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Oxytocin cells in the paraventricular nucleus receive excitatory synaptic inputs from the contralateral paraventricular and supraoptic nuclei in lactating rats

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HIGHLIGHTS

- Electrical stimulation of contralateral SON or PVN orthodromically excited OT cell.
- Proportion of excited OT cell was greater than that of vasopressin cell.
- Train stimulation of contralateral SON or PVN increased firing rate of OT cell.

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ABSTRACT

The present experiments were undertaken to examine whether oxytocin cells in the paraventricular nucleus receive synaptic inputs from the contralateral supraoptic or paraventricular nucleus. Using urethane-anesthetized lactating rats, extracellular action potentials were recorded from single oxytocin or vasopressin cells in the paraventricular nucleus. Electrical stimulation was applied to the contralateral supraoptic nucleus or paraventricular nucleus, and responses of oxytocin or vasopressin cells were analyzed by peri-stimulus time histogram or by change in firing rate of oxytocin or vasopressin cells. Electrical stimulation of the contralateral supraoptic nucleus or paraventricular nucleus did not cause antidromic excitation in oxytocin or vasopressin cells but caused orthodromic responses. Although analysis by peri-stimulus time histogram showed that electrical stimulation of the contralateral supraoptic nucleus or paraventricular nucleus caused orthodromic excitation in both oxytocin and vasopressin cells, the proportion of excited oxytocin cells was greater than that of vasopressin cells. Train stimulation applied to the contralateral supraoptic nucleus or paraventricular nucleus at 10 Hz increased firing rates of oxytocin cells and decreased those of vasopressin cells. The results of the present experiments suggest that oxytocin cells in the paraventricular nucleus receive mainly excitatory synaptic inputs from the contralateral supraoptic nucleus and paraventricular nucleus. Receipt these synaptic inputs to oxytocin cells may contribute to the synchronized activation of oxytocin cells during the milk ejection reflex

Oxytocin (OT) cells in the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON) project to the posterior pituitary and release OT into blood circulation from their axon terminals in the posterior pituitary. In lactating rats, OT is released intermittently as a result of periodic and synchronized activation of OT cells distributed in the bilateral SON and PVN, whereas pups suck nipples continuously [1–4,20].

The synchronization of the bursting activity of OT cells during the milk ejection reflex may include two types of synchronization, i.e., intranuclear and internuclear synchronization. The former was suggested to occur by local glutamatergic drive onto OT cells [9]. The latter mechanisms, which underlie the coordinated synchronized activation of OT cells distributed in 4 separate magnocellular nuclei, are not fully known at present. Our previous studies on the DMH showed that the cells in one side of the DMH projected to the SON in both sides of the hypothalamus and that some of such cells showed bursting activity preceding milk ejection [5–7]. These findings suggest that projections to OT cells in the bilateral SON from unilateral DMH may contribute to the synchronization of bursting activity of OT cells in the bilateral hypothalamus. Although such

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type of projections from the DMH to the SON may contribute to the synchronized activation of OT cells during the milk ejection reflex, the projections of this type were not many [7]. It seems likely that other mechanisms also contributed to synchronized activation of OT cells preceding milk ejection. Our previous study showed that OT cells in the SON receive excitatory synaptic inputs from contralateral SON or PVN and suggested such synaptic input may contribute to the synchronization of bursting activity of OT cells in bilateral hypothalamus [8]. The present experiments were undertaken to examine whether OT cells in the PVN also receive excitatory input from the SON or the PVN in the contralateral hypothalamus.

Animals were handled in accordance with the Guidelines for the Care and Use of Laboratory Animals of Fukui Prefectural University. Adult female Wistar rats (250–320 g B.W.) were used on days 8–12 of lactation under urethane–anesthesia (1.1 g/kg, i.p.). An inguinal mammary gland was cannulated with a polyethylene tube to measure intramammary pressure, which was used to detect milk ejection. A silicone cannula was inserted into the right atrium through the right jugular vein for injection of oxytocin, which was used to check whether measurement of intramammary pressure worked well. The rat was then fixed prone in a stereotaxic frame. Three small holes were drilled in the skull for insertion of recording and stimulating electrodes. The stereotaxic coordinates of Paxinos and Watson [12] were used for insertion of electrodes. A side-by-side stimulating electrode comprised of stainless steel wire (200 μm diameter), which was slanted laterally at an angle of 6 degrees to the vertical line, was inserted into the neurohypophysis (4.0 mm anterior to the interaural line, midline, 0–0.2 mm dorsal to the interaural line) in order to antidromically identify neurons in the PVN projecting to the neurohypophysis. After securing the stimulating electrode inserted into the neurohypophysis with acrylic resin and self-tapping screws in the skull, the same type of stimulating electrodes were inserted into the right SON (7.8 mm anterior to the interaural line, 1.7 mm lateral to the midline, 0.1 mm dorsal to the interaural line) or PVN (7.2 mm anterior to the interaural line, 0.5 mm lateral to the midline, 2.5 mm dorsal to the interaural line). These stimulating electrodes were also secured in place with acrylic resin and self-tapping screws in the skull.

A glass micropipette (tip diameter, 1 μm; impedance, 20–30 MΩ) filled with 0.5 M sodium acetate containing 2% Pontamine sky blue 6B (Tokyo Chemical Industry Co., Ltd., Japan) was introduced into the left PVN. Pontamine sky blue 6B was used to mark the recorded site when it was necessary. Extracellular recordings were then made from single neurons. Recorded neurons were further identified as projecting to the neurohypophysis by their antidromic responses to electrical stimulation of the neurohypophysis. Identified PVN neurons were further divided into two groups according to their response to suckling. Eight to 11 pups were applied to a mother's nipples, and the milk ejection reflex was induced. Neurons that showed a brief high frequency burst of action potentials approximately 10–20 s before milk ejection that was detected by a sharp increase in intramammary pressure were designated as putative OT cells [1,10,11,13,20] and neurons that did not show bursts before milk ejection were classified as putative vasopressin (VAP) cells [13]. Then neurons were tested for their response to electrical stimulation of the contralateral SON or PVN. To collect data for peri-stimulus time histograms (PSTH), a hundred sets of electrical stimulus pulses (2 monophasic pulses with a 5-ms interval; current intensity, 1 mA; pulse duration, 0.5 ms) were applied to the contralateral SON or PVN at 2-s intervals. When the averaged number of spikes for 25 ms after stimulation increased by more than 100% compared with the averaged number before stimulation, the response was regarded as orthodromic excitation (OD+). When the silent period continued for more than 25 ms after electrical stimulation, the response was regarded as orthodromic inhibition (OD−). When the two above-mentioned responses were observed consecutively, the response was regarded as orthodromic inhibition followed by excitation (OD±). In some of the cells analyzed by PSTH, the effects of train electrical stimulation of the contralateral SON or PVN on firing rate were also analyzed. Electrical stimulus pulses (monophasic pulse; current intensity, 1 mA; pulse duration; 0.5 ms) were applied to the contralateral SON or PVN at 10 Hz, 5 Hz and 2 Hz for 100 s. Mean firing rates for 100 s before stimulation and those during stimulation were compared.

After the end of each experiment, a direct current of 1 mA was passed through each stimulating electrode for 5 s to mark

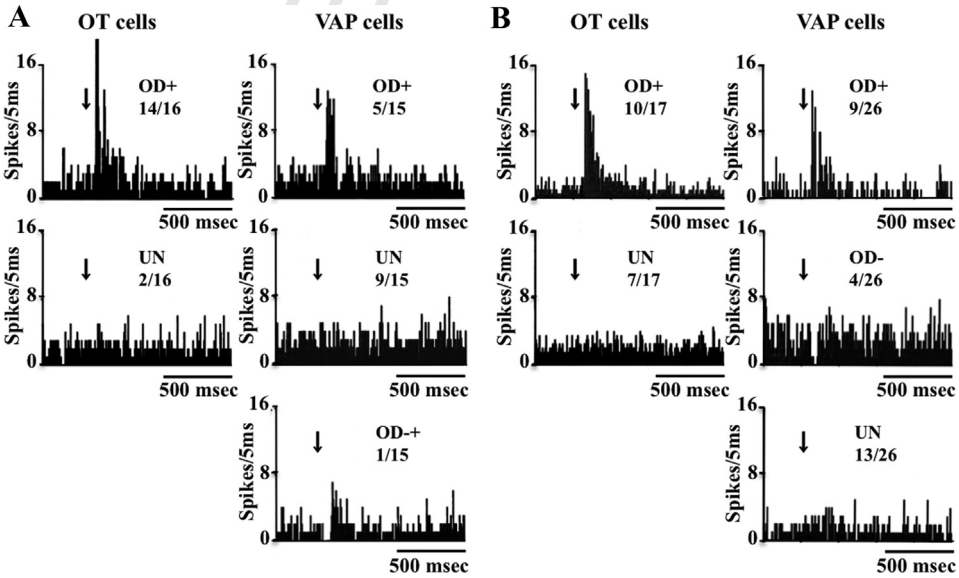

Fig. 1. Examples of PSTH analysis of responses in putative OT and VAP cells in the SON to electrical stimulation of the contralateral SON (A) and PVN (B). Electrical stimulation with a current intensity of 1 mA was applied at each allows. OD+, orthodromic excitation; OD−, orthodromic inhibition; OD±, orthodromic inhibition followed by excitation; and UN, unresponsive. The number in each histogram indicates the number of cells that showed each type of response.

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