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The hypothalamic magnocellular neurosecretory system in developing rats

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SUMMARY

Studies concerning the development of the magnocellular system are scarce and discordant in literature. We carried out an immunohistochemical study on supraotic and paraventricular hypothalamic nuclei using antivasopressin and antioxytocin antibodies in developing rats between the 15th day of intrauterine life and the 6th day of postnatal life. In addition, we performed RT-PCR experiments to establish the stage at which these hormones appear and neurosecretory activity commences. The results showed that supraoptic and paraventricular nuclei appear, respectively, on the 16th and the 18th day of intrauterine life and both immediately synthetize vasopressin neurohormone. By contrast, synthesis of oxytocin takes place from the 2nd day after birth. Probably, these nuclei synthetize oxytocin in conjunction with the decline of placental maternal oxytocin.

INTRODUCTION

The hypothalamic anterior area plays an important functional role. In this area, besides the parvocellular nuclei, nuclei of the magnocellular system can be found: supraoptic (SO), paraventricular (PV) and accessory. These nuclei synthetize and release two neurohormone: vasopressin and oxytocin (Dierickx and Vandesande, 1979; Sofroniew and Glassmann, 1981); and studied were performed in various mammalian species: guinea pig (Sofroniev *et al.*, 1979), monkey (Antunes and Zimmermann, 1978), rat (Vandesande and Dierickx, 1975) and man (Dierickx and Vandesande, 1979).

By contrast, only few studies were carried out on the hypothalamic magnocellular nuclei during development, and discordant results were found. Ifft (1972), using an autoradioghraphic technique, observed that hypothalamic nuclei arose from the 10th to the 22nd day of pregnancy, according to lateromedial and dorsoventral gradients. Indeed, in relation to the III ventricle, the more laterally placed nuclei generally arose on the 14th day of gestation, while the medial nuclei arose on the 16th day of gestation. Ifft (1972), furthermore, observed that the parvocellular part of the PV nucleus arose on the 16th day, while the magnocellular portion of the same nucleus arose on the 14th day, simultaneously with the appearance of the supraoptic nucleus.

Choy and Watkins (1979), from immunohistochemical studies performed in rats, showed that vasopressin is present in the SO and PV nuclei starting from the 19th day in foetuses, and oxytocin from the 4th day in newborns. Recently, Lazcano *et*

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al. (1990) carried out a histochemical study on the developing SO nucleus and observed that vasopressin appears from the 18th day of development, while oxytocin is present from the 4th day in newborns. In the present study, we used immunohistochemical techniques and molecular methods to establish the time of appearance of the magnocellular nuclei, the time when their neurosecretory activity begins, and whether the neurosecretory activity of each nucleus is related to the simultaneous secretion of both vasopressin and oxytocin neurohormones or to secretion of a single hormone.

MATERIALS AND METHODS

Tissue preparation

Ten pregnant female Wistar rats were kept under standard conditions of housing. They were deeply anaesthetized with pentobarbital and were perfused via the aorta with Bouin's solution. The embryos at 15, 16, 18 and 21 days of gestation were removed in toto and fixed by immersion in Bouin's solution. Newborns from 0 to 6 days old were fixed by immersion in Bouin's solution. Brains were removed and fixed in the same fixative, dehydrated in a graded series of ethanol and embedded in paraffin. Six micra thick serial sections were prepared; some specimens were stained with the Giemsa method, others immunostained with anti-vasopressin (1:1000) and antioxytocin (1:200) polyclonal antibodies (Chemicon) in 0.05 M TRIS-HCl saline buffer pH 7.2 for 12 hours at 4°C, and detected with the avidin-biotin complex or the streptavidin system. The negative controls were carried out as a) treatment with non immunized antiserum ; b) omission of the primary antiserum.

RNA isolation and purification

Tissue samples were homogenized in Trizol reagent (Life Technologies, Gibco) using a glass Potter. Lysates were incubated for 5 min at RT and were twice extracted with phenol/chloroform. RNA was precipitated with isopropyl alcohol for 10 min at RT and the resulting pellet was washed with 75% ethanol. The dry RNA pellet was resuspended with DEPC water and incubated for 10 min at 60°C. RNA was quantified at 260nm and its integrity was determined with 1.4% denaturating agarose gel.

Reverse transcription-PCR

Total cellular RNAs were extracted from rat hypothalamic areas at different stages of development (16, 18, 21 days and adult) and were used as template in a reverse transcriptase reaction (Retroscript, Ambion) for the corresponding cDNA synthesis. 2ug RNA, dNTP, and oligo dT primers were mixed and heated for 3 min at 80°C; then placental Rna-ase inhibitor and M-MLV reverse transcriptase were added. Finally, this reaction was inactivated at 92°C for 10 min. The primers used for vasopressin and oxytocin DNA amplification were, respectively:

5'-d CTCACCTCTGCCTGC-3'and 5'-d CTCGACGACGAATCC-3;

5'-d GCTCTGACCTCCGCC-3'and 5'-d CCGTAGACGACATCG-3'.

We used the following thermocycle profile with a Hybaid PCR Express Thermal Cycler:

1. Heat 3 min at 95°C
2. Cycle 35: (95°C for 30 sec ; 52°C for 30 sec; 72°C,for 40 sec)
3. Final extension: 10 min at 72°C.
4. Amplified products were quantified at 260nm and visualized by ethidium bromide staining of 1.5% agarose gel.

RESULTS

The SO and PV nuclei are morphologically invisible by light microscopy in the rat embryos at the 15th day. Moreover, the corresponding hypothalamic area is immunonegative for vasopressin and oxytocin.

By contrast, the SO nucleus is visible laterally to the optic chiasma in the rat embryos at the 16th day. This nucleus shows few neurons and an immunopositivity for vasopressin (Fig. 1A), while it is immunonegative for oxytocin. The PV nucleus is invisible.

The SO nucleus becomes well defined in the rat at the 18th day. It shows an intense immunoreactivity for vasopressin (Fig. 1B). In addition, the PV nucleus becomes visible but is immunonegative for vasopressin (Fig. 1E). However, both nuclei are immunonegative for oxytocin.

Newborn rats (0 days) present vasopressin-immunopositive and oxytocin- immunonegative SO and PV nuclei (Figs. 1F; 1C).

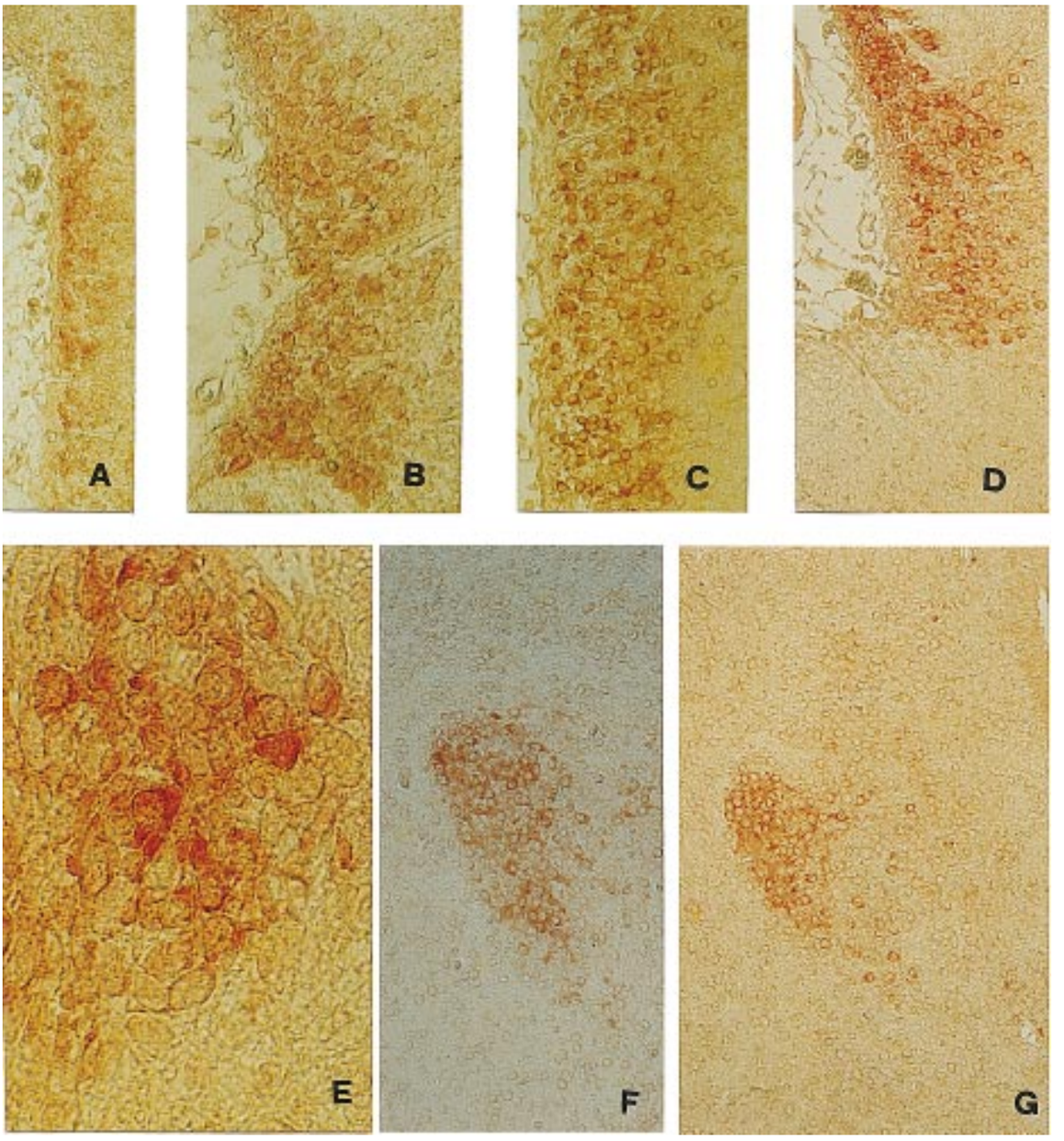

Fig. 1 - Rat hypothalamic immunopositive nuclei for vasopressin. Supraoptic nuclei: A) 16th day embryo; original magnification x10; B) 18th day embryo x10; C) 0th day newborn x10; D) 2 day newborn x40. Paraventricular nuclei: E) 18th day embryo x40; F) 0th day newborn x10; G) 2 day newborn x10.

The SO nucleus of 2-day-old rats shows an intense immunopositivity for vasopressin (Fig. 1D),while a small number of neurons appear weakly immunopositive to oxytocin (Fig. 2A); moreover, the PV nucleus presents a considerable number of vasopressin-immunopositive neurons (Fig. 1G), while few neurons are oxytocin-immunopositive (Fig. 2D).

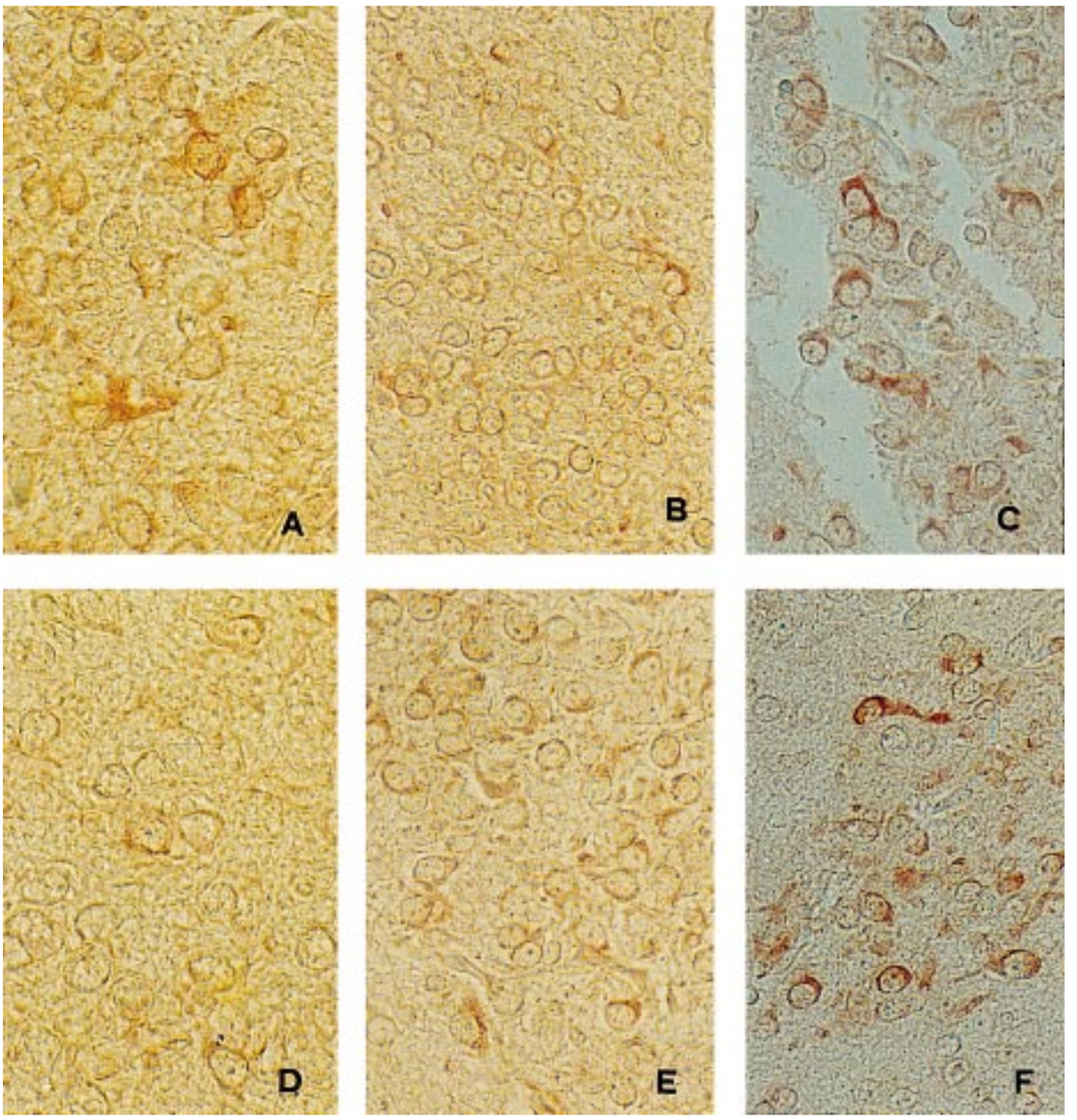

Fig. 2 - Rat hypothalamic immunopositive nuclei for oxytocin. Supraoptic nuclei: A) 2 day newborn; original magnification x10; B) 4th day newborn x10; C) 6th day newborn, x10. Paraventricular nuclei: D) 2 day newborn x25; E) 4th day newborn x25; F) 6th day newborn x10.

Finally, the SO (Fig. 2B) and PV nuclei (Fig. 2E) in the 4-day-old rats show a weak immunopositivity for oxytocin although more intense than in the previous stage, while in the 6-day-old rats, both the SO (Fig. 2C) and PV nuclei (Fig. 2F) present strongly intense oxytocin immunopositivity.

In addition, we studied the expression pattern of vasopressin and oxytocin transcripts in the hypothalamic areas. RT-PCR experiments show low levels of oxytocin (Fig. 3A) and vasopressin (Fig. 3B) transcripts in the observed early developmental stages, while their concentration increases in the adult rat.

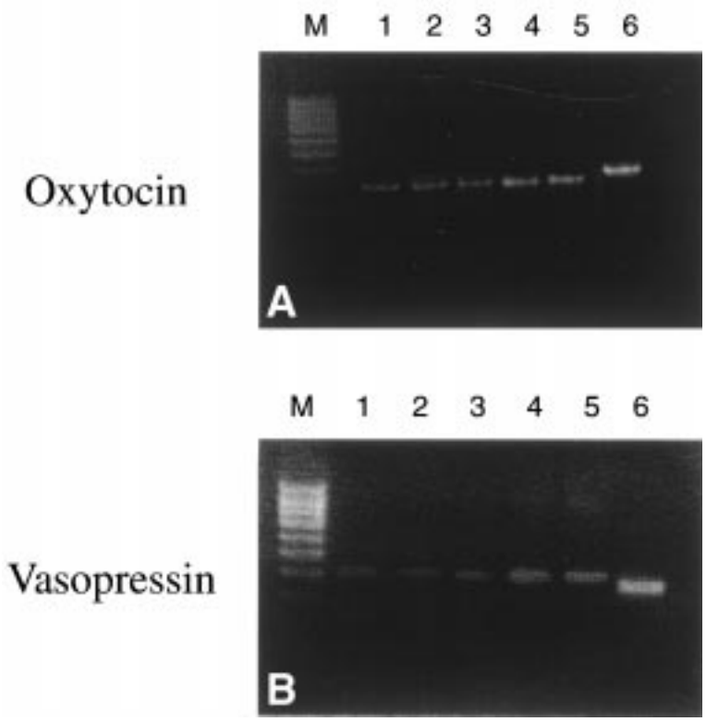

Fig. 3 - Agarose gel electrophoresis of RT-PCR products. The figure shows oxytocin (A) and vasopressin (B) transcripts level in 16, 18, 21-day-old rat embryos (lanes 1-3) and in adults (lanes 4, 5). Lane 6 represents the positive control, a highly conserved region of a constitutively expressed housekeeping gene rig/S15. DNA, subjected to electrophoresis on 1.5% agarose gel, was visualized by ethidium bromide staining. Lane 1 represents a DNA ladder (1kb).

DISCUSSION

This study, carried out on rat embryos from the 15th day of intrauterine life to the 6th day of postnatal life, demonstrates the progressive morphological and functional development of hypothalamic SO and PV nuclei at different stages. The SO nucleus is morphologically visible in its definitive position starting from the 16th day in rat foetuses and appears earlier than the PV nucleus, present from the 18th day. These findings disagree with the results obtained by Ifft (1972) who affirmed that the SO nucleus and the magnocellular portion of PV nucleus appear from the 14th day of gestation. It is known that SO and PV nuclei release vasopressin and oxytocin, but the neurosecretory activity during development is different in time, both in nuclei appearance and cellular secretion. Indeed, the SO nucleus is able to synthesize vasopressin from the 16th day, while the PV nucleus starts its activity on the 18th day of intrauterine life. This result is in disagreement with that reported by Choy and Watkins (1979), whose observations on the 18th day rat foetuses showed no differences concerning the start of secretory activity in the two nuclei. We are also in disagreement with Lazcano *et al.* (1990), who showed that vasopressin neurosecretion in the SO nucleus started from the 18th day in rat foetuses. Our results concerning the PV nucleus confirm the observations of Choy and Watkins (1979). Oxytocin synthesis, as is evident from immunohistochemical data, starts simultaneously in both SO and PV nuclei from the 2nd day after birth. The latter datum is slightly discordant with that of Choy and Watkins (1979) who affirmed the presence of oxytocin in the hypothalamus on the 3rd day of postnatal life. This is also discordant with Lazcano *et al.* (1990) who showewd that the SO nucleus synthetizes oxytocin starting from the 4th day of postnatal life. Comparing immunohistochemical and RT-PCR results, it is evident that genes encoding vasopressin and oxytocin are already transcribed on the 16th day of intrauterine life, but corresponding proteins are synthetized at different development stages. Indeed, vasopressin transcript appears on the 16th embryonic day and increases during development, along with its protein expression. By contrast, oxytocin RNA is present at early develop-

mental stages, while the corresponding protein is not detected by immunohistochemical experiments.

We suggest that the gene encoding foetal oxytocin is regulated mainly at the post-transcriptional level during rat hypothalamic nuclei development. In this way, gene transcription is activated, but the protein appears at a specific time of development. Growing evidence demonstrates the importance of a post-transcriptional mechanism, including regulation of splicing, vectorial transport of mature mRNAs, regulation of mRNA stability and availability for translation, in control of gene expression both in developing and differentiated cells.

We retain that during intrauterine life the placental oxytocin inhibits synthesis and secretion of foetal hypothalamic oxytocin. After birth, placental oxytocin apport terminates and hypothalamic SO and PV nuclei are stimulated to synthetize neurohormone.

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