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Hypertrophy of Oxytocinergic Magnocellular Neurons in the Hypothalamic Supraoptic Nucleus from Gestation to Lactation

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ABSTRACT—In the present experiments, we examined the changes in cell size (profile area) of oxytocinergic magnocellular neurons during reproductive states and dehydration with quantitative immunohistochemistry. During lactation, hypertrophy was observed in oxytocinergic magnocellular neurons but not in vasopressinergic ones in the supraoptic nucleus. In virgin rats, chronic dehydration increased the cell size in both oxytocinergic and vasopressinergic neurons. After normal weaning time, the cell size decreased, returning to virgin level within 20 days. However, if the mothers were deprived of their litters immediately after parturition, the cell size rapidly returned to virgin level within 5 days. Furthermore, the increase in the cell size of the mothers was not affected by the size of their nursing litters.

INTRODUCTION

Evidence has been accumulated, indicating that magnocellular neurons in the supraoptic nucleus (SON) of the hypothalamus reveal striking morphological changes, called structural plasticity, in response to chronic physiological stimuli such as lactation and dehydration (Hatton, 1990). Many of these neurons are contacted by the same presynaptic terminals (multiple synapses) in lactating animals, although they are rare in non-lactating virgin females (Montagnese *et al.*, 1987; Theodosis and Poulain, 1984). Concurrently, the surface membranes of magnocellular neurons are extensively in direct membrane contact (juxtaposition) without the intervening of glia or neuropiles (Hatton and Tweedle, 1982; Montagnese *et al.*, 1987; Theodosis and Poulain, 1984). During lactation, it has been shown that these morphological changes occur in oxytocinergic magnocellular neurons, but not in vasopressinergic ones (Theodosis *et al.*, 1986). The ratio of juxtaposition in oxytocin magnocellular neurons was low two weeks after the beginning of pregnancy, but it was elevated just prior to parturition, retained during lactation, and returned to virgin level again one month after post-weaning (Montagnese *et al.*, 1987; Theodosis and Poulain, 1984). At present, this structural plasticity is considered to contribute to increase (directly or indirectly) the excitability of oxytocinergic magnocellular neurons so that sufficient oxytocin can be released to trigger milk-let down from the mammary gland in response to suckling stimulation (Theodosis and Poulain, 1992).

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In our previous study, it has been shown that time course changes of the ratio of membrane juxtaposition are similar to that of the cell size of the magnocellular neurons during dehydration and rehydration (Miyata *et al.*, 1994a). However, no study was made on the time course changes of the cell size during reproductive states and dehydration in virgin rats. Therefore, in present experiments, we examined the time course changes of the cell size of oxytocinergic and vasopressinergic magnocellular neurons during gestation, parturition, lactation, and post-weaning. Further, we examined the effect of the removal of litters and the size of nursing litters on the increase of the cell size during lactation.

MATERIALS AND METHODS

All experiments were performed on female Wistar rats (15 – 20 weeks), housed in controlled environments (light on 07:00 to 21:00, 22 ± 2°C) with food and water *ad libitum*. The immunohistochemistry was performed following reproductive states: 1) non-stimulated virgin (at diestrous); 2) gestation 10 and 20 days; 3) parturition (2 hr after the first pup was expelled); 4) lactation 10 and 20 days; 5) post-weaning 10, 20, and 30 days; 6) arrested lactation (rats were allowed to give birth but all their pups were removed at parturition) 5, 10, and 20 days. In this experiment, the size of nursing litters was fundamentally adjusted to 10 pups just after parturition. In another experiment, the size of the nursing litters was adjusted to 2 or 20, or virgin rats were deprived of water for 7 days. Four animals were examined at each time point except for the controls (n=10) or those undergoing dehydration (n=5).

The rats were anesthetized with pentobarbital(50 mg/kg, ip) and perfused through the heart with phosphate buffer saline (PBS) containing heparin followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The blocks containing the hypothalamus were dissected out and further fixed in the above fixative for 48 hr at 4°C. Twenty micron coronal sections were cut using a freezing microtome. Freely floating sections were incubated either with anti-

oxytocin rabbit antibody (UCB Bioproduct, Belgium,SA, diluted 1:120,000) or with anti-vasopressin rabbit antibody (Chemicon Int. Inc., Temecula, CA, diluted 1:40,000 in PBS containing 10^{-7} M oxytocin, Sigma) for 48 hr at 4°C. The primary antibodies were then reacted with biotin-labeled anti-rabbit immunoglobulins and finally with an avidin-biotin peroxidase complex (Vector, Burlingame, CA). The peroxidase was visualized with 3, 3'-diaminobenzidine (Sigma Chemical Co. Ltd., St. Louis) as chromogen. Quantitative analysis of the soma size was performed according to our previous method (Miyata *et al.*, 1994b; Miyata *et al.*,1995). All data were expressed as mean ±SD. The values of group means were derived from animal means. Statistical differences were analyzed via an analysis of variance (ANOVA) and Newman-Keuls tests.

RESULTS AND DISCUSSION

Oxytocinergic magnocellular neurons tend to localize more dorsally in the SON, but vasopressinergic neurons tend to distribute more ventrally in the SON (Fig. 1A, B). In lactating females, hypertrophy was seen only in oxytocinergic magnocellular neurons. It was not observed in vasopressinergic neurons (Fig. 1E, F) compared to virgin ones (Fig. 1C, D).

The time course changes in the cell size of oxytocinergic magnocellular neurons during gestation, parturition, lactation, and post-weaning are shown in Figure 2. The cell size did not significantly increase during gestation. But it enlarged significantly from parturition to post-weaning 10 days, and returned to virgin level at post-weaning 20 days. When mothers were allowed to give birth, but all their litter was removed at parturition, the cell size

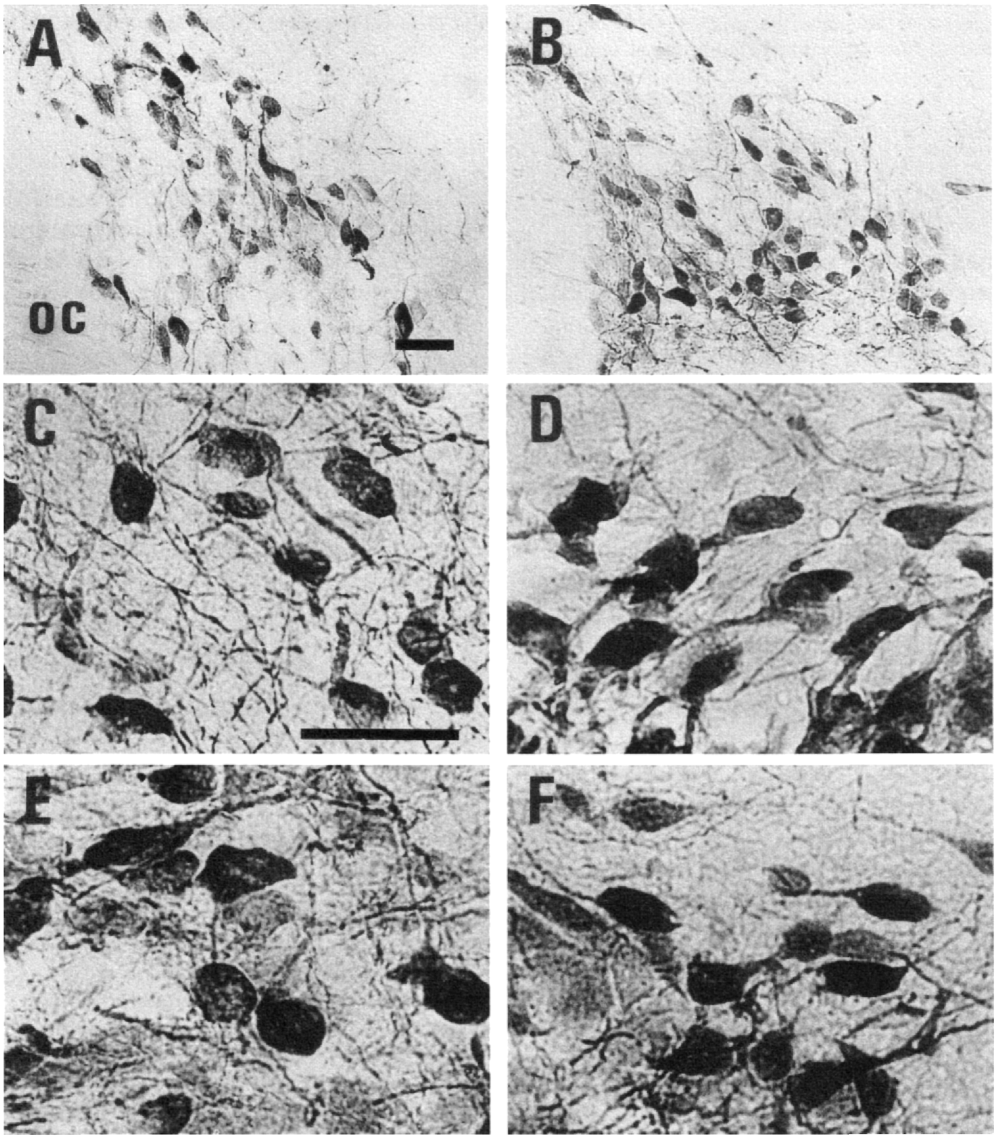

Fig. 1. Photographs showing oxytocinergic (A, C and E) and vasopressinergic (B, D and F) magnocellular neurons in the SON of virgin (A, B, C, and D), and lactating (E and F). Oxytocinergic magnocellular neurons in lactating females reveal hypertrophy, but vasopressin ones did not show. OC; optic chiasma. Scale bar = 50 μm.

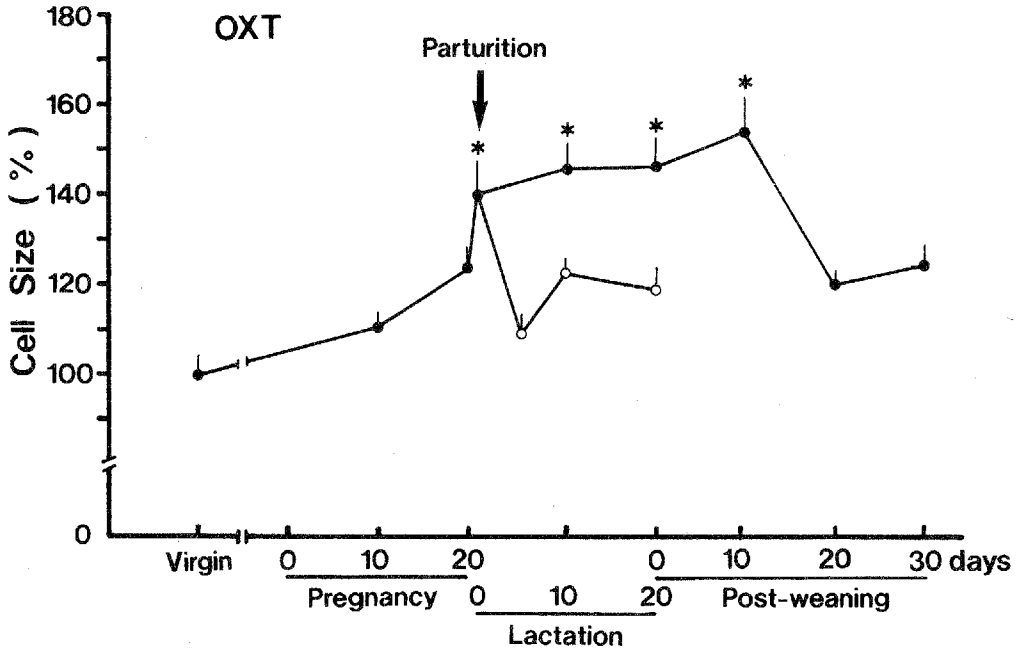

Fig. 2. Changes in the cell size of oxytocinergic magnocellular neurons in the SON during gestation, parturition, lactation, and postweaning. The soma size was significantly increased from early lactation to post-weaning 10 days, but it returned to virgin level at post-weaning 20 days (—●—). Removal of nursing litter immediately after parturition rapidly reduced the sell size to return to virgin level (—○—). Statistically significant vs. virgin (* $p<0.01$, ** $p<0.001$ by ANOVA and Newmann-Kleus). OXT = oxytocin.

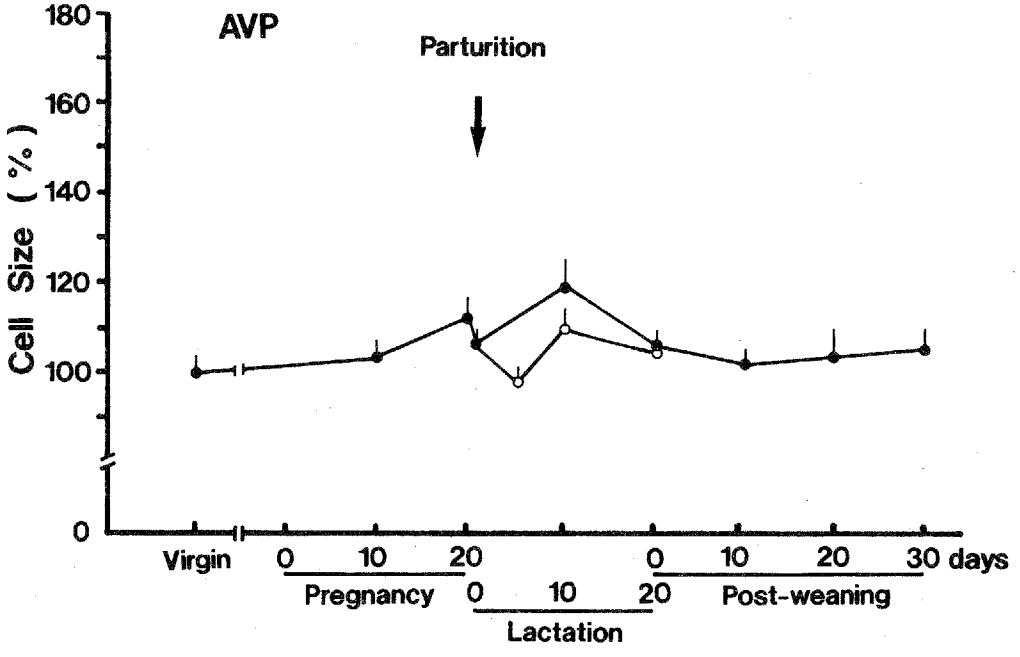

Fig. 3. Changes in the soma size of vasopressinergic magnocellular neurons in the SON during gestation, parturition, lactation, and post-weaning. There was observed no significant hypertrophy in vasopressinergic magnocellular neurons at any reproductive states. AVP = vasopressin.

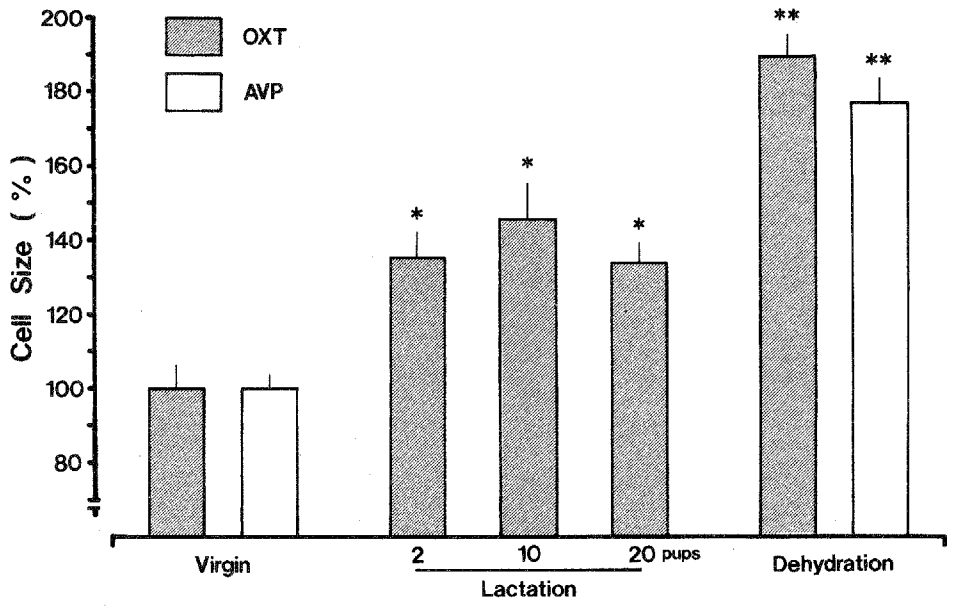

Fig. 4. Comparison of increase in the cell size of oxytocinergic and vasopressinergic magnocellular neurons when the number of nursing litter was changed to 2, 10, or 20 litters, and comparison of them between lactating and dehydrated females. Statistically significant vs. virgin (*$p<0.01$, **$p<0.001$ by ANOVA and Newmann-Kleus). OXT = oxytocin, AVP = vasopressin.

immediately returned to the virgin level within 5 days. By contrast, the cell size of vasopressinergic magnocellular neurons did not significantly increase in any reproductive state (Fig. 3), although the cell size in both oxytocinergic and vasopressinergic magnocellular neurons increased in virgin rats during dehydration (Fig. 4).

To examine the effects of intensity of suckling stimulation on the increase in cell size of oxytocinergic magnocellular neurons, the number of nursing litters were changed to adjust to 2, 10, or 20 after parturition (Fig. 4). The increase in the cell size of the oxytocinergic magnocellular neurons in the mothers was not affected by the size of their nursing litters during lactation.

In the present study, it is apparent that the cell size of the oxytocinergic magnocellular neurons increased during lactation, but vasopressinergic ones did not change. The present time course changes of cell size in oxytocinergic magnocellular neurons is very similar to that of an ultrastructural change such as membrane juxtaposition reported by others (Theodosis and Poulain, 1984). Moreover, the present result strongly coincides with the ultrastructural observation that the plastic changes are restricted in oxytocinergic neurons during lactation. During dehydration, we have already shown that the time course changes of cell size strongly correlate with that of membrane juxtaposition. Therefore, it is concluded that the analysis of the soma size in the magnocellular neurons is a convenient and useful indicator to predict the plastic changes of the ultrastructures (Miyata *et al.*, 1995).

In this experiment, when the mothers were deprived of their litters immediately after parturition, the cell size of the oxytocinergic magnocellular neurons rapidly returned to their virgin level. Therefore, suckling stimulation is seen as the most important factor in maintaining the hypertrophy of the oxytocinergic magnocellular neurons during lactation (Miyata *et al.*, 1994b; Theodosis *et al.*, 1986). Similar results are found in the ultrastructural changes of lactating rats (Theodosis and Poulain, 1984). Moreover, the present results reveal that the size of nursing litters does not affect the increase in the cell size of oxytocinergic magnocellular neurons during lactation; Only two litters had the same ability as 20 litters in maintaining the increase in cell size. During chronic dehydration, the cell size of the magnocellular neurons gradually increased in response to daily elevation of the plasma osmolarity (Miyata *et al.*, 1994a). However, pulsatively increase in the level of plasma oxytocin triggers the milk-let down from the mammary gland in response to suckling stimulation during lactation. The hypertrophy of the magnocellular neurons is owing to the development of intracellular organelles, which are involved in the synthesis of hormones, by the increased demands for oxytocin during lactation, and for oxytocin and vasopressin during dehydration (Lightman and Young, 1987; Vantol *et al.*, 1988). Therefore, we suppose that the suckling stimulation may be transmitted through the spinal cord, and integrated as an input information in the central pacemaker region such as a population of limbic neurons in the bed nuclei of the stria terminalis (Ingram *et al.*, 1995), to induce the hypertrophy of oxytocinergic magnocellular neurons.

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