ZOOLOGICAL SCIENCE 5: 1019–1032 (1988)

Fetal and Postnatal Development of Arginine Vasopressin-Immunoreactive Neurons in the Mouse

TOMOO YAMASHITA[1], KEIICHI KAWAMOTO[2] and SEIICHIRO KAWASHIMA[3]

Zoological Institute, Faculty of Science, Hiroshima University, Hiroshima 730, and [3]Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113, Japan

ABSTRACT—The ontogeny of arginine vasopressin (AVP)-producing neurons in the hypothalamo-neurohypophysial system (HNS) was immunocytochemically studied in fetal and postnatal mice. Presumptive AVP neurons underwent proliferation in the vicinity of the third ventricle and migrated to settle in the final loci by fetal age of 18 days (FA 18). AVP-immunoreactive neurons were first detected on FA 14 in the presumptive supraoptic (SON) and retrochiasmatic nuclei (RCN). AVP-immunoreactive axons and terminals were present in the median eminence and pars nervosa of the neurohypophysis on FA 15, but not on FA 14. Immunoreactive neurons were recognized in the paraventricular nucleus (PVN) on FA 15 and their terminals in the external layer of the median eminence became immunoreactive on FA 18. In the suprachiasmatic nucleus (SCN) AVP-immunoreactive perikarya appeared on FA 16. The number of AVP neurons in the SON and RCN markedly increased during fetal life. Postnatal increase in the number of immunoreactive neurons in the PVN and SCN as well as that of the SON and RCN was apparent. To sum up, the present study shows that cytodifferentiation of AVP-producing neurons in the HNS takes place during early days of last trimester of pregnancy and that the HNS completes the general morphological changes before birth.

INTRODUCTION

Arginine vasopressin (AVP) is synthesized mainly in the magnocellular neurons in the supraoptic (SON), retrochiasmatic (RCN) and paraventricular nuclei (PVN) of the hypothalamus in mammals [1–5]. AVP is transported through the fiber layer of the median eminence (ME) to the pars nervosa (PN) of the pituitary and to the external layer of the ME [6]. Some other AVP-producing neurons are present among the parvocellular neurons in the suprachiasmatic nucleus (SCN), the axons originating from which projecting to the brain regions other than the PN and ME [7, 8]. Besides the vasopressinergic pathways from the PVN to the PN and ME [9–12], extrahypothalamic projections have also been documented, for example, to the forebrain [8, 12], the brain stem [8, 13] and the spinal cord [8, 13, 14]. Therefore, AVP has been proposed to act not only as the antidiuretic hormone but also as a neurotransmitter.

During the last ten years the ontogeny of the hypothalamo-neurohypophysial system (HNS) has been investigated in some species of rodents. Histological staining [15], electron microscopy [16–20], immunocytochemistry [21–23] and radioimmunoassay [19, 24, 25] were applied for the study of development of the HNS. These studies have demonstrated that AVP synthesis in the magnocellular neurons begins to occur and rapidly elevates during the late gestation period and that the HNS completes its maturation by the end of the first month of life. Compared to the studies on other species of mammals, however, developmental study on the mouse HNS is scanty.

Therefore, in the present study the development of AVP-producing neurons of the HNS in fetal and postnatal mice was examined by means of AVP immunocytochemistry.

Accepted January 5, 1988
Received December 5, 1987

* Fetal ages in the cited paper were rearranged for convenience of comparison with the present study, so that the day when vaginal plug was observed was designated as FA 0.

[1] Present Address: Numata High School, Tomo 161-1, Numata-cho, Asaminami-ku, Hiroshima 731-31.

[2] Present Address: Department of Neurosurgery, School of Medicine, Hiroshima University, Hiroshima 734.

[3] Reprint requests to: Dr. S. Kawashima.

MATERIALS AND METHODS

Animals

Mice of the C57BL/6NCrj strain maintained in this laboratory were used in the present study. They were housed in a temperature-controlled room at 12-hr light (06:00–18:00 hr) and 12-hr dark cycle with free access to laboratory chow (CA-1, Japan Clea Inc.) and tap water.

Female mice were placed in a cage with males in the evening and separated from males in the next morning. The day was designated as fetal age of 0 day (FA 0) for successful pregnancy. Most pregnant mothers delivered their pups in the morning on FA 19. The day of birth was designated as postnatal age of 0 day (PA 0).

Tissue preparation

Fetuses between FA 14 and FA 18 regardless of sexes were taken out by Caesarean cut at 13:00 hr from at least two different litters at each fetal age. After birth, in order to exclude any possible sex difference, only male mice were chosen and killed at 13:00 hr on PA 2, 14, 30 and 90. Five animals were used for each fetal and postnatal age groups.

Animals were killed by decapitation, and the brains were taken out and fixed in Bouin's fluid for two days. After trimming all the brains were kept in 70% ethanol overnight. Dehydration in a graded series of ethanol and embedding in paraplast were completed within the following day. Serial frontal sections were cut at 6 μm in thickness, and every fifth sections were mounted on albumin-coated glass slides for immunocytochemical staining. The adjacent sections were stained with Ehrlich's haematoxyline-eosin (fetuses) or thionine (postnatal mice) for general histological changes during development of the HNS.

Immunocytochemical procedures

Immunocytochemistry for AVP was performed by means of the avidin-biotin-peroxidase complex (ABC) technique [26]. Deparaffinized sections were reacted with the following sequence of solutions: (1) rabbit anti-AVP serum (RV-1K, raised in this laboratory) (1:6,400) for 24 hr at 4°C, (2) biotinylated goat anti-rabbit IgG serum (Vector Laboratories, California) (1:200) for 30 min at room temperature (RT), (3) 0.3% H_2O_2 for 30 min at RT, (4) ABC reagents (avidin DH and biotinylated horseradish peroxidase H) of the ABC kit (Vector Laboratories, California) (1:100) for 30 min at RT and (5) 0.015% 3,3′-diaminobenzidine in 0.05 M Tris-HCl buffer (pH 7.4) containing 0.01% H_2O_2 for 10 min at RT. Sections were washed three times with 0.01 M phosphate buffered saline (pH 7.4) at 4°C, 5 min each, between each step. The characterization of anti-AVP serum was reported previously [27].

After washing in three changes of distilled water, the preparations were dehydrated with graded series of ethanol and mounted with balsam.

Morphometry

The right halves of the hypothalamus were used for morphometry. In immunocytochemically stained sections, only those cells that showed distinct nucleoli and contained brown reaction products were counted as AVP-producing neurons. The total number of cells (N) per animal was calculated from the total number of cells (n) in every fifth sections by the formula: $N=2\times5\times n$.

RESULTS

1. Development of the HNS

FA 14. General morphology of the hypothalamus was extremely different from that in the adults (Fig. 1a). Presumptive magnocellular neurons appeared to be produced in the diamond-shaped region around the third ventricle at the junction of the ventral and medial lobes of the diencephalic neuroepithelium. Ependymal cells were spindle-like in shape, forming two or three layers.

The condensation of the SON, RCN and PVN was not completed on FA 14. However, in the presumptive region of the SON, the magnocellular neurons were a little more concentrated than the surrounding area. The presumptive PVN contained the cells migrating from the neuroepithelium to the final locus in the hypothalamus. The SCN was not yet demarcated from the adjacent area. The ependymal cells along the ventral floor of the third ventricle were still undergoing mitosis.

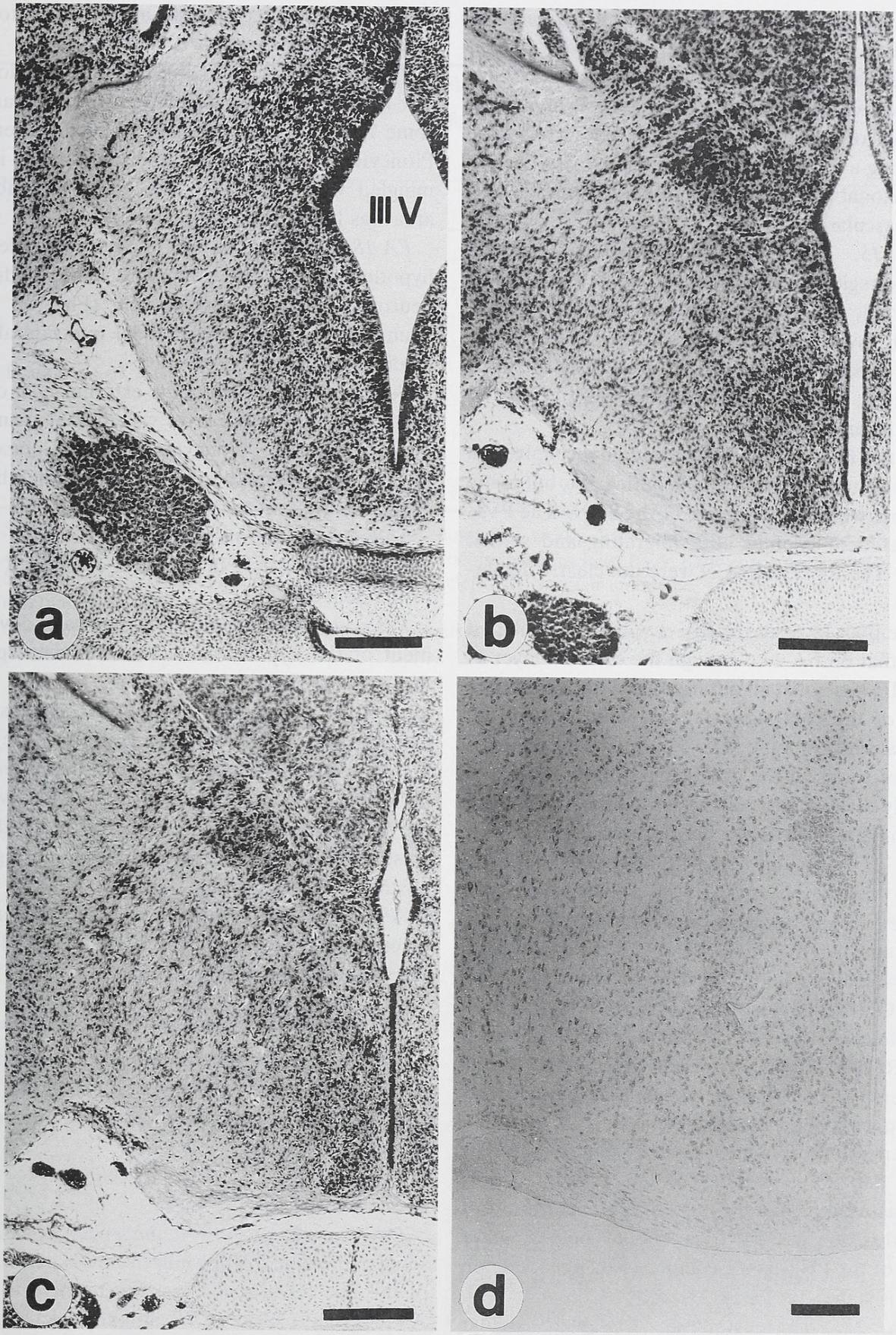


FIG. 1. Frontal sections of the hypothalamus (right side) during development. Stained with haematoxyline and eosin (a-c), or thionine (d). a, FA 14; b, FA 16; c, FA 18; d, PA 14. IIIV, third ventricle. Bars: 200 μm.

The ME was consisted of the ependymal and fiber layers, and the external layer was not yet differentiated. Scattered cells of the pars tuberalis were visible under the ME. The PN was immature and full of glial cells (primordial pituicytes) surrounding a central cavity and possesed very few, if any, axonal termini. The periphery of the PN was well vascularized.

FA 15. Ependymal cells in the diamond-shaped region around the third ventricle continued to divide, and some of them might differentiate into the hypothalamic cells, because the migratory paths of cells from the matrix layer along the third ventricle to the ventro-lateral region of the hypothalamus was observed.

At this fetal age the SON could be recognized as the cell cluster laterally to the optic tract, but many neurons were still migrating to the SON. The PVN appeared to contain both migrating and settled cells. The RCN and SCN were not clearly demarcated.

The fiber layer of the ME was better developed than that on FA 14. Though the external layer of the ME was not at all differentiated, the number of cells of the pars tuberalis was increased on the ventral surface of the ME. Because of the penetration of axons to the outer area of the PN, it was clearly divided into two areas; the inner pituicyte-rich area and the outer fibrous area.

FA 16. By this day, most ependymal cells lining the third ventricle appeared to cease any further mitotic divisions, and the neurons migrating to the ventrolateral regions of the hypothalamus were very few (Fig. 1b). Morphogenesis of the SON was almost completed on FA 16, but a few neurons were still migrating to this nucleus. The RCN and PVN were clearly recognized as cell clusters. The SCN showed itself as a slightly dense area on the optic chiasma.

General morphology of the neurohypophysis (ME and PN) did not show much difference as compared to that on FA 15. The penetration of blood vessels into the pars tuberalis and the inner area of the PN was the characteristic phenomenon at this stage.

FA 17. The cytoplasm of magnocellular neurons in the SON and PVN was not well-developed. The parvocellular SCN formed a discrete structure, locating as a pair close to the medial optic chiasma.

The external layer of the ME was developed. The PN was markedly developed on FA 17, and in some fetuses the central cavity had disappeared. Pituicytes dispersed all around the PN and intermingled with axons, so that exclusively fibrous area was left only at the periphery.

FA 18. On this last day of embryonic life, the hypothalamic nuclei containing AVP-producing neurons completed development (Fig. 1c). The adult-type partition of the RCN into lateral and medial groups was observed.

Further development of the external layer of the ME was seen, but the layer was thinner than that in adult animals. Pituicytes further dispersed in the PN, and among these cells a greater number of axons was present than on FA 17.

These observations indicate that the morphogenesis of the HNS is almost completed prior to the delivery.

Postnatal development. The postnatal development of the hypothalamus was characterized by the hypertrophy of each neuron (Fig. 1d). The nucleoli in the neuronal nuclei became distinct. Though the hypothalamic neurons were still undergoing condensation on PA 2, the intercellular spaces became spread after PA 14. However, the hypothalamic nuclei were obviously distinguished from the surrounding area during the postnatal maturation.

After birth, the development of the fiber and external layers of the ME further advanced, and the growth of the PN was apparent.

2. *Development of AVP-producing Neurons*

The changes of AVP immunoreactivity of the hypothalamus and neurohypophysis during fetal and postnatal development are summarized in Table 1.

Fetal life

Development of the hypothalamus. On FA 14, some neurons of the presumptive SON in three out of the five fetuses were weakly reactive to anti-AVP serum. Weak AVP immunoreactivity was also detected in the ventro-caudal part of the RCN in all animals. The number of immunoreactive

TABLE 1. Résumé of AVP immunoreactivity of the mouse hypothalamus and neurohypophysis during fetal and postnatal development

Age (days)	Hypothalamus					Neurohypophysis		
	SON	RCN	PVN	SCN	Acc.	ME		PN
						f.l.	e.l.	
Fetal age								
14	±	+	−	−	−	−	−	−
15	+	+	+	−	±	+	−	+
16	+	+	+	±	+	+	−	+
17	++	++	+	±	++	++	+	++
18	++	++	+	±	++	++	+	++
Postnatal age								
2	++	++	++	+	++	++	++	++
14	+++	+++	+++	++	++	+++	++	+++
30	+++	+++	+++	++	+++	+++	+	+++
90	+++	+++	+++	++	+++	+++	+	+++

Staining intensity was arbitrarily graded as: −, none; +, weak; ++, moderate; +++, intense.
Abbreviations: SON, supraoptic nucleus; RCN, retrochiasmatic nucleus; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; Acc., accessory nuclei; ME, median eminunce; f.l., fiber layer; e.l., external layer; PN, pars nervosa.
Accessory nuclei are consisted of the anterior commissural nucleus, nucleus circularis, and anterior and posterior fornical nuclei.

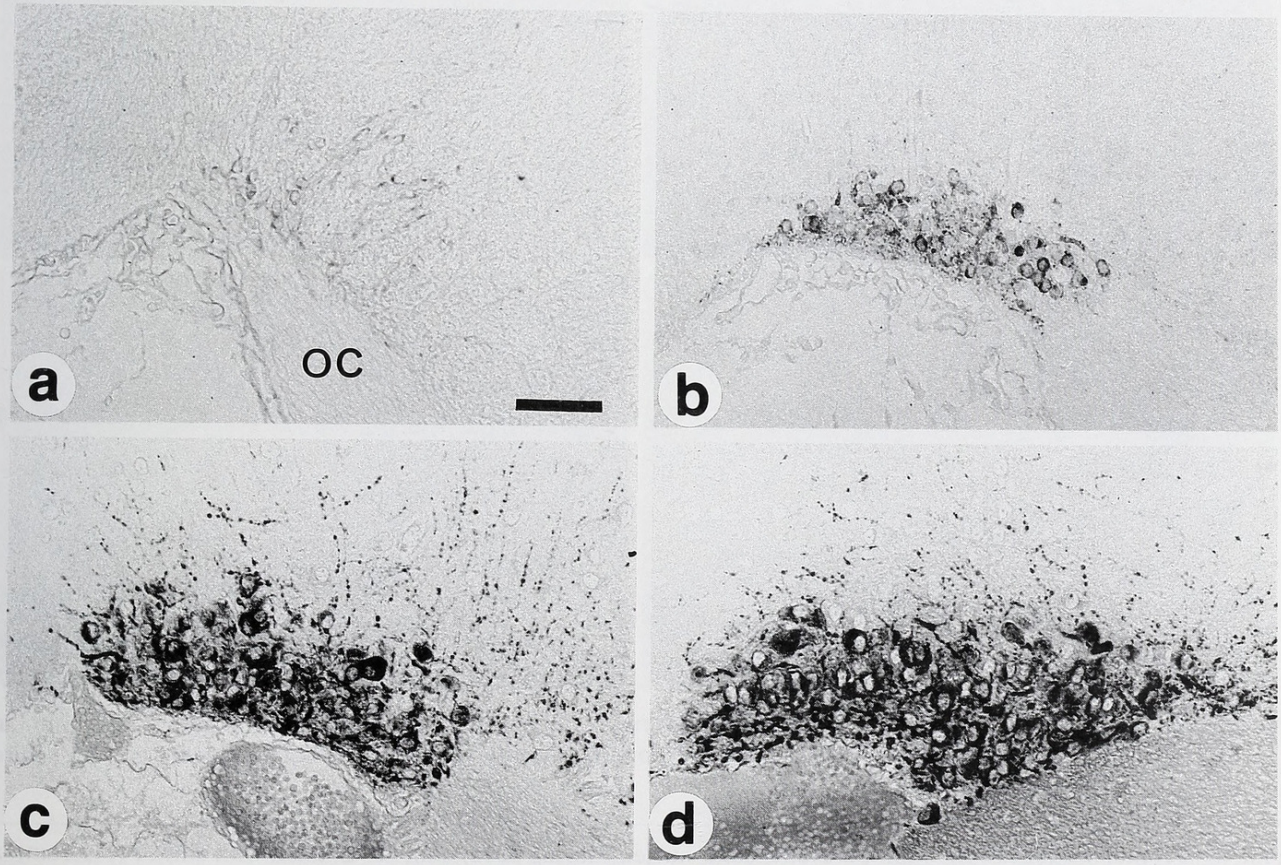


FIG. 2. Frontal sections of the right supraoptic nucleus during development. Stained with anti-AVP serum. a, FA 15; b, FA 18; c, PA 14; d, PA 90. OC, optic chiasma. Bar: 50 μm.

neurons in the RCN was more than in the SON. There was no AVP immunoreactivity in other nuclei of the hypothalamus.

On FA 15, the AVP immunoreactivity of the SON and RCN neurons became apparent, though the stainability was still weak (Figs. 2a and 3a). Each cell nucleus was enclosed in a thin rim of cytoplasm. There were some neurons which did not completely settle in the SON. A few neurons in the PVN and internuclear magnocellular (accessory) nuclei between the SON and PVN became first stainable on this day. But no immunoreactive material was detected in the SCN.

AVP immunoreactivity in the PVN on FA 16 was almost the same as that on FA 15 (Fig. 4a). Weak AVP immunoreactivity was first detected in the SCN in one of five FA 16 fetuses. Adult-type partition of immunoreactive neurons in the RCN into medial and lateral groups was observed as early as this fetal day.

Immunostainability of the SON and RCN neurons increased on FA 17 (Table 1). The SCN neurons were only weakly stainable with the antiserum in three fetuses on FA 17 (Fig. 5a).

On FA 18, many neurons in the SON and RCN accumulated the AVP-immunoreactive material in their cytoplasmic rim. Axonal fibers from the neurons with the bead-like deposits were encountered in these nuclei (Figs. 2b and 3b).

Numbers of immunoreactive neurons (Fig. 8). During the fetal life, magnocellular neurons in the SON, RCN and PVN did not grow as large as those of the adult. The number of immunoreactive neurons generally increased during the gestation period. In the SON and RCN, the number of AVP-immunoreactive neurons rapidly increased between FA 14 and FA 18. More immunostained cells were counted in the SON than in the RCN,

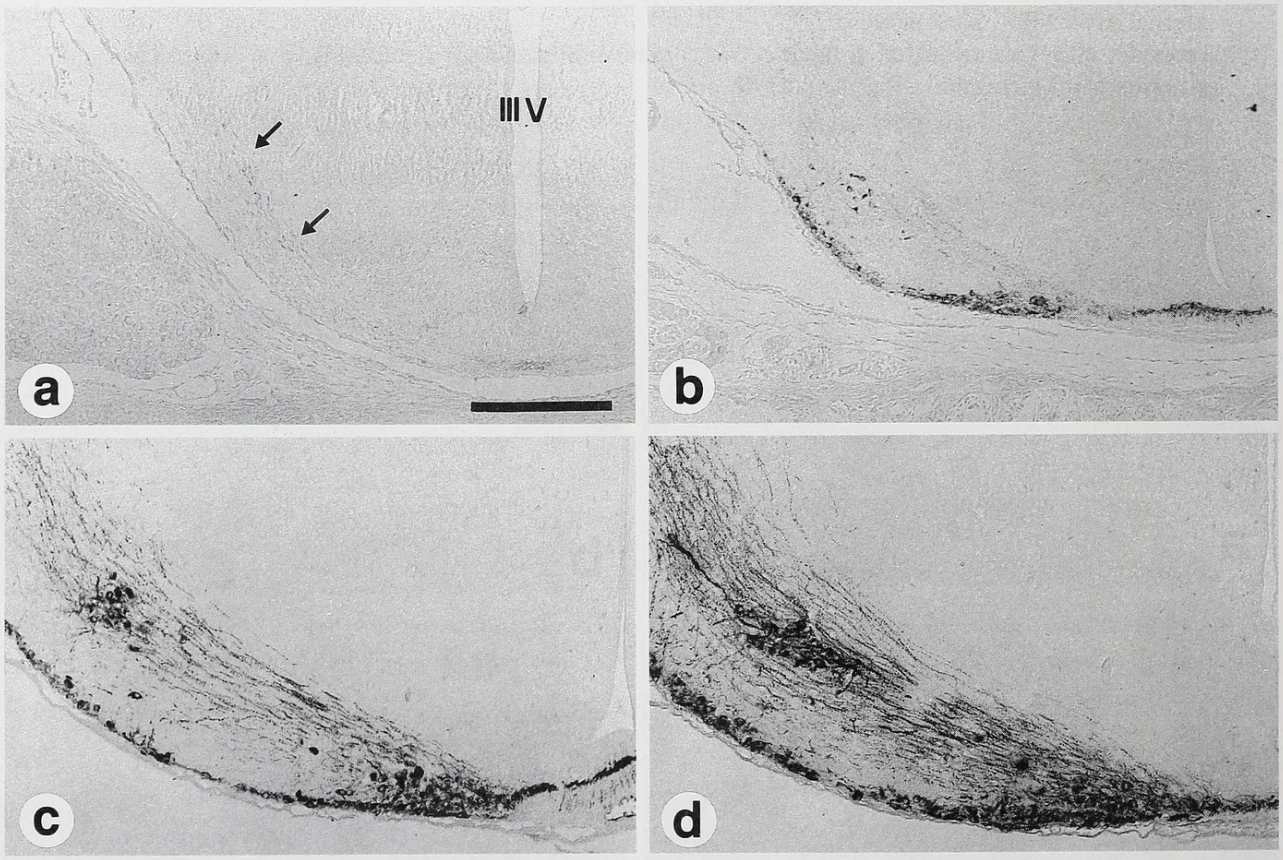
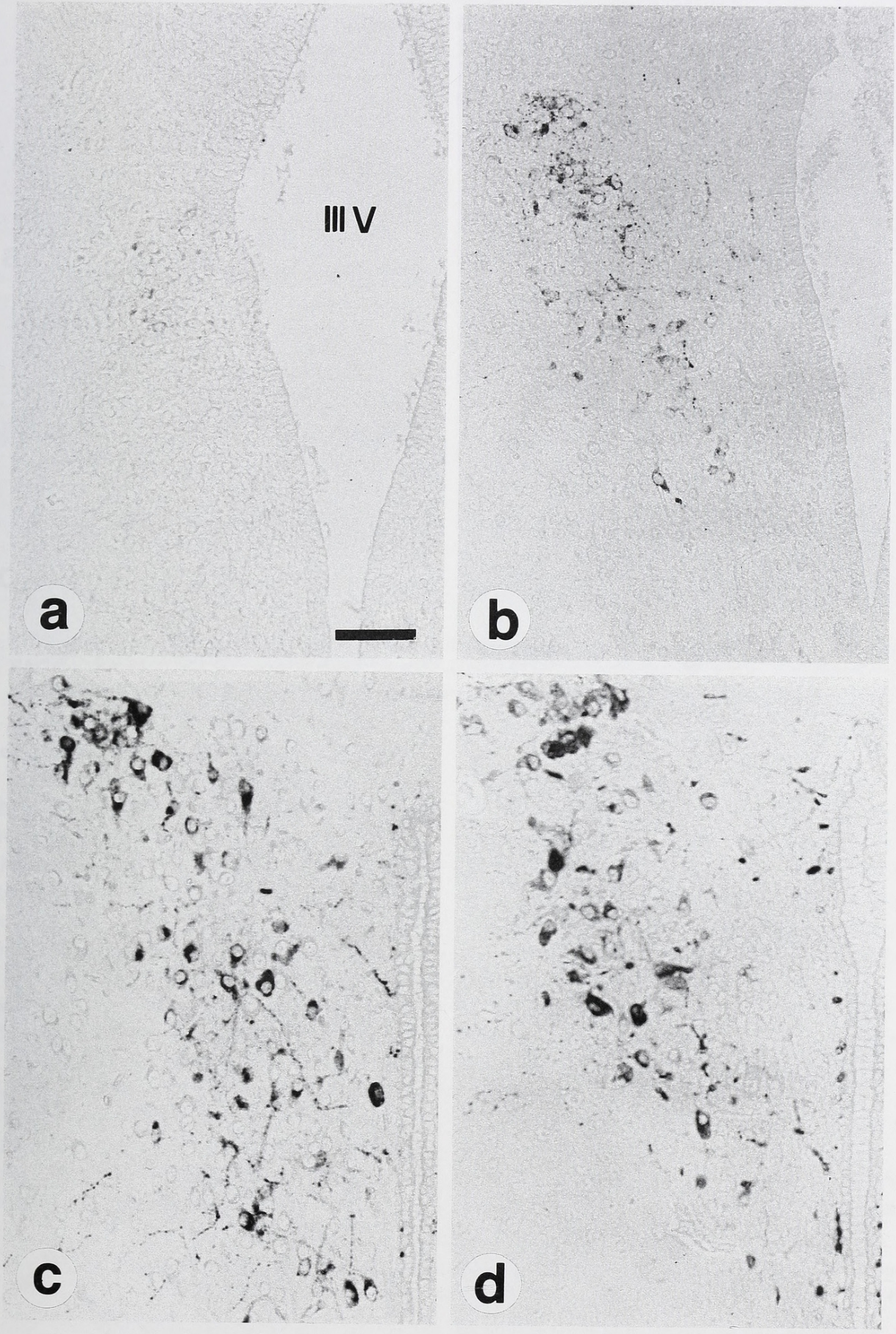
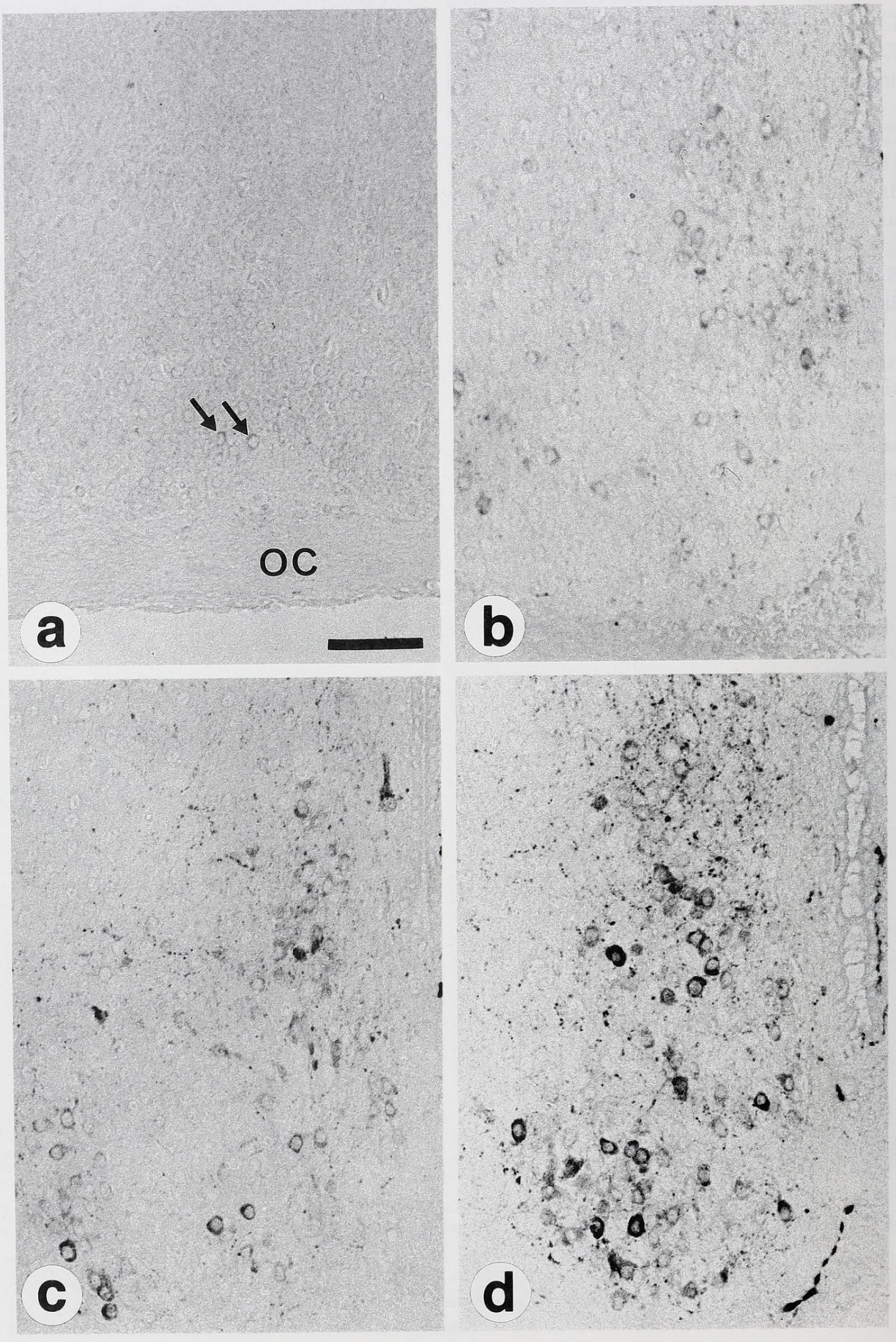


FIG. 3. Frontal sections of the right retrochiasmatic nucleus during development. Stained with anti-AVP serum. a, FA 15; b, FA 18; c, PA 14; d, PA 90. Arrows show the weakly AVP-immunoreactive neurons. IIIV; third ventricle. Bar: 200 μm.

FIG. 4. Frontal sections of the right paraventricular nucleus during development. Stained with anti-AVP serum. a, FA 16; b, PA 2; c, PA 14; d, PA 90. IIIV, third ventricle. Bar: 50 μm. (in page 1025)


III V
a
b
c
d


OC
a
b
c
d

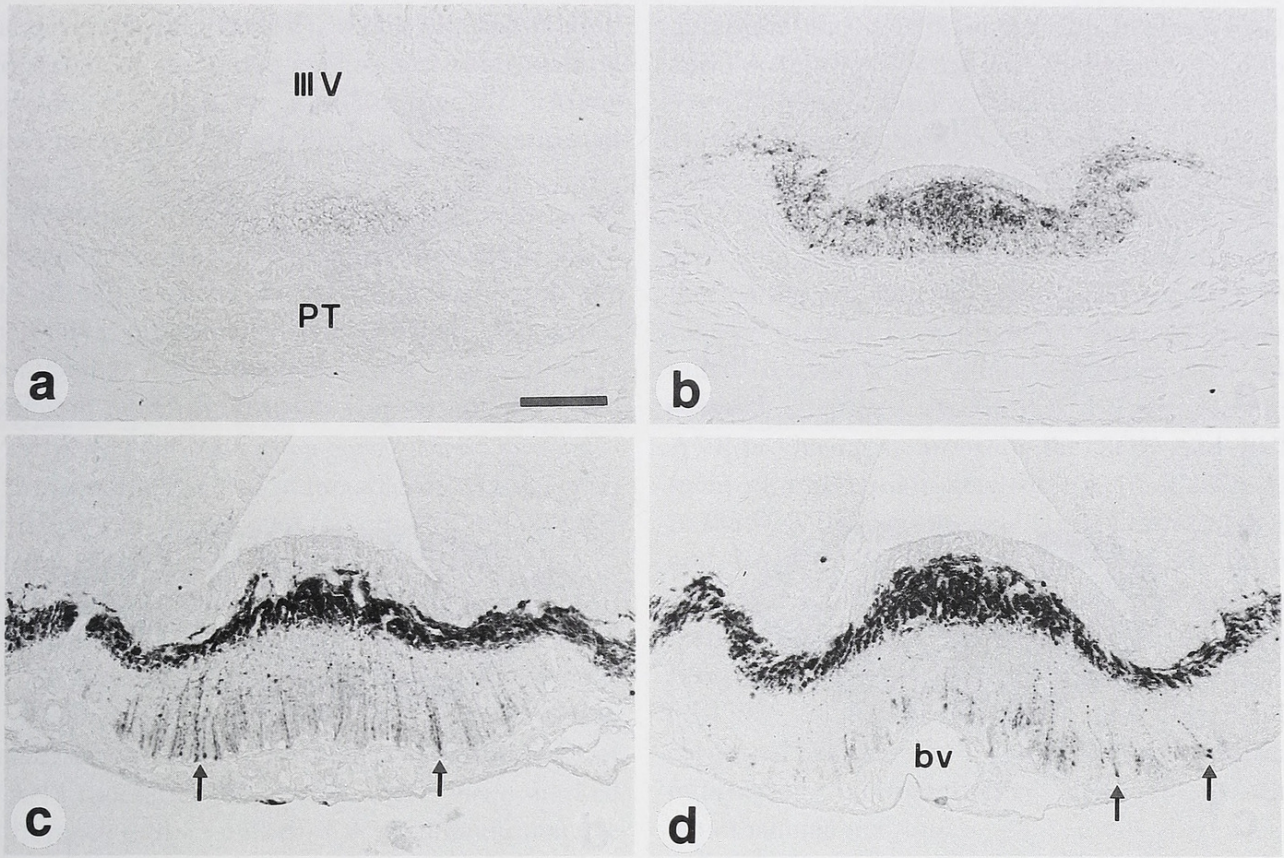


FIG. 6. Frontal sections of the median eminence during development. Stained with anti-AVP serum. a, FA 15; b, FA 18; c, PA 14; d, PA 90. Arrows show the AVP-immunoreactive axonal termini in the external layer of the median eminence. IIIV, third ventricle; PT, pars tuberalis; bv, blood vessel. Bar: 50 μm.

except on FA 14. In comparison with these nuclei, the numbers of immunoreactive neurons in the PVN, SCN and accessory nuclei showed a slight increase during the fatal life.

Development of the neurohypophysis. With the maturation of AVP-producing neurons, stainability of the ME and PN was also enhanced. AVP immunoreactivity was first detected on FA 15 in the ME and the peripheral edge of the PN (Figs. 6a and 7a). AVP-immunoreactive axonal termini in the external layer of the ME first appeared on FA 18 (Fig. 6b). Immunoreactive area in the PN indicated the preferential peripheral localization at the early stage of development (Fig. 7ab), and it gradually expanded to the central area. However, even on FA 17 and 18 strong immunoreactivity was confined to the peripheral area (Fig. 7cd).

Postnatal life

Development of the hypothalamus. After birth, the maturation of magnocellular neurons in the SON, RCN and PVN further advanced. The cells were hypertrophied and became oval in shape. The hypertrophy was generally accompanied by the accumulation of AVP-immunoreactive material (Figs. 2cd, 3cd and 4d).

On PA 2, immunoreactive area of the PVN became triangular in shape like that of the adult. With the rapid increase in the number of immunoreactive cells in the PVN between PA 2 and PA 14, stainability of the cells also increased (Fig. 4bc).

In the parvocellular SCN the increase in AVP immunoreactivity occurred mainly during postna-

FIG. 5. Frontal sections of the right suprachiasmatic nucleus during development. Stained with anti-AVP serum. a, FA 17; b, PA 14; c, PA 30; d, PA 90. Arrows show the weakly AVP-immunoreactive neurons. OC, optic chiasma. Bar: 50 μm. (in page 1026)

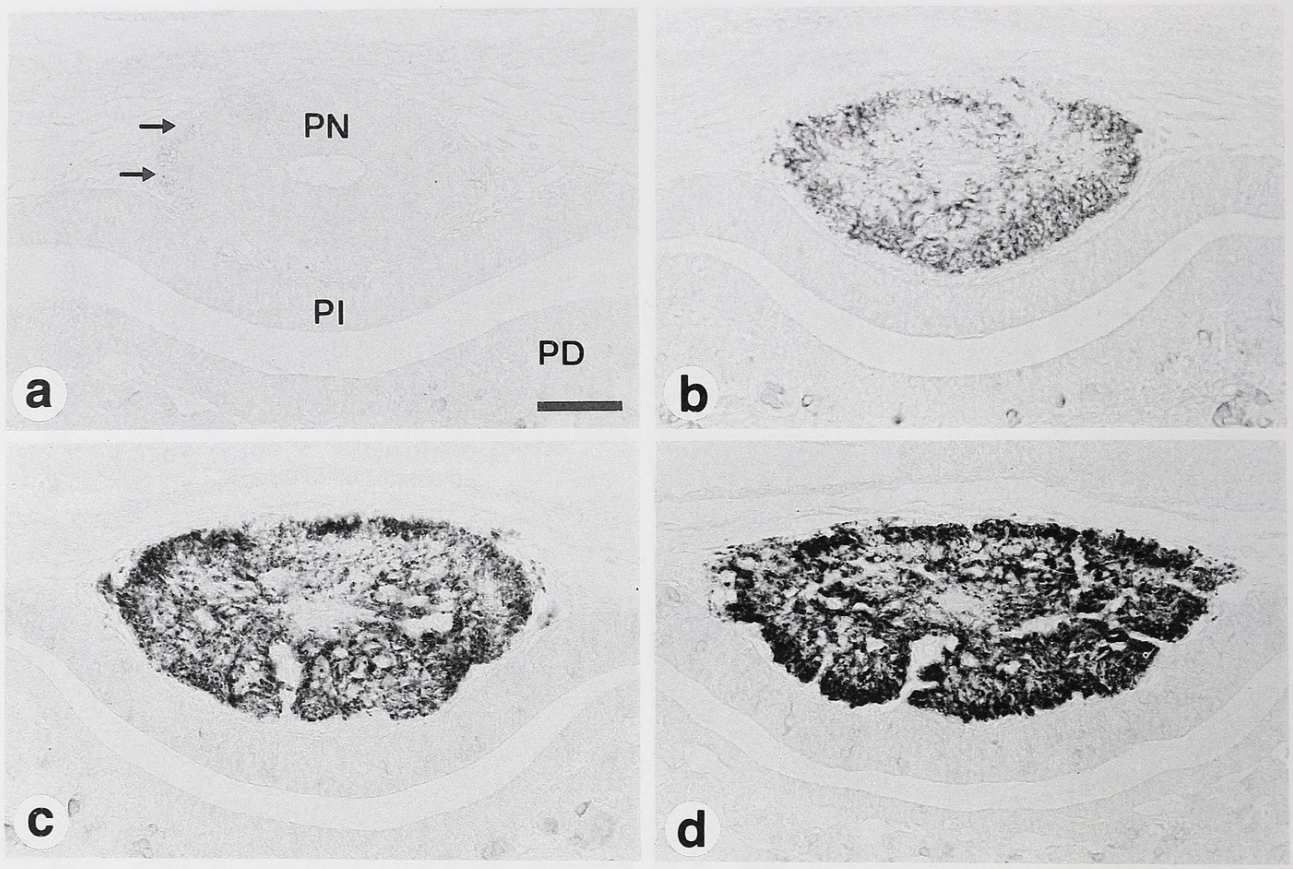


FIG. 7. Frontal sections of the pars nervosa (PN) during fetal development. Stained with anti-AVP serum. a, FA 15; b, FA 16; c, FA 17; d, FA 18. Arrows show the AVP-immunoreactive area in the periphery of the PN. Weak non-specific reactivity is seen in the pars intermedia (PI) and pars distalis (PD). Bar: 50 μm.

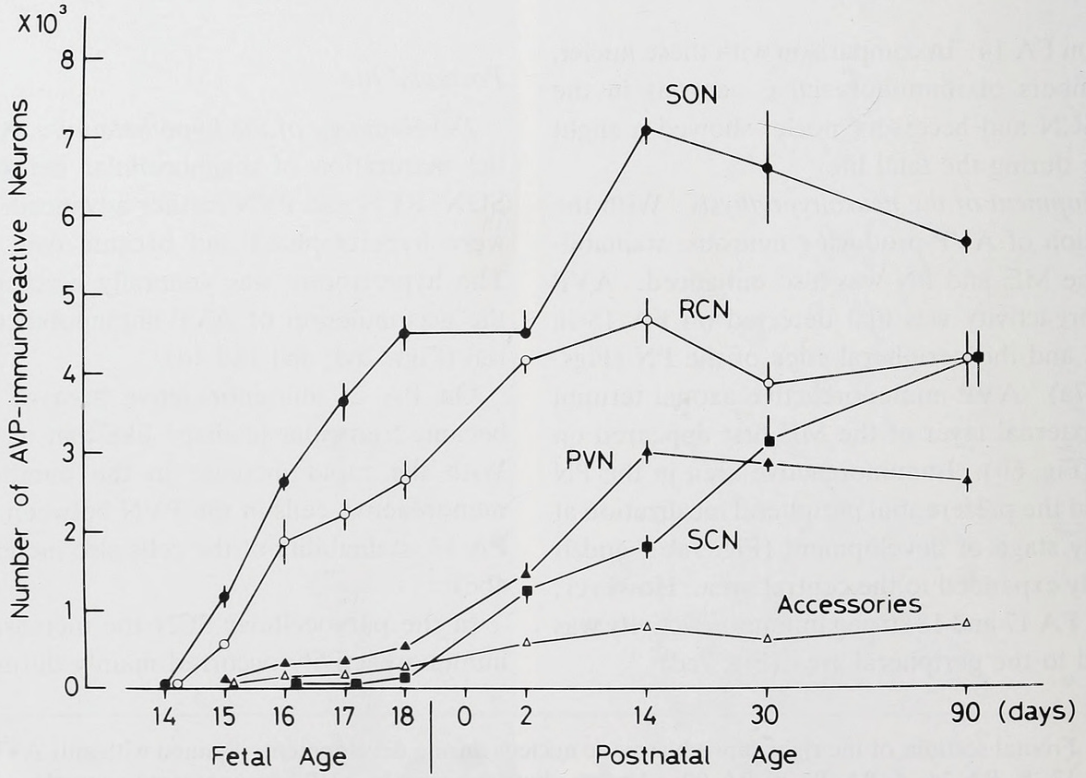


FIG. 8. Total number of AVP-immunoreactive neurons in the mouse hypothalamus during fetal and postnatal development. Vertical bars indicate the standard errors of the means. The number of animals was five in all age groups. For abbreviations, see the footnotes of Table 1.

tal development (Table 1). Adult-like distribution consisted of the dorso-medial and ventro-lateral groups was seen on PA 30 (Fig. 5c). Many neurons were weakly stained with the antiserum (Fig. 5d).

Bead-like structure of immunoreactive axons became more frequently encountered in the vicinity of the neurons as the accumulation in the perikarya proceeded. For example, the axons containing AVP-immunoreactive material were found in the PVN and SCN (Fig. 5b) on PA 2 and PA 14, respectively.

Most of the neurons of the accessory nuclei were strongly immunoreactive to anti-AVP serum after birth (Table 1).

Numbers of immunoreactive neurons (Fig. 8). The numbers of AVP-immunoreactive neurons in the RCN and accessory nuclei were stable after PA 2. The numbers in the SON and PVN continued to increase until PA 14 and maintained the level of PA 14 thereafter. However, there was a marked difference in the number of AVP-immunoreactive neurons between the SON and PVN. On the other hand, the number of immunoreactive neurons of the SCN continued to increase until PA 90.

Development of the neurohypophysis. AVP immunoreactivity in the fiber layer of the ME and the PN on PA 14 was stronger than on PA 2, but after PA 14 it was almost constant (Table 1). Postnatal development of the external layer of the ME was marked (Fig. 6c). Many AVP-immunoreactive axons with the bead-like deposits grew out from the fiber layer down to the external layer of the ME, and their termini were settled on the primary capillary plexus (Fig. 6d). AVP immunoreactivity in the external layer of the ME has decreased concomitantly with the development of the ME (Table 1).

DISCUSSION

Several investigators have reported the developmental changes of neurons in the hypothalamic nuclei by means of [^{3}H]-thymidine autoradiography [28, 29]. In the rat, Ifft [28]* showed that the neurons of the SON became visible between FA 11 and FA 15, those of the RCN between FA 11 and FA 17, those of the PVN between FA 11 and FA 16, and those of the SCN between FA 11 and FA 17 in the matrix layer surrounding the third ventricle. On the other hand, in the mouse of which gestation period is about two days shorter than that of the rat, Okamura *et al.* [29] reported the time of origin of AVP-producing neurons by combined technique of immunocytochemistry and autoradiography. According to their results, AVP-producing neurons in the SON and PVN seem to differentiate between FA 10 and FA 12. In the SCN the neurons proliferate between FA 10 and FA 14, and most of them appear to be produced in the latter half of this period. After the final division in the matrix layer, hypothalamic neurons migrate to occupy the final loci within 24 hr [30]. In addition, Altman and Bayer [31] suggested that there is a general lateral-to-medial internuclear differentiation gradient of the hypothalamic neurons.

In the present study, AVP-immunoreactive neurons were first detected on FA 14 in the SON and RCN but not in other nuclei of the mouse hypothalamus. Evidently, there was a time lag between the stage of neuronal proliferation and the stage of AVP synthesis. Since we could not observe immunoreactivity in the neurons migrating to the SON and RCN, the settlement of cells in the final loci may have some positive effects on the initiation of AVP synthesis. Silverman *et al.* [32], however, reported that on FA 13 some presumptive SON neurons synthesized the AVP carrier protein, neurophysin, while they were still migrating. Because of the limit of sensitivity of immunocytochemical reaction, it is possible that there was an earlier onset of AVP synthesis in the hypothalamic neurons and/or that many more cells contained subdetectable levels of AVP. In fact, a very small amount of AVP was detected by radioimmunoassay in the mouse brain on FA 13 (our unpublished observation). If immunocytochemistry using antibodies to the hormone precursor or autoradiography using the labelled oligonucleotide probe to AVP messenger RNA was performed, the time of cytodifferentiation of AVP neurons might be found at earlier embryonic life.

* Fetal ages in the cited paper were rearranged for convenience of comparison with the present study, so that the day when vaginal plug was observed was designated as FA 0.

Magnocellular neurons developed rapidly during the fetal life. Especially in the SON and RCN, the number of AVP-immunoreactive neurons increased linearly during the fetal life and reached the adult level on PA 2. On the other hand, the PVN appeared to develop later than the SON and RCN, although the number of AVP-immunoreactive neurons of the PVN reached the adult level on PA 14. These results are consistent with the previous studies [21, 22, 32]. Since the neurons of the SON, RCN and PVN cease to divide and start migration almost at the same time [28, 29, 31, 33], it needed to be clarified why the SON and RCN neurons should be so advanced in increasing their numbers. The SCN neurons became immunoreactive in one animal on FA 16. The later accumulation of AVP in the parvocellular neurons may be related with their late withdrawal from the mitotic cycle as compared to the magnocellular neurons [28, 29, 31, 33]. This delay might imply a less significant physiological importance of the SCN than the SON, RCN and PVN during perinatal period. Because parvocellular neurons are known to project their axons to the forebrain and brain stem [7, 8], but not to the PN and ME, they may not be related to the regulation of water and electrolyte metabolism. It is likely that AVP in the SCN neurons begin to function as a neurotransmitter after birth, concomitant with their increase in AVP immunoreactivity.

The adult-type distribution pattern of SON neurons completes during fetal life, that is, more AVP neurons are present in the caudal region of the SON than the rostral region. This particular distribution pattern has also been shown in the rat [34].

The occurrence of bead-like deposits along the axons implies the active transport of neurosecretory material from the hypothalamus to the PN and ME. After birth, many large-sized deposits or Herring bodies could be observed in the axons of the hypothalamo-neurohypophysial tracts. Axonal outgrowth from the PVN neurons also occurred relatively quickly after the appearance of immunoreactivity in the perikarya. AVP-immunoreactive cell bodies were encountered in the SON and PVN on FA 15, and their termini in the external layer of the ME, which recieves projections of AVP-immunoreactive fibers almost exclusively from the PVN [11, 12], were detected on FA 18.

Immunoreactive axons were first observed in the peripheral area of the PN on FA 15. The age of the fetus at which AVP-immunoreactive axons were first detected in the present study was older as compared with the result using neurophysin immunocytochemistry by Silverman *et al.* [32]. The findings in the present study were in harmony with those by Eurenius and Jarskär [17] in that small fiber bundles were found in the PN, primarily in the region bordering the intermediate lobe on FA 14. Their data suggest that axonal outgrowth is initiated at least in some cells very shortly after their arrival at the presumptive locus of the hypothalamic nuclei (the arrival is between FA 13 and FA 14). Our results seem to show that the transport of the AVP begins after the projection of axonal termini reaches the PN. At present we don't know how long the time lag for the transport of AVP from the perikarya to the termini last, but if it is very short, then it is possible that the hormone is present in the growth cone, the very front of the axonal growth. Previous studies suggested that the fetal PN is a heterogeneous structural entity and that there are distinct territories for ingrowing fibers [17, 32]. Present observations also demonstrated that in the fetal PN AVP-immunoreactive axons were exclusively present in the peripheral area. The fact that the central area of the developing PN was occupied by proliferating pituicytes may have some is closely related to the peripheral distribution of growing fibers.

The present study clarified the developmental events of AVP-immunoreactive neurons of the mouse HNS. To sum up, the present results show that (1) there may be a time lag between the withdrawal from mitotic cycle and the initiation of peptide synthesis in AVP neurons, that (2) the HNS develops rapidly to attain the adult-like pattern during fetal life, and that (3) the HNS nearly attains complete maturity by the time of weaning.

REFERENCES

1 Vandesande, F. and Dierickx, K. (1975) Identification of the vasopressin producing and of the oxyto-

cin producing neurons in the hypothalamic magnocellular neurosectetory system of the rat. Cell Tissue Res., **164**: 153–162.

2 Choy, V. J. and Watkins, W. B. (1977) Immunocytochemical study of the hypothalamo-neurohypophysial system. II. Distribution of neurophysin, vasopressin and oxytocin in the normal and osmotically stimulated rat. Cell Tissue Res., **180**: 467–490.

3 Sofroniew, M. V., Weindl, A., Schinko, I. and Wetzstein, R. (1979) The distribution of vasopressin-, oxytocin-, and neurophysin-producing neurons in the guinea pig brain. Cell Tissue Res., **196**: 367–384.

4 Rhodes, C. H., Morrell, J. I. and Pfaff, D. W. (1981) Immunohistochemical analysis of magnocellular elements in rat hypothalamus: Distribution and numbers of cells containing neurophysin, oxytocin, and vasopressin. J. Comp. Neurol., **198**: 45–64.

5 Kawamoto, K. (1985) Immunohistochemical localization of vasopressin and oxytocin neurons in the mouse hypothalamus. J. Sci. Hiroshima Univ., Ser. B, Div. 1, **32**: 25–33.

6 Brownstein, M. J., Russell, J. T. and Gainer, H. (1980) Synthesis, transport, and release of posterior pituitary hormones. Science, **207**: 373–378.

7 Hoorneman, E. M. D. and Buijs, R. M. (1982) Vasopressin fiber pathways in the rat brain following suprachiasmatic nucleus lesioning. Brain Res., **243**: 235–241.

8 Zimmerman, E. A., Nilaver, G., Hou-Yu, A. and Silverman, A. J. (1984) Vasopressinergic and oxytocinergic pathways in the central nervous system. Fed. Proc., **43**: 91–96.

9 Vandesande, F., De Mey, J. and Dierickx, K. (1974) Identification of neurophysin producing cells. I. The origin of the neurophysin-like substance-containing nerve fibres of the external region of the median eminence of the rat. Cell Tissue Res., **151**: 187–200.

10 Dierickx, K., Vandesande, F. and De Mey, J. (1976) Identification, in the external region of the rat median eminence, of separate neurophysin-vasopressin and neurophysin-oxytocin containing nerve fibres. Cell Tissue Res., **168**: 141–151.

11 Vandesande, F., Dierickx, K. and De Mey, J. (1977) The origin of the vasopressinergic and oxytocinergic fibers of the external region of the median eminence of the rat hypophysis. Cell Tissue Res., **180**: 443–452.

12 Antunes, J. L., Carmel, P. W. and Zimmerman, E. A. (1977) Projections from the paraventricular nucleus to the zona externa of the median eminence of the rhesus monkey: An immunohistochemical study. Brain Res., **137**: 1–10.

13 Lang, R. E., Heil, J., Ganten, D., Hermann, K., Rascher, W. and Unger, Th. (1983) Effects of lesions in the paraventricular nucleus of the hypothalamus on vasopressin and oxytocin contents in brainstem and spinal cord of rat. Brain Res., **260**: 326–329.

14 Hawthorn, J., Ang, V. T. and Jenkins, J. S. (1985) Effects of lesions in the hypothalamic paraventricular, supraoptic and suprachiasmatic nuclei on vasopressin and oxytocin in rat brain and spinal cord. Brain Res., **346**: 51–57.

15 Rinne, U. K. and Kivalo, E. (1965) Maturation of hypothalamic neurosecretion in rat, with special reference to the neurosecretory material passing into the hypophysial portal system. Acta Neuroveget., **27**: 166–183.

16 Paull, W. K. (1973) A light and electron microscopic study of the development of the neurohypophysis of the fetal rat. Anat. Rec., **175**: 407–408.

17 Eurenius, L. and Jarskär, R. (1974) Electron microscopy of neurosecretory nerve fibres in the neural lobe of the embryonic mouse. Cell Tissue Res., **149**: 333–347.

18 Galabov, P. and Schiebler, T. H. (1978) The ultrastructure of the developing neural lobe. Cell Tissue Res., **189**: 313–329.

19 Gash, D., Sladek, C. and Scott, D. (1980) Cytodifferentiation of the supraoptic nucleus correlated with vasopressin synthesis in the rat. Brain Res., **181**: 345–355.

20 Kawamoto, K., Yamada, Y. and Kawashima, S. (1984) Immunohistochemical and electron microscopic studies on the development of the mouse posterior lobe. Zool. Sci., **1**: 980 (Abstract).

21 Silverman, A. J. (1975) The hypothalamic magnocellular neurosecretory system of the guinea pig. II. Immunohistochemical localization of neurophysin and vasopressin in the fetus. Am. J. Anat., **144**: 445–460.

22 Wolf, G. and Trautmann, B. (1977) Ontogeny of the hypothalamo-neurohypophysial system in rats — an immunohistochemical study. Endokrinologie, **69**: 222–226.

23 Choy, V. J. and Watkins, W. B. (1979) Maturation of the hypothalamo-neurohypophysial system. I. Localization of neurophysin, oxytocin and vasopressin in the hypothalamus and neural lobe of the developing rat brain. Cell Tissue Res., **197**: 325–336.

24 Sinding, C., Robinson, A. G., Seif, S. M. and Schmid, P. G. (1980) Neurohypophyseal peptides in the developing rat fetus. Brain Res., **195**: 177–186.

25 Sinding, C., Seif, S. M. and Robinson, A. G. (1980) Levels of neurohypophyseal peptides in the rat during the first month of life. I. Basal levels in plasma, pituitary, and hypothalamus. Endocrinology, **107**: 749–754.

26 Hsu, S.-M., Raine, L. and Fanger, H. (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem., **29**: 577–580.

27 Kawamoto, K. and Kawashima, S. (1986) Effects of glucocorticoids and vasopressin on the regeneration of neurohypophyseal hormone-containing axons after hypophysectomy. Zool. Sci., **3**: 723–736.

28 Ifft, J. D. (1972) An autoradiographic study of the time of final division of neurons in the rat hypothalamic nuclei. J. Comp. Neurol., **144**: 193–204.

29 Okamura, H., Fukui, K., Koyama, E., Tsutou, H. L. O., Tsutou, T., Terubayashi, H., Fujisawa, H. and Ibata, Y. (1983) Time of vasopressin neuron origin in the mouse hypothalamus: Examination by combined technique of immunocytochemistry and [^{3}H]thymidine autoradiography. Dev. Brain Res., **9**: 223–226.

30 Shimada, M. and Nakamura, T. (1973) Time of neuron origin in mouse hypothalamic nuclei. Exp. Neurol., **41**: 163–173.

31 Altman, J. and Bayer, S. A. (1978) Development of the diencephalon in the rat. II. Correlation of the embryonic development of the hypothalamus with the time of origin of its neurons. J. Comp. Neurol., **182**: 973–994.

32 Silverman, A. J., Goldstein, R. and Gadde, C. A. (1980) The ontogenesis of neurophysin-containing neurons in the mouse hypothalamus. Peptides, **1**: 27–44.

33 Anderson, C. H. (1978) Time of neuron origin in the anterior hypothalamus of the rat. Brain Res., **154**: 119–122.

34 Swaab, D. F., Nijveldt, F. and Pool, C. W. (1975) Distribution of oxytocin and vasopressin in the rat supraoptic and paraventricular nucleus. J. Endocrinol., **67**: 461–462.