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Responses to Osmotic Stimulation of Presumed Vasopressin and Oxytocin Neurons in the Supraoptic Nucleus of Ovariectomized Rats

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Summary. **This study re-evaluated the osmosensitivity of the supraoptic (SON) vasopressin (AVP) and oxytocin (OXT) neurons in rats *in vitro*. Brain slice preparations made from ovariectomized (OVX) rats were incubated in 300 mOsm/kg H_2O artificial cerebrospinal fluid (ACSF) with low-Ca^{2+}(0.2 mM), and high-Mg^{2+}(5.0 mM), to block neuronal activation by way of synaptic inputs. Identified in this study by their discharge patterns according to Poulain and Wakerely were AVP neurons which had a phasic pattern, and OXT neurons, which showed a tonic pattern. In the phasic SON neurons (n=29, 1.74±0.34 Hz, mean±SE), a significant (P<0.0005) increase in the mean discharge rate (5.59±0.77 Hz) was detected in the hyperosmolar condition (340 mOsm/kg H_2O). In contrast to the phasic neurons, the SON neurons (n=14) with a continuous discharge pattern (7.25±1.11 Hz), did not show any significant change in the mean discharge rate in the same hyperosmolar condition (7.27±1.07 Hz). These results suggest that the OXT neuron but not the AVP neuron is much less sensitive to increased osmolality in OVX rats.**

Key words—vasopressin neuron, oxytocin neuron, osmosensitivity, ovariectomy, female rat.

INTRODUCTION

The mechanism of the osmotic release of vasopressin (AVP) and oxytocin (OXT) has been studied using immunoassay, electrophysiology and *in situ* hybridization C-Fos in male subjects. The view is widely held currently that the hypothalamic magnocellular neurons have an osmosensitivity based on recent electrophysiological studies[13,14] in the male sex. The female sex, however, has been often excluded from studies on the central osmoregulatory mechanism in the neurohypophyseal hormone release because cyclic variation in the plasma contents of ovarian sex steroids may exert subtle effects on the functions of the neuronal mechanism in osmoregulation. In fact, past studies have suggested that ovarian steroids affect the basal release of AVP[10,11,18] and induce the release of OXT[19].

In the present study, therefore, we re-examined the osmo-sensitivity of the magnocellular neurons in the supraoptic nucleus (SON) in the female sex following an ovariectomy (OVX) in order to eliminate possible endocrinal effects.

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METHODS AND MATERIALS

Animals and treatment

Twenty-eight Wistar female rats (200-230 g) were used. All experiments were carried out in OVX rats. An OVX was done under ether anesthesia between 2 and 5-weeks before the experiment. The animals were housed in a controlled environment at 22±1°C and 50±5% humidity, with a 14:10 h light-dark cycle (light on at 7: 00 a.m.) . Food and water were provided *ad libitum*. All aspects of the present experiments were conducted in conformity with the guiding principles for the care and use of animals as approved by the Japanese Association for Laboratory

Animal Science (1987).

Brain slice preparation

Animals were decapitated with a guillotine under light anesthesia with ether to obtain extracellular recordings from SON neurons in the brain slice preparations. The whole brain was rapidly removed and placed in cold artificial cerebrospinal fluid (ACSF) at 4°C, and kept in this during the cutting procedures. The hypothalamic block was sliced along the frontal plane to obtain tissue slices 350-450 μm thick. The slices were further trimmed to isolate the SON from surrounding neuronal structures. Recording sites and the isolation of the SON in the slices is shown in Fig. 1. The slices were incubated for at least 1.5 h in aerated (95% O_2-5% CO_2)ACSF to prior recording. The recording procedures were similar to those used in the whole animal preparation[4], except that no antidromic identification of SON neurons was attempted.

ACSF

The regular ACSF was contained in mM: 125 NaCl, 5 KCl, 1.24 KH_2PO_4, 1.3 $MgSO_4$, 26 $NaHCO_3$, 2.0 $CaCl_2$, and Glucose 10.0. Additional sodium chloride was applied for convenience. This solution had an osmolality of 301±2 mOsmol/kg H_2O (N=10). The perfusion medium was continuously buddled with 95% O_2-5% CO2 and had a pH of 7.2-7.4. The medium was perfused into the recording chamber at a flow rate of 2.0 ml/min through polyethylene tubing in a warm bath to maintain its temperature at 37± 0.5 °C. The osmolality of the perfusate as determined with the NaCl content was changed to hypertonic at 320, 340 or 360 mOsmol/kg H_2O. Osmotic stimuli were always started at 200 sec after the control recording. Synaptic transmission was blocked by replacing the standard ACSF perfusate with one containing reduced Ca^{2+}(0.2 mM) and increased Mg^{2+} (5.0 mM), as described by Alger et al.[6] and Qu et al. [16]. Additional sodium chloride was also applied for osmotic adjustment (293-305 mOsm/kg H_2O). This type of ACSF is designated as a low-Ca^{2+}, high-Mg^{2+} -ACSF in the interatue.

Electrical stimulation

Synaptic inputs to the recorded SON neurons were evaluated with constant current stimulation (500 μs duration, 0.82Hz) of the perinuclear regions of the SON and by a coaxial bipolar stimulating electrode made from stainless steel wire and tubing. All tests for blocking the synaptic inputs were examined under the threshold stimulus intensity (80-125 μA) to avoid the induction of a compound and complicated orthodromic response. Coaxial bipolar electrodes were constructed from 0.8 mm outer diameter hypodermic tubing and 120 μm thick stainless-steel wire. A peristimulus-time histogram (PSTH) was constructed on- and off-line by using a MacLab system (ADInstruments Corp.) .

Statistical analysis

After stabilization of the neuronal activity, the subsequent 10-min discharge was defined as mean discharge rate of the particular neuron. The effects of the osmitic stimuli on the neuronal discharge were determined by measuring the mean discharge rate in the 50-150 sec following the onset of stimuli. The data on the effects of the osmotic stimuli on the spontaneous discharge of the neurons were analyzed with the randomization test for paired samples[12].

Histological localization of neurons

Positions of the recording electrode were marked by electrophoretically applied dye (Pontamine Sky Blue 6B) in the slice preparation. Frozen serial sections of the formalin-fixed brain tissue were made at 25 μm. Locations of the recording site were determined in cresyl violet-stained sections (see Fig. 1).

RESULTS

Characteristics of SON neurons

Extracellular activity of a total of 67 neurons was recorded from the SON in twenty-nine brain slice preparations of the OVX rat (n = 28). Among them, 32 neurons (48%) had a clear phasic pattern in the spontaneous discharge. An important criterion for classification as the phasic type was that the neuronal discharge activity was interrupted by distinct periods of complete silence which began and terminated abruptly. Other SON 17 neurons (25%) had a discharge pattern with a constant discharge rate. These neurons were designated as a continuous type in this study. According to Poulain and Wakerley[15], the former type of neuron was considered as"vasopressin (p-AVP) neurons", and the latter as the" oxytocin (p-OXT) neurons" . Both types of neurons were recorded well within the nuclear boundary of the SON (Fig. 1). The neurosecretory products (AVP and OXT) of these neurons can be detected and measured in the plasma, and several physiological reflexes cause a selective activation of these neurons, resulting in the release of detectable quantities of the hormones, AVP and OXT.

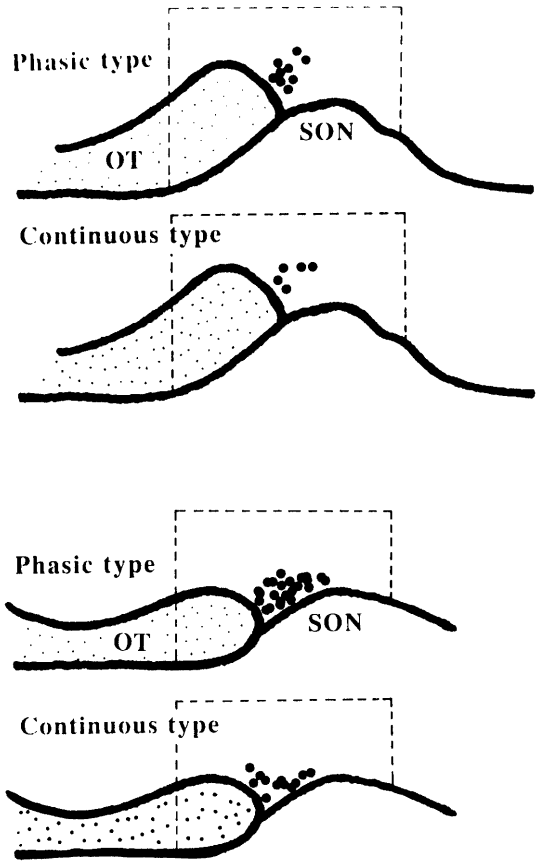

Fig. 1. Histological mappings of the recorded neurons (*black circles*) identified as a p-AVP (Phasic type) and p-OXT (Continuous type) neuron in the SON. The SON was isolated from surrounding structures at the broken lines. Upper and lower paired parts showed two different sections in the coronal atlas of the rat hypothalamus according to the atlas of the brain by Albe-Fessard et al[5].

The remaining 18 neurons (27%) could not be identified because of their irregular and random discharge patterns. Although their locations were completely confined to the SON, they were not involved in the analysis in this study.

Blockade of synaptic inputs

Three phasic SON neurons and two SON neurons of the continuous type were examined for the blockade of synaptic volley following a single stimulus (80–125 μA intensity, 0.5 ms duration, 0.82 Hz) to the perinuclear region by replacing the standard ACSF (Ca^{2+}, 2.0 mM and Mg^{2+}, 1.3 mM) perfusate with ACSF containing reduced Ca^{2+}(0.2 mM) and increased Mg^{2+} (5.0 mM) . Fig. 2 shows two examples of PSTH in which excitatory (A) and inhibitory (B) synaptic effects with short latency (less than 20 msec) were completely blocked by low-Ca^{2+}, high-Mg^{2+} ACSF perfusion. In all phasic neurons and a neuron of a continuous type of neuron, the basal discharge rate of the SON neurons was elevated slowly from 20 to 30 min after the perfusion of low-Ca^{2+}, high-Mg^{2+} -ACSF. The remaining one neuron of the continuous type did not show any change in discharge rate. Synaptic blockade was reversed by replacing low-Ca^{2+}, high-Mg^{2+} ACSF with the standard ACSF, and basal activity returned to its initial level as shown in Fig. 2A and B. In two phasic neurons showing excitation, recovered orthodromic responses were obviously much greater than those of the initial ones (Fig. 2A) .

Effects of osmotic stimulus

The extracellular activity of 29 phasic and 14 continuous types of neurons was recorded from the SON for more than 25 min, during which period their stability and the pattern of spontaneous discharge were established. Their mean discharge rates were 1.74 ± 0.34 and 7.25 ± 1.11 Hz (mean$\pm$SE) , respectively, in the neutral (300 mOsm/kg H_2O) low-Ca^{2+}, high-Mg^{2+}-ACSF.

In the phasic SON neurons (n=29), the mean discharge rate increased significantly ($P<0.0005$, $5.59\pm$ 0.77 Hz, mean$\pm$SE) in the hyper-osmotic condition (340 mOsm/kg H_2O) as shown in Fig. 3. In contrast to this, in the continuous neurons (n = 14), the mean discharge rate was not significantly changed in the same hyperosmolar state (7.27 ± 1.07 Hz) (Fig. 3).

DISCUSSION

In this study, neurons were recorded within the SON, and the discharge patterns of spontaneous activity were classified into p-AVP and p-OXT neurons by the criteria proposed by Poulain and Wakerley[15] in the female rat and consolidated by Yamashita et al.[20], Cobbett et al.[9] and Armstrong et al.[7] in the male rat by a combination of electrophysiology and immunocytochemistry.

It is now generally accepted that the neural inputs from the peripheral and central osmoreceptors converge on the AVP and OXT neurons in the hypothalamus. Therefore, even in this *in vitro* study, osmosensitive neuronal elements might remain in the slice preparation examined. In order to avoid that possibility, we tried to record the osmosensitivity of the SON neurons in the modified ACSF which has

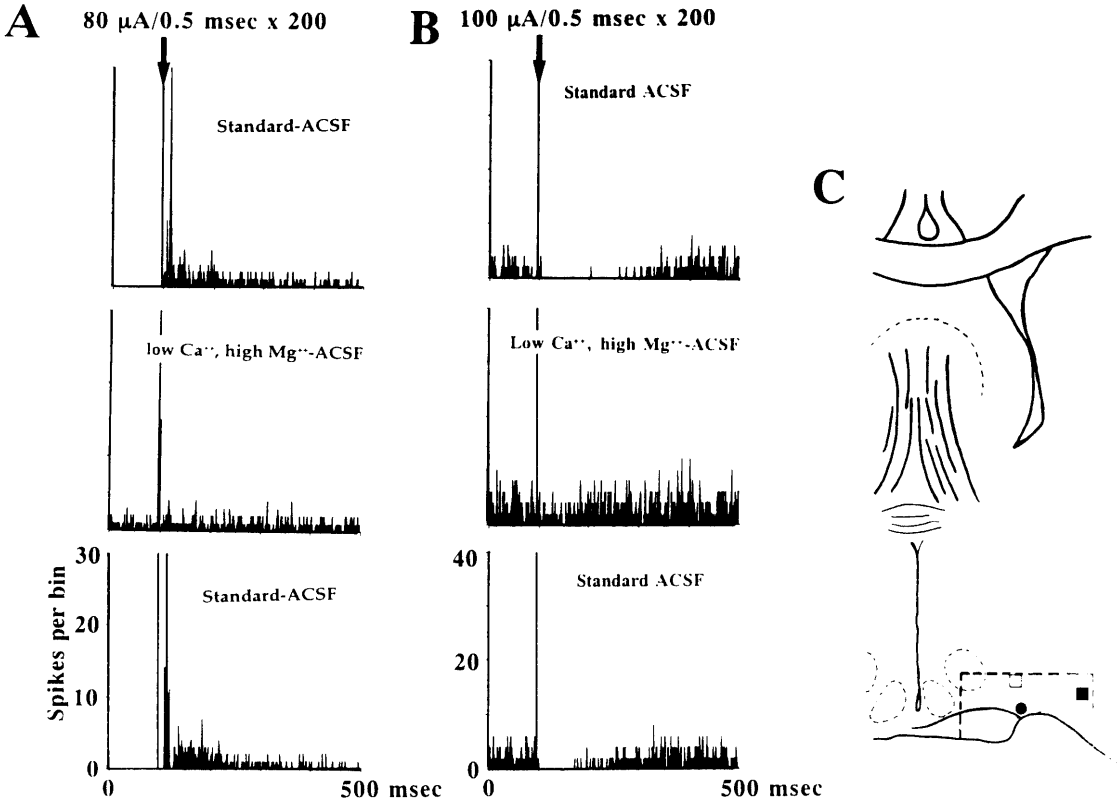

Fig. 2. Peristimulus time histograms (200 sweeps, 1 ms per bin) of two SON neurons that were excited (**A**; p-AVP neuron) and inhibited (**B**; p-OXT neuron) by threshold stimulation of the perinuclear region of the SON. Recording (*closed circle,* **A**; *open circle,* **B**) and stimulation (*closed square,* **A**; *open square,* **B**) sites are illustrated in **C**. Respective excitatory (**A**) and inhibitory (**B**) responses induced by threshold stimuli were blocked during perfusion of low-Ca^{2+}, high-Mg^{2+} ACSF.

been confirmed to block synaptic transmission in the mammalian central nervous system[6,16]. Mean discharge rates in SON neurons of both the phasic and continuous types, p-AVP and p-OXT neurons, seemed to be higher than those in our previous *in vitro* studies carried out in OVX rats[1,2,3]. This may depend mainly upon the removal of synaptic effects by using a low-Ca^{2+}, high-Mg^{2+} -ACSF. Because the discharge rates in some phasic and continuous types of SON neurons were elevated by replacing the standard ACSF by low-Ca^{2+}, high-Mg^{2+} -ACSF, it is suggested that inhibitory effects predominate in the perinuclear and/or intranuclear neuronal mechanisms. Another possibility is that concentrations of the ions, especially low-Ca^{2+}, might influence the membrane potential mechanisms governing the threshold for generation of the spontaneous discharge in the neuron. The higher osmolality (300 mOsm/kg H_2O) of ACSF may also affect membrane potential in the AVP neurons because they are osmosensitive[8,13,14].

In the male rat, Bourque and Renaud[8]has shown that the SON magnocellular neurosecretory neurons, either with phasic, continuous, or irregular discharge patterns, increased their discharge activity in response to osmotic elevation of the Mg^{2+} content in the perfused medium up to 12 mM. Mason[14] also has revealed that, in an *in vitro* study using elevated Mg^{2+}, SON neurons were osmosensitive in the male rat. In these studies, presynaptic elements were successfully removed by increasing the Mg^{2+} content up to 15 mM in the perfused medium. These above studies[8,14] have very clearly established that the SON neurons with both a phasic and continuous discharge pattern were sensitive to osmotic elevation of the perfused medium. The SON p-AVP neurons showing a phasic discharge in this study were certainly osmosensitive as has been designated in past studies. However, in the present study, the SON p-OXT neurons with a continuous discharge pattern were relatively insensitive to the osmotic elevation.

The discrepancies in osmosensitive neuronal populations between ours and past studies[8,14] may be due

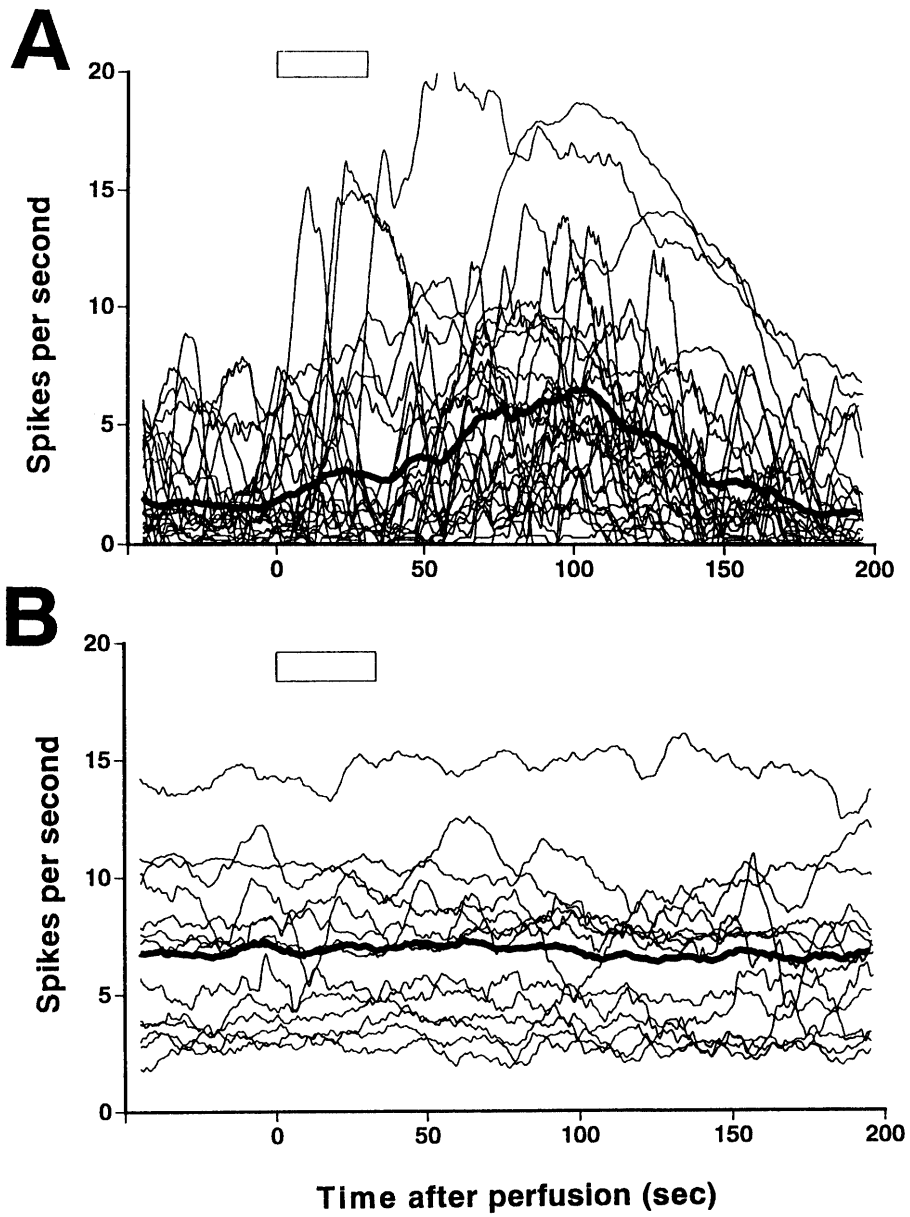

Fig. 3. Responses obtained from the p-AVP and p-OXT neurons in the SON. Averaged and smoothed (by simplified least squares procedures of back-and-forth 101 points) ratemeter recordings of all p-AVP (phasic: *upper, thinner lines*) and p-OXT (continuous: *lower, thinner lines*) neurons examined. Thicker lines are the mean responses of the p-AVP (*upper*) and p-OXT (*lower*) neurons. Perfusion of the hypertonic ACSF (340 mOsm/kg H_2O) was done for 30 sec (*square*) .

to sexual differences between males and females. However, this point remains to be elucidated because no comparative study under the same experimental conditions has been previously made concerning sexual differences in osmosensitivity in the AVP and OXT neurons *per se*. Furthermore, gonadal endocrine functions would affect the osmosensitivity in female OXT neurons. In fact, some past studies using female subjects have suggested that the endocrine function of the ovary may affect the osmosensitive mechanisms in the central nervous system[3,10,11]. All rats used in this study were ovariectomized to exclude possible endocrinal effects of the ovary on the hypothalamic mechanisms. The ovariectomy may affect the osmosensitivity of the hypothalamic OXT neurons because it is widely accepted that they are estrogen-concentrating[17], and ovarian hormones affect the electrophysiological characteristics of the p-OXT neuron in the hypothalamus[1]. A further estrogen-supplemented study will be necessary for a final answer to this question.

Another possibility is that the discrepancy in the osmosensitivity of p-AVP and p-OXT neurons between ours and previous studies[8,14] may depend upon the ion composition of the perfused medium. In previous studies, the concentration of Mg^{2+}(12-15 mM) , but not Ca^{2+}, was increased to block orthodromic volleys to the SON from other neuronal structures in the brain slice recorded. Different ion components in the perfusate may possibly produce a different response in the p-OXT neuron.

An additional possibility that a higher discharge rate in the p-OXT neurons may mask the further increase in response in the hyperosmotic state is unlikely because even some of p-OXT neurons which showed a relatively low discharge rate did not increase their activities in the hyperosmotic state as shown in Fig. 3B.

In conclusion, in the OVX rat, the p-OXT neuron with a continuous discharge pattern in the brain slice preparation was less sensitive to elevation in the osmolality of the perfused medium than the p-AVP neuron. On this point, the female OXT neuron would have a different mechanism from that in the male sex in their osmotic release of OXT.

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