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Increased Activity of Phasic Vasopressinergic Neurons in the Hypothalamic Paraventricular Nucleus, as Elicited by NaCl Injection into the Hepatic Portal Vein in the Rat

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Summary. **Magnocellular neurosecretory cells were antidromically identified in the hypothalamic paraventricular nucleus of urethane-anesthetized, ovariectomized female rats following electrical stimulation of the neurohypophysis. The vasopressinergic cells with a phasic pattern of spontaneous discharge and the oxytocinergic cells with a tonic pattern of discharge were distinguished and their response to hypo-, iso- and hypertonic NaCl solutions injected into the portal vein was examined. The hypertonic NaCl injection produced an increase in the discharge of vasopressinergic cells, while there was no appreciable change in the discharge after the hypo- and isotonic NaCl injections. The discharges in the oxytocinergic neurons were unchanged after these injections of NaCl. The stimulatory response in the vasopressinergic neurons caused by the hypertonic NaCl solution was not reproduced following hepatic branch vagotomy. Because neural discharge in the vasopressinergic cell has been shown to be linked to vasopressin secretion, these findings suggest that hypertonic hepatic vagus signals may modulate fluid balance through vasopressin at the paraventricular level.**

INTRODUCTION

Electrophysiological studies have shown that afferent hepatic vagus discharges are modified by changes in osmolality or in the sodium concentration in the hepatic portal circulation,[1-3] and the activation mechanism of the sodium receptor has been clarified.[4,5] Moreover, it has been shown that injections of hypo- or hypertonic solutions into the portal vein elicit changes in diuresis.[6] Recently, it was proved that there is a functional correlation between the hepatic vagus signal and the activation of the supraoptic neurosecretory cells (SONs). The SONs have been identified as neurons which secrete antidiuretic hormone, vasopressin, and regulate urination.[7] However, the direct relation of the vasopressin secreting cell activity to the hepatic vagus signal has not yet been examined. On the other hand, it has been established that the paraventricular nucleus (PVN) in the hypothalamus contains neurosecretory neurons which secrete vasopressin and oxytocin,[8,9] and electrophysiological criteria have been used to identify these neurons.[10,11]

This experiment was designed to investigate whether an omsotic solution containing NaCl injected into the hepatic portal vein affects the discharge of these neurosecretory neurons in the PVN.

MATERIALS AND METHODS

All experiments were carried out in forty-two adult female rats of the Wistar strain, ovariectomized 10-14 days before recording in order to prevent possible endocrine effects. The animals were housed individually with free access to tap water and food.[12] Their body weight during the experiment was between 240 and 300 g. Each animal was anesthetized with urethane (1.2 g/kg, i.p.) and put in a stereotaxic frame in a prone position according to the atlas of Albe-Fessard et al. (1966).[13] The exposed cerebral cortex was covered with warm agar solution in saline. The rectal temperature was maintained between 36.0 and 37.0℃ with a heating pad.

Side-by-side bipolar stimulating electrodes were constructed from 200 μm diameter stainless steel wire, and the electrodes, insulated except for their

tips, had a DC resistance of approximately 100 kΩ in saline. These electrodes were implanted in the neurohypophysis (NHP) and fixed to the skull with dental cement.[12]

The PVN was systematically explored while isolated rectangular pulses (0.5 ms duration) were applied at 0.8 Hz to the NHP. The intensity of the constant current pulses was distributed between 40 and 150 μA; active and indifferent poles of the stimulation electrode were determined to obtain a lower activation threshold. Following determination of the threshold for each response, stimulation was delivered routinely at 20% above the threshold unless otherwise stated. Recording of extracellular potentials was made by means of glass micropipettes. The recording barrel was filled with a 2% (W/V) solution of Pontamine Sky Blue 6B dye in 0.5 M sodium acetate buffer. The DC resistance of the recording electrode ranged from 10 to 20 MΩ. The location of the recording was determined by applying Pontamine Sky Blue 6B dye. Wave forms of the antidromic action potentials and neuronal discharge were observed on a digital oscilloscope (Nihon-Kohden, ATAC-150) and were plotted on an X-Y recorder (National, VP-6412A).

The units were identified antidromically as those of neurosecretory cells when the unit spike evoked by posterior pituitary stimulation showed constant latency followed by high frequency stimulus (200 Hz), and was cancelled by collision with a spontaneously occurring spike.

The mean of antidromic spike latency was 12.1 ms. NaCl, D-glucose, urea and mannitol dissolved in distilled water kept at 36.0℃ were used as test solutions. A fine catheter (0.6 mm, o.d.) was introduced into the hepatic portal vein before the brain operation, and through this blood for osmolality estimation was drawn and test solutions were injected. Four test solutions, 25 μl each, were injected in random order, each injection lasting 5 sec. In some animals, a catheter was inserted into the right jugular vein for test injection.

A loose thread was looped around the hepatic branch of the vagus nerve with both ends of the thread taken outside of the body through a plastic tube so that the nerve could be cut by pulling the thread.[14]

Blood (50 μl) obtained from the portal vein was used for the measurement of plasma osmolality.[15] Osmolality was estimated by freezing point depression with an OS osmometer (Fiske Associates).

At the end of the experiment, a 0.25 mA anodal

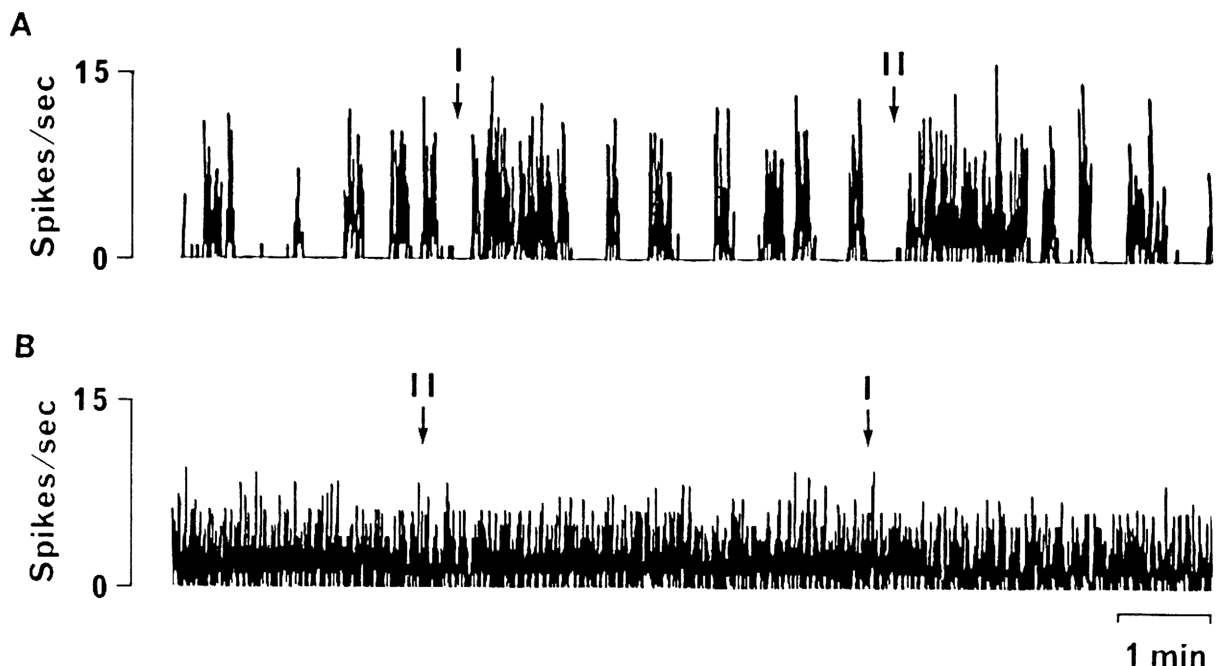

Fig. 1. Alterations in discharge of paraventricular cells following NaCl injection into the hepatic portal vein. Vasopressinergic discharges with a phasic pattern (A, upper trace) and oxytocinergic discharges with a tonic pattern (B, lower trace) were identified, then 164 mM (I; 305 mOsm/kg) or 170 mM (II; 315 mOsm/kg) NaCl solution was injected into the vein. Arrows indicate the time of injection.

current was passed through the stimulation electrode for 10 sec to deposit a small quantity of iron from the tip. The position of the recording electrode was marked by the passage of a cathodal 3-5 μA current for 15 min. The rats were perfused with a 10% solution of potassium ferrocyanide in 10% formalin, which was followed by 10% formalin. Prussian Blue reaction product was used to identify the position of the stimulation electrode tip. The brain was fixed in formalin, and 100 μm frozen serial sections were cut from the frontal plane and stained with Cresyl Violet.

The number of discharges every second was counted during the 4 min immediately before and after the portal injection of the test solution, and the significance of the difference between the mean discharge rates before and after the injection was examined in each unit by t-test. The differences between several groups of data were then evaluated by ANOVA after checking the homogeneity of variance between the groups.[16] Specific results were analysed by Duncan's multiple range test. The average of 5 or more discharges was determined.

RESULTS

Data were collected from 59 magnocellular paraventricular neurosecretory cells which were antidromically driven from the NHP. Twenty-two of them had a tonic pattern of oxytocinergic discharge, while 37 of them had a phasic pattern of vasopressinergic discharge when the NHP stimulation was suspended. Data from those without spontaneous activity were not included in the present analysis. Plasma osmolality just before the portal test injections was 294.7±0.1 mOsm/kg (n=42), and it was noted that 20 of 37 vasopressinergic discharges recorded showed a significant increase 1 min after 164 mM NaCl (305 mOsm/kg) injection into the portal vein (t-test, $p<0.01$ - $p<0.001$).

Representative changes in vasopressinergic and oxytocinergic discharges following NaCl injection into the portal vein are shown in Fig. 1. When four different concentrations of NaCl (148, 158, 164 and 170 mM) were injected into the portal vein, vasopressinergic discharges among times and among groups were significantly different: F (9,279)=34.836, $p<0.002$ and F (4,279)=114.827, $p<0.002$, respectively. The osmolalities in these solutions were 275, 295, 305 and 315 mOsm/kg, respectively (Fig. 2).

Although the hypo- and isotonic NaCl solutions (148 and 158 mM) injected into the portal vein failed to cause a change in the discharge, hypertonic NaCl solutions (164, 170 and 180 mM) produced a significant

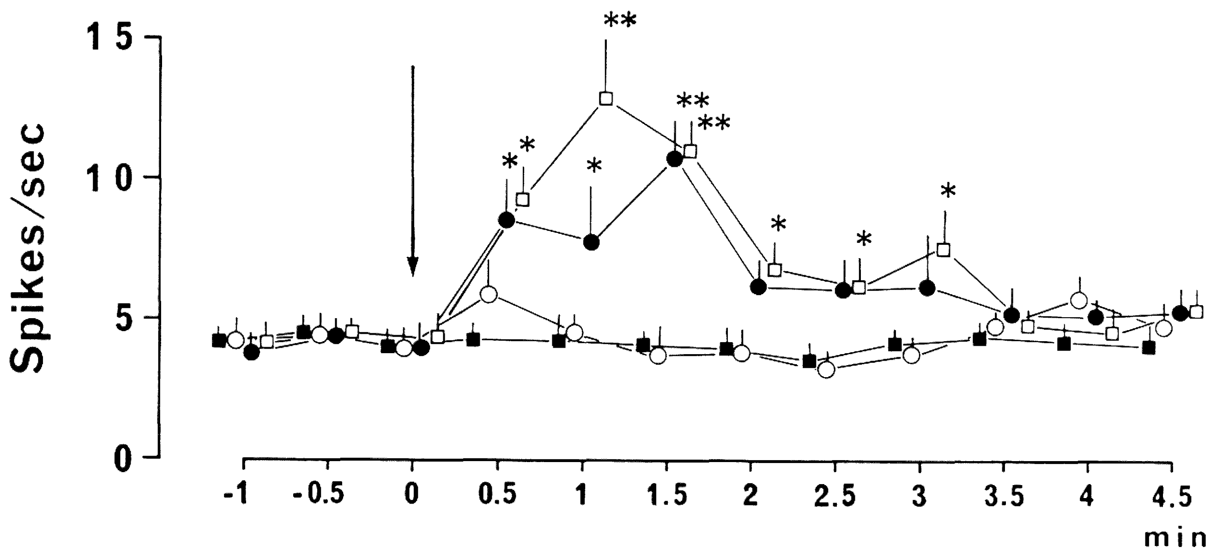

Fig. 2. Changes in the discharge of vasopressinergic cells in the paraventricular nucleus after NaCl injection into the hepatic portal vein. Four different concentrations of NaCl (■, 148 mM; ○, 158 mM; ●, 164 mM; □, 170 mM) were portally injected. Osmolalities of these solutions were 275, 295, 305 and 315 mOsm/kg, respectively. An arrow shows the time of injection. There are 7 samples from each group; values are the mean ± SEM. *$p<0.05$ and **$p<0.01$ vs. ○.

increase in the discharge. The excitatory response in the discharge reached its peak about 1 min after the 170 mM NaCl injection, then recovered to the control level within another 2 min. When the integrated discharges of vasopressinergic cells 30 sec after four different concentrations of NaCl were compared, the difference in the discharge among groups was significant: $F(4,34) = 73.996$, $p < 0.001$ (Fig. 3); it was also noted that the excitatory response was dose dependent. On the other hand, hypo-, iso-, and hypertonic NaCl injections into the portal vein produced no appreciable change in the discharge of the oxytocinergic neurons; ANOVA revealed no difference in the discharge among groups, $F(2,20) = 3.78$, $p > 0.05$ (Fig. 3). Both vasopressinergic and oxytocinergic neurons showed no significant change in the discharge when three different concentrations of NaCl were injected into the right jugular vein; the differences in the discharges among the groups were $F(2,20) = 1.304$, $p > 0.05$ and $F(2,20) = 1.021$, $p > 0.05$, respectively. The response in the vasopressinergic neurons associated with the portal injection of hypertonic 170 mM NaCl was not reproduced after hepatic branch vagotomy (Fig. 3).

D-glucose, urea and mannitol solutions which were equitonic to 180 mM NaCl solution (335 mOsm/kg) produced an increase in the vasopressinergic discharge when injected into the portal vein. However, the NaCl solution was most potent in increasing the discharge (Fig. 4). ANOVA showed a difference among the groups, $F(7,55) = 69.684$, $p < 0.001$.

DISCUSSION

A hypertonic solution injected into the portal vein has been shown to affect urination and the neuronal activity of the SONs.[6,7] Based on these findings, it has been presumed that afferent osmotic signals from the portal vein influence urination through vasopressin,

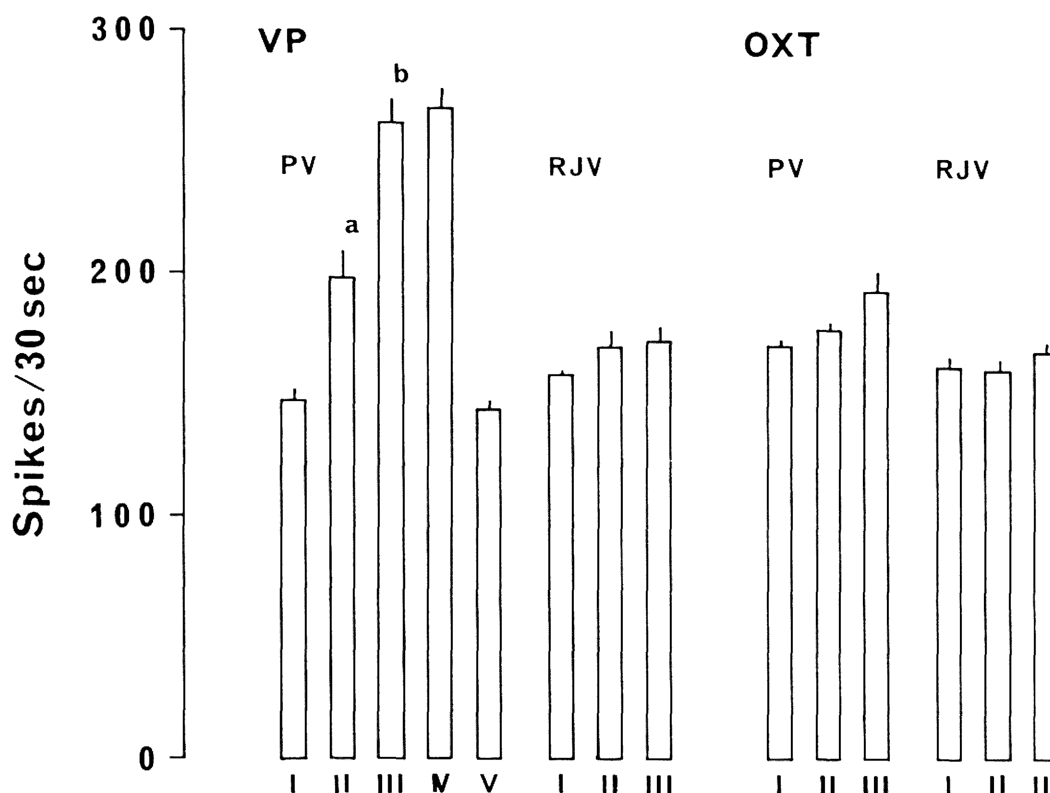

Fig. 3. Responses in the discharge of vasopressinergic (VP) and oxytocinergic (OXT) neurons in the paraventricular nucleus following NaCl injection into the portal vein (PV) and into the right jugular vein (RJV). The number of discharges occurring in the 30 sec after the injection is shown. Different concentrations of NaCl (I, 158 mM; II, 164 mM; III, 170 mM; IV, 180 mM) were injected, and their osmolalities were 295, 305, 315 and 335 mOsm/kg, respectively. NaCl (170 mM) injection with hepatic branch vagotomy is indicated in V. There are 7 samples for each bar. Values are the mean ± SEM. a, $p < 0.05$ vs. I. b, $p < 0.05$ vs. II.

especially since the SONs contain vasopressin secreting cells.[7] However and unfortunately, in the above studies, neither identification of vasopressinergic cells nor hepatic vagal branch contribution was attempted. In the present study, vasopressinergic neurons in the PVN were identified by their phasic discharge pattern,[10,11] and it was noted that the discharge was increased when hypertonic NaCl solution was injected into the portal vein (Figs. 1, 2). This seems to be specific to vasopressinergic neurons, because putative oxytocinergic neurons with a tonic discharge did not respond to the NaCl injection (Fig. 3). Because stimulation of the vasopressinergic discharge did not occur when NaCl solution was injected into the right jugular vein (Fig. 3), the response may be attributed to the portal areas.

The vasopressinergic neuronal response evoked by portal NaCl injection produced an increase in discharge in a dose dependent manner (Fig. 3). This response is considered specific to NaCl. A single afferent unit obtained from the hepatic vagus branch of rats has been documented to change within several seconds when NaCl is injected, the response lasting for several minutes.[2] In this study, the response in the vasopressinergic neurons caused by NaCl injection occurred 30 sec after the injection, and continued for several minutes (Figs. 1, 2). Thus, the duration of the vasopressinergic neuronal response seemed to be determined at the peripheral portal level. However, it is not easy to explain the difference in latency obtained from the hepatic vagus and vasopressinergic neuronal responses. Further work needs to be done.

Electrophysiologically, it has been demonstrated that there are two kinds of fibers in the afferent hepatic vagus nerve; one fiber is sensitive to hyperosmolality, and the other to hyposmolality.[17] Signals conveyed to the brain by these two fibers types were detected at the medullary level.[18,19] In contrast to this, hypotonic NaCl solution injected into the portal vein did not affect the vasopressinergic neuronal activity (Fig. 2); the PVN cells received signals only related to the hyperosmolality. It is possible that there might be a mechanism differentiating the signal between the medulla and PVN.

Considering the report that vasopressin release is dependent on the frequencies of the neuronal discharge[8,9] together with the fact that portal osmotic signals were effective in stimulating vasopressin

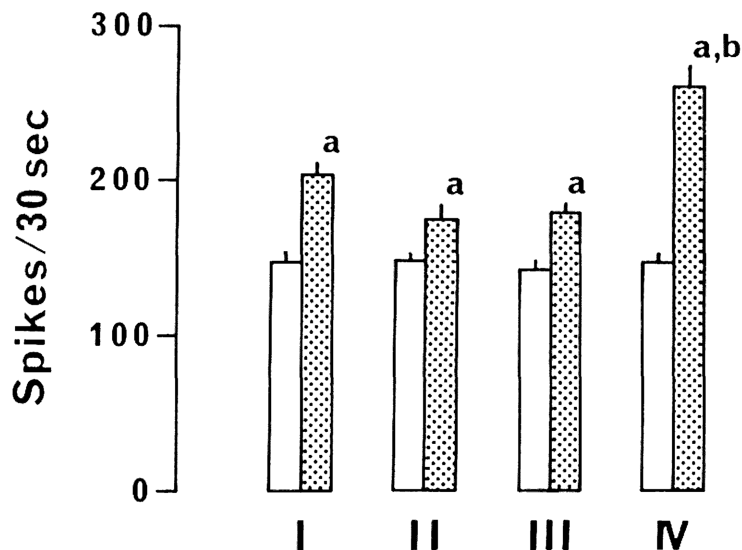

Fig. 4 Responses in vasopressinergic discharges of the paraventricular nucleus after portal injection of four different osmotic solutions. The integrated discharges at 30 sec before (open bar) and after (shaded bar) the injection are compared. D-glucose (I; 318 mM), urea (II, 335 mM), mannitol (III, 319 mM) and NaCl (IV; 180 mM) were used. The osmolality of these solutions was 335 mOsm/kg. There are 7 samples for each bar. Values are the mean ± SEM. a, $p<0.01$ vs. the value before injection. b, $p<0.01$ vs. the value after injection of I, II or III.

secretion from the SONs,[7] our data can be interpreted as indicating that portal hypertonic signals can stimulate vasopressin release by activating neurons in the PVN. This could explain the finding that hypertonic solution injected into the portal circulation resulted in reduced urination,[6] because the increased secretion of vasopressin would decrease urination. However, the increased urination associated with the portal hypotonic signal[6] can not be explained by our results; areas in the central nervous system other than the PVN may be involved.

Because stimulation of the vasopressinergic discharge associated with portal NaCl injection did not occur after sectioning of the hepatic branch of the vagus nerve was performed[20] (Fig. 3), the NaCl signal may be principally transmitted via the vagus branch, even though an osmotic signal from the portal areas has also been believed to be sent to the brain through a branch of the spinal cord.[21,22]

Four different substances producing almost the same osmolality in solution brought a stimulatory response in the vasopressinergic neuron when injected into the portal vein (Fig. 4). However, the magnitude of the response was not the same for all four substances, and it was noted that the NaCl solution cleary stimulated the neuronal activity. Therefore, it is doubtful that all these responses were derived from the osmoreceptor. Activation of the vasopressinergic cells by the portal signal would be influenced by sodium receptors rather than by osmoreceptors.

These observations lead us to suggest that hypertonic NaCl signals from the portal vein modulate vasopressin release at the hypothalamic PVN level.

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