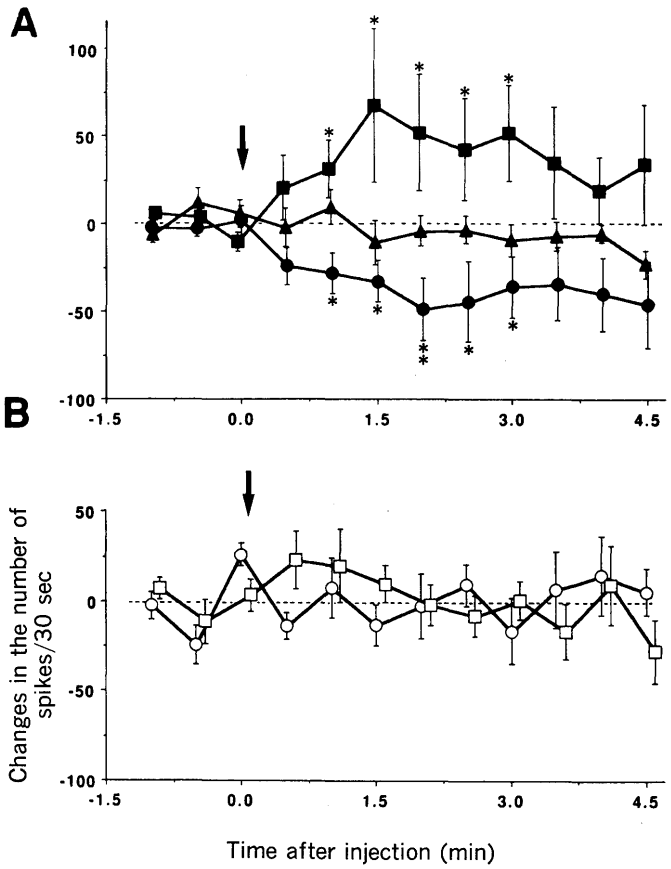

Fig. 2. A. Mean changes in the number of spikes every 30 sec after the application of water (N=11, closed circles), 0.15 M NaCl (N=10, closed triangles) and 0.3 M NaCl (N=13, closed squares). The ordinate and abscissa are the same format as shown in Fig. B. **B.** Effects of 0.3 M NaCl applied to the tongue (N=8, open squares) and of 0.3 M choline chloride to the pharynx (N=9, open circles). Each solution was applied at the arrow. **, $P<0.01$ (vs. preapplication level, ANOVA); *, $P<0.05$.

activity showed a minimum value for the change in the number of spikes (-48.7 ± 17.6 spikes/30 sec, $P<0.01$). A significant fall in their activity was maintained for 2.5 min (from 0.5 to 3.0 min after the onset of stimulation). On the other hand, 0.3 M saline induced an increase in the firing activity. The maximum value (67.9 ± 44.1 spikes/30 sec, $P<0.05$) was obtained between 1.0 and 1.5 min after the pharyngeal application of 0.3 M saline. A statistically significant acceleration of the firing activity was also maintained for 2.5 min (from 0.5 to 3.0 min after application) (Fig. 2A). Throughout the molarity range (0-0.3 M) for the sodium chloride, the percent change in the firing rate during the initial 3 min following the application of each test solution was associated with the molarity of sodium chloride in the solution applied (-17.1 ± 4.5, water, $P<0.01$; -3.4 ± 2.5, 0.15 M NaCl, N.S.; $41.7\pm26.0\%$, 0.3 M NaCl, $P<0.05$; mean$\pm$SEM) (Fig. 3).

Effects of test solutions on mean blood pressure

The effects of the application of test solutions on the mean blood pressure were examined in six animals. A slight decrease (less than 5 mmHg) in the mean blood pressure was transiently detected following the pharyngeal/laryngeal application of each test solution. The time courses of these changes were, however, not associated with those of each evoked discharge response induced by the test solutions. This indicates that evoked responses are not due to influences derived from the baroreceptors.[1,17]

Effects of amiloride

The next step in this study examined the effects on evoked responses in AVP cells of amiloride, which has recently been shown to reduce the perceived intensity of sodium chloride at the level of the receptor site.[6,16] Each test solution was made in 0.05 mM amiloride. The application of 0.05 mM amiloride (N=10) caused a significant ($P<0.05$) decrease in the firing activity during the initial 3 min, as shown in Fig. 3. Interestingly, hypertonic saline (0.3 M, N=12) in amiloride caused an insignificant change in their activity, that is, the evoked response was partially inhibited. Isotonic saline (N=11) in amiloride had no effect. Percent changes in the firing rate during the

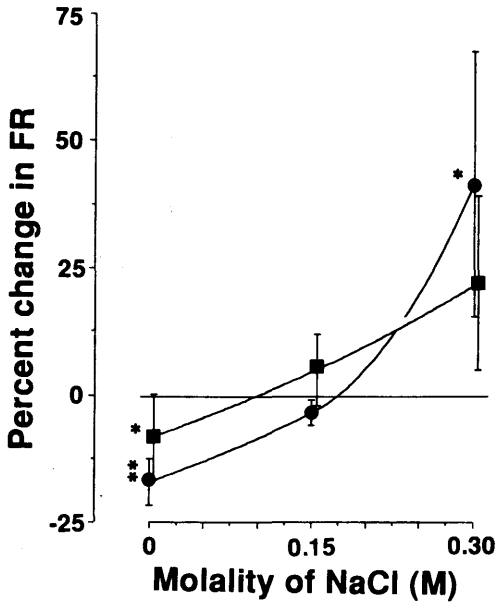

Fig. 3. Relationships between percent change in the firing rate (FR) during the initial 3 min following the application of test solutions and the molarity of sodium chloride in the solutions. Solid line with closed circles, control; closed squares, 0.05 mM amiloride; **, $P<0.01$; *, $P<0.05$ (vs. preapplication level, ANOVA).

initial 3 min following application were still clearly associated with the molarity of the sodium chloride in the test solution (-8.2±8.4, water; 5.0±7.1, 0.15 M NaCl; 22.3±17.1%, 0.3 M NaCl) (Fig. 3).

Effects of application on the tongue

The effects of the application of the same volume (0.15 ml/kg) of 0.3 M saline to the dorsal tongue were examined in some AVP neurons (N=8), and compared with those induced by pharyngeal application. As shown in Fig. 2B, an insignificant change in the discharge activity was detected following the application of 0.3 M saline to the dorsal tongue during observation.

Effects of choline chloride

The effects of pharyngeal application of 0.3 M choline chloride on the discharge activities of AVP cells (N=9) were also examined for comparison with those of the same molar sodium chloride solution. During observation, an insignificant change in the spontaneously generated activity was detected, as shown in Fig. 2B.

DISCUSSION

It is now clear that a change in the osmolality of extracellular fluid following the intake of a considerable volume of water or hypertonic saline promotes the firing activity of hypothalamic AVP neurosecretory cells.[8] In this study, however, it was not probable that the application of water or saline affected the osmolality of the extracellular fluid, since the volume (0.15 ml/kg, b. wt.) was very small and, in addition, the esophagus of the animals was ligated to eliminate gastric factors.[7] Oropharyngeal and laryngeal neural mechanisms have been proposed as first-order factors determining the amount of water drunk.[3-5,7,9,13-15] The present study confirmed our previous results[2] suggesting a change in AVP release in human subjects after drinking the same volume (0.15 ml/kg, b.wt.) of water or hypertonic saline. An interesting point in this study is that the firing activity of AVP neurosecretory cells is accompanied by the molarity of the sodium chloride in the solution applied. This suggests the existence of a mechanism which detects the molarity of the sodium chloride on the mucosa stimulated, and that this influences the firing activity of AVP cells. The neuronal responses induced rapidly and transiently suggest that these effects have the nature of a neuroendocrine reflex mediated by afferent nerves which innervate the mucosa of the pharynx and/or the larynx.

It has been shown in the unanesthetized monkey, that the supraoptic neurosecretory neurons can be transiently inhibited and the discharge activity facilitated during and after drinking the water[4,14] and hypertonic saline,[4] respectively. However, the time course of the evoked neurosecretory responses in this study clearly differs from that in the unanesthetized monkey, a difference which seems mainly due to the anesthesia. It should be always taken into account that, in a study of unanesthetized subjects, it is quite difficult to neglect the psychological (psychosomatic) factors. Any psychological factors in our data could be deleted because of the anesthesia.

Nevertheless, this may reflect the difference in the stimulation site. It is quite conceivable that the mucosae of the oral cavity involving the tongue, pharynx, larynx, esophagus and stomach were exposed by the solution following drinking. In this study of esophagectomized animals, a very small volume of each solution was applied to the pharynx via tubing inserted previously. Although these procedures may enable us to predominantly stimulate the

pharynx/larynx, this does not exclude the possibility that the upper part of esophagus is also stimulated and that this affected the present results.

Saphier[10] has recently reported that an immediate acceleration was observed in the discharge activity of the AVP cell in the rat paraventricular nucleus following the oropharyngeal application of 0.80-0.83 ml/kg b.wt. (more than five times the volume we applied) of 2-4 M saline. This discrepancy would depend upon the different volume and molarity of the saline applied. A stronger stimulus extending over a wider mucous area may cause nonspecific reaction, such as a noxious response. On the basis of our observations, we became convinced that influences originating in the tongue were not involved in the accelerating or inhibiting mechanism of the discharge activity of AVP cells following the application of test solutions to the pharynx in the present series of experiments.

The question of the nature of this receptive mechanism in the mucosa arose in our previous study.[2,3] Shingai and Beidler[12] have reported that in mice there are fibers sensitive to saline, in both the glossopharyngeal and superior laryngeal nerves which are known to be major afferent pathways from the pharynx and larynx to the central nervous system. It is therefore very likely that these types of nerves, at least in part, mediate information on sodium chloride reception at the mucosa. Interestingly, an evoked response in neuronal activity was partially blocked by the simultaneous application of amiloride, which is widely reported to be a sodium transport blocker,[6] and the same concentration (0.05 mM) of amiloride has reported to eliminate taste responses in the afferent nerve (chorda tympani nerve) to sodium ion.[16] Apparently these data mean that gustatory information from the mucosa is involved in the oropharyngeal/laryngeal mechanism regulating AVP release. An incomplete blockade of the evoked response, however, may indicate the participation of other amiloride insensitive, unknown mechanisms located in the pharyngeal/laryngeal mucosa. The fact that the application of 0.3 M choline chloride had an insignificant effect suggests that the sodium ion is essential for this reaction.

In 1980, Shingai[11] reported the existence of fibers in the super laryngeal nerve of the rat which were accelerated by application of water on the laryngeal surface of the epiglottis. The decreased activity after water application in AVP cells examined in this experiment may therefore depend upon the laryngeal nerve fibers. This point should be confirmed in a further study using a denervated animal. Another possibility is that the applied water dilutes the salivary components. As a result of a lower concentration of sodium chloride at the level of the receptor site, applied water might result in an inhibition of the discharge activity of AVP cells. These details will be the subjects of further studies.

The present study suggests: 1) that pharyngeal/laryngeal afferents are involved in the regulating mechanism of the firing activity of AVP cells; 2) that this mechanism depends on the molarity of the sodium chloride in the solution applied; and also 3) that amiloride sensitive gustatory components are involved in the pharyngeal/laryngeal mechanism regulating AVP release.

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REFERENCES

1) Akaishi T, Homma S: Baroreceptor control of vasopressin producing cells in streptozotocin diabetic rats. *Brain Res Bull* **31:** 719-722, 1993.
2) Akaishi T, Shingai T, Miyaoka Y, Homma S: Antidiuresis immediately caused by drinking a small volume of hypertonic saline in man. *Chemical Senses* **16:** 277-281, 1991.
3) Akaishi T, Shingai T, Miyaoka Y, Homma S: Hypotonic diuresis following oropharyngeal stimulation with water in humans. *Neurosci Lett* **107:** 70-74, 1989.
4) Arnauld E, du Pont J: Vasopressin release and firing of supraoptic neurosecretory neurones during drinking in the dehydrated monkey. *Plügers Arch* **394:** 195-201, 1982.
5) Geelen G, Keil LC, Kravik SE, Wade CE, Thrasher TN, Barnes PR, Pyka G, Nesvig C, Greenleaf JE: Inhibition of plasma vasopressin after drinking in dehydrated humans. *Am J Physiol* (Reg Int Comp Physiol 16) **247:** R968-R971, 1984.
6) Heck GL, Mierson S, DeSimone JA: Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science* **223:** 403-405, 1984.
7) Nicholaides S: Early systemic responses to orogastric stimulation in the regulation of food and water balance: functional and electrophysiological data. *Ann NY Acad Sci* **157:** 1176-1203, 1969.
8) Poulain DA, Wakerley JB: Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neuroscience* **7:** 773-808, 1982.
9) Salata RA, Verbalis JG, Robinson AG: Cold water stimulation of oropharyngeal receptors in man inhibits release of vasopresssin. *J Clin Endocrinol*

Metab **65:** 561-567, 1987.
10) Saphier D: Vasopressin-secreting neurones of the paraventricular nucleus respond to oropharyngeal application of hypertonic saline. *Neurosc Lett* **109:** 97-101, 1992.
11) Shingai T: Water fibers in the superlaryngeal nerve of the rat. *Jpn J Physiol* **30:** 305-307, 1980.
12) Shingai T, Beidler L: Response characteristics of three taste nerves in mice. *Brain Res* **335:** 245-249, 1985.
13) Shingai T, Miyaoka Y, Shimada K: Diuresis mediated by the superior laryngeal nerve in rats. *Physiol Behav* **44:** 431-433, 1988.
14) Thrasher TN, Nistal-Herrera JF, Keil LC, Ramsay DJ: Satiety and inhibition of vasopressin secretion after drinking in dehydrated dogs. *Am J Physiol* (Endocrinol Metab 3) **240:** E394-E401,1981.
15) Vincent JD, Arnauld E, Bioulac B: Activity of osmosensitive single cells in the hypothalamus of the behaving monkey during drinking. *Brain Res* **44:** 371-384, 1972.
16) Yamamoto T, Matsuo R, Fujimoto Y, Fukunaga I, Miyasaka A, Imoto T: Electrophysiological and behavioral studies on the taste of umami substances in the rat. *Physiol Behav* **49:** 919-925, 1991.
17) Yamashita H, Koizumi K: Influence of carotid and aortic baroreceptors on neurosecretory neurons in supraoptic nuclei. *Brain Res* **170:** 259-277, 1979.