Development of tyrosine hydroxylase-immunoreactive neurons in the human paraventricular and supraoptic nucleus

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Tyrosine hydroxylase-immunoreactive (TH-IR) neurons were found in the developing human paraventricular and supraoptic nucleus. In the preterm infants only few, small, incompletely differentiated TH-IR neurons were evident in a minority of the cases. In the full-term infants a considerable but strongly variable population of morphologically mature TH-IR perikarya was observed in these neuroendocrine nuclei in most subjects.

Tyrosine hydroxylase (TH) is the rate limiting enzyme in the synthesis of catecholamines (CA) converting L-tyrosine to L-DOPA¹². The immunocytochemical demonstration of TH has been used in the literature to obtain information about the development of TH immunoreactive (TH-IR) perikarya in the brainstem of human fetuses^{17,18,22}.

Previous studies have indicated that in the adult human hypothalamus most of the TH-IR perikarya have been localized in the paraventricular (PVN) and supraoptic (SON) nuclei^{10,16}. However, in the fetal human brain neither TH-IR^{17,18,24} nor CA-fluorescent neurons^{6,13,14,20} have been reported in the neurosecretory nuclei. The purpose of the present study was, therefore, to investigate the development of the TH-IR perikarya in the PVN and SON.

The brains of 17 human neonates (13 males and 4 females), from fetal week 28 (calculated from the first day of the last menstrual period) to 14 months after birth, were obtained by autopsy. Six of them had been born preterm (< 37 weeks of gestation) and 11 at full term (> 37 weeks of gestation). Four of the newborns had died on the day of birth, while the majority of them remained in the intensive care unit of the hospi-

tal for 1–6 days. The clinical and pathological data of the infants studied are indicated in Table I. The neuropathology report showed no evidence of primary neurological diseases. The brains were weighed and fixed in 10% neutral buffered formalin for 14 to 35 days. Adjacent 6 μ m paraffin frontal sections²¹ of each subject were alternately stained for TH or for vasopressin in order to determine the boundaries of the neurosecretory nuclei. After rehydration the sections were (a) rinsed in Tris-buffer saline (TBS: 0.05 M Tris buffer with 0.5 M NaCl, pH 7.6); (b) incubated overnight at 4°C with anti-TH (Jacques Boy Institute, France)¹⁶ or anti-vasopressin (Truus, 10/4/1986)²¹ antibodies, 1:1000 in incubation buffer consisting of TBS pH 7.6 with 0.25% gelatin and 0.5% Triton X-100; (c) washed in TBS (2 $\times$ 10 min); (d) incubated for 30 min in goat anti-rabbit serum (Betsie) 1:50 in incubation buffer; (e) rewashed in TBS (2 $\times$ 10 min); (f) incubated for 1 h in peroxidase-antiperoxidase (PAP) 1:1000 in incubation buffer; (g) washed in TBS (2 $\times$ 10 min); (h) rinsed in 0.05 M Tris-HCl buffer pH 7.6 for 15 min; (i) incubated for 15–20 min in 0.5 mg/ml 3,3'-diaminobenzidine (Sigma) in 0.05 M Tris-HCl buffer pH 7.6 containing 0.2% Nickel ammonium sulphate and

0.01% H₂O₂; (j) washed in distilled water; (k) dehydrated in ethanol and mounted in Entellan.

The specificity of the immunocytochemical reaction was checked by incubating adjacent sections in anti-TH serum adsorbed with the pure enzyme TH (kindly donated by Prof. J. Thibault, College de France, Paris) or with pre-immune serum.

The application of the peroxidase–antiperoxidase method and nickel intensification revealed the presence of TH-IR neurons in the PVN and SON of the human infant brain. In the control sections, i.e. those incubated with the antibody adsorbed with the pure enzyme (TH) or with pre-immune serum, no peroxidase reaction appeared in any cell body or fiber in the hypothalamus.

TH-IR perikarya were evident in 13 of the 17 subjects, while vasopressin-immunoreactive neurons were observed in the PVN and SON of all the cases studied, thus confirming previously reported findings on the same material (see ref. 2). In the preterm infants only few, lightly stained TH-IR perikarya were evident in 2 out of 6 cases and in 3 out of 6 cases in the PVN and the SON, respectively (Table I). The TH-IR neurons in the PVN and SON of the preterm infants appeared small, ovally shaped and usually without visible processes (Fig. 1a, short arrow). Only in the largest TH-IR

neurons was a short thick process evident (Fig. 1a, long arrow).

From 37 weeks of gestation onwards, a large number of TH-IR perikarya was observed in the infant PVN and SON (Table I). In most of the full-term newborns the TH-IR neurons were numerous and distributed across the entire PVN and SON (Fig. 1b,c) as these nuclei were delineated in the vasopressin-stained adjacent sections (Fig. 1d). In these cases the TH-IR perikarya showed an intense peroxidase reaction over the entire cytoplasm and processes (Fig. 1b,c). In the vasopressin-stained perikarya, however, the reaction product was preferentially accumulated around the nucleus (Fig. 1d). Most of the TH-IR perikarya were large in size but also some smaller, intensely stained, TH-IR neurons were found within the PVN (Fig. 1b). Many varicose processes were observed crossing the field. Although most of the full-term newborn infants showed a considerable population of TH-IR neurons in the PVN and SON, two subjects (of 40 and 42 weeks of gestation, respectively; see Table I) displayed no staining for TH-IR neurons or fibers in the plane of the section.

Among the infants who had died 3, 6 and 14 months after birth, a large variation was observed in the relative number of TH-IR neurons in the PVN and SON.

TABLE I

Clinical and pathological data of the infants studied

w = weeks of gestation; m = months after birth; ND = not determined; M = male; F = female; c = delivered by caesarian section; d = died the day of birth. Symbols indicative of the relative differences in TH-IR cell numbers: + + + large number; + + many; + some; ± a few; – none. * See ref. 9.

Case no.	Age	Sex	Cause of death	Body weight (g)	Brain weight (g)	* Percentile	Postmortem delay (hrs)	Fixation (days)	TH-IR neurons	
									PVN	SON
88.321	28w	M	Immaturity, E. coli sepsis, meningitis, convulsions, hyperbilirubinemia, open duct of Botalli	1060	125	25–50	24	31	+	±
90.263.3c	29w	M	Dysmaturity, hyaline membrane	760	160	2.3–5	24	28	–	±
89.188.5	29w	M	Prematurity, dysmaturity, pneumonia, open duct of Botalli, hyaline membrane	650	110	< 2.3	24	30	–	–
82.009	30w	M	Prematurity, pneumothorax, acidosis, ventricular haemorrhage	1690	210	50–75	24	33	±	–
90.253.3cd	31w	M	Convulsions due to ischaemia	1300	206	10–25	6.5	28	–	±
86.335.4	31w	F	Prematurity, hyaline membrane, hypoxia	1730	200	50–25	24	33	–	–
89.153.3	37w	M	Pneumothorax, streptococcus sepsis, bronchopneumonia, hypoxia	2930	360	25–50	48	30	++	++
87.361.3	39w	F	Perinatal asphyxia, brain oedema	1630	300	< 2.3	5.5	24	+++	+++
88.381.4d	40w	M	Asphyxia, acidosis	3450	430	25–50	12	32	–	–
88.353.2d	40w	M	Asphyxia, meconium aspiration	2530	360	< 2.3	24	33	+++	+++
86.358.6	40w	F	Cerebral hypoxia	3720	375	50–75	24	19	++	++
87.280.3	40w	M	Hypoplastic left heart syndrome, anoxia	2995	400	2.3–5	24	35	+	±
90.259.1	42w	M	Pneumothorax, hyaline membrane	3400	390	25–50	24	28	++	+
87.152.5d	42w	M	Unknown, small bleeding in pons and nucleus dentatus	2790	350	2.5–5	24	35	–	–
85.002.3	3m	F	Bronchopneumonia, myocarditis, small sub-arachnoidal haemorrhage	ND	610	–	24	33	+++	+++
86.411.0	6m	M	Sudden infant death syndrome, brain oedema	ND	800	–	14	14	++	±
88.209.2	14m	M	Pneumococcal meningitis, pericarditis, endocarditis, brain oedema	ND	990	–	14	28	+	±

The largest number of TH-IR neurons was found in a 3-month-old infant. In this case numerous TH-IR perikarya were evident throughout the PVN, whereas in the SON TH-IR neurons were located in the dorsal part (Fig. 2). In the hypothalamus of the children who had died 6 and 14 months after birth, relatively few TH-IR perikarya were found, especially in the SON, where only few isolated neurons were localized in the dorsal part of this nucleus.

Our results clearly show the presence of TH-IR neurons in the PVN and SON of the human infant

brain in conventionally fixed paraffin-embedded material, indicating that after Nickel intensification the PAP method is sensitive enough to reveal TH in such tissues. TH-IR perikarya were evident even in the youngest of our cases, i.e. as early as 28 weeks of gestation. Since our sample did not include cases of shorter gestational age than 28 weeks, the time of the first appearance of TH-IR neurons in the human PVN and SON might even be earlier. TH-IR neurons in the human hypothalamus have indeed been reported to appear earlier in development. A small group of TH-IR

Fig. 1. a: PVN of a fetus of 28 weeks of gestation stained for TH. Note the presence of small, incompletely differentiated TH-IR neurons (short arrow). A short thick process is evident in one of the largest TH-IR neurons (long arrow). v, third ventricle. Bar = 100 μ m. b: PVN of a fetus of 39 weeks of gestation stained for TH. Note the large number of intensely stained mature TH-IR neurons and their processes. v, third ventricle. Bar = 100 μ m. c,d: SON of a fetus of 39 weeks of gestation stained for TH (c) and vasopressin (d). Note the large number of TH-IR neurons distributed over the entire SON. Bar = 100 μ m.

perikarya has been observed in the ventral hypothalamic anlage as early as 5 weeks of gestation²⁴. TH-IR perikarya have also been demonstrated in the hypothalamus of fetuses of 17–21 weeks¹⁸, but the exact location of these neurons has not been reported.

A clear difference was observed between the preterm and the full-term infants of our sample in the number of TH-IR neurons within the PVN and SON as well as in the intensity of the immunocytochemical reaction

for TH. In the pre-term infants only few, small, incompletely differentiated TH-IR neurons were evident in the PVN and SON in a minority of the cases studied. In the full-term infants a considerable population of morphologically mature TH-IR neurons was observed in the neurosecretory nuclei of most subjects.

From the above observations it appears that in the human PVN and SON the final development of TH-IR neurons takes place in the late gestational period. This

Fig. 2. a,b: PVN of a 3-month-old child stained for TH (a) and vasopressin (b). The TH-IR neurons are numerous, large and intensely stained over the entire cytoplasm and processes. Some smaller TH-IR perikarya were also evident. Bar = 100 μ m. c,d: SON of a 3-month-old child stained for TH (c) and vasopressin (d). Note the large number of TH-IR neurons concentrated in the dorsal part of the SON. OT: optic tract. Bar = 100 μ m.

is not the case for the brainstem TH-IR systems, which have been reported to develop earlier. The essential features of the nigrostriatal dopaminergic pathway of the human brain appear to be established before the end of the first trimester¹⁷. In 5- to 6-week-old human fetuses, the locus coeruleus neurons also contain TH²⁴. Furthermore, the TH-IR neurons of the brainstem appear morphologically mature, with intensely stained large perikarya and numerous processes as early as 15–27 weeks of gestation²². In the PVN and SON of the 28-week-old infant of our sample, however, only few incompletely differentiated neurons were found. The above observations indicate that a temporal difference is present in the maturation of the TH-IR systems between the neurosecretory nuclei (PVN and SON) and the brainstem in humans.

The full-term infants displayed a large variation in number of TH-IR perikarya, especially in the SON, that could not be attributed to sex, postmortem delay or fixation time. The level of TH-immunoreactivity in the magnocellular neurosecretory neurons has been shown to be regulated by extracellular factors, probably afferent inputs⁷. In the rat brain under normal conditions only very few TH-IR neurons are detectable in the magnocellular subnuclei of the PVN^{5,23} while in the SON TH-IR perikarya are found only occasionally²³. However, magnocellular neurons in both PVN and SON of the rat become TH-immunopositive after experimental manipulations that affect the water balance in the body, i.e. under stimuli that are supposed to affect the vasopressin production⁷. After the expulsion phase of labor in humans, vasopressin levels reach values that are in the highest range ever found in physiology^{1,4,15}. Vasopressin levels in cord blood after normal delivery are much higher than after elective caesarian section not in labor¹, indicating an active secretion of vasopressin by the fetus during labor. It has been proposed that the increased vasopressin secretion by the fetus during labor is induced by stress, asphyxia or rises in intracranial pressure associated with delivery^{1,4}. It is considered to be an adaptive mechanism intended to redistribute cardiac output to vital organs such as the brain, heart and adrenals¹⁹. Since all the full-term neonates of our sample were delivered vaginally and since most of them died of asphyxia or hypoxia related to the delivery, the question may then be raised whether the presence of a large number of TH-IR neurons found in these cases represents a primary developmental phenomenon or partially reflects a secondary phenomenon related to the activation of the vasopressinergic systems during the process of labor.

As is shown in the present report, the PVN and

SON of the human full-term infants contain cells with TH which is indicative of CA synthesis. Catecholamines per se, however, have not been reported in these nuclei in 3 human fetuses of 7–9 months of gestation²⁰ by the monoamine fluorescence methodology. In the brain of experimental animals it has been shown that the number of hypothalamic TH-IR neurons is higher than that of the CA-containing perikarya^{3,8}. In view of the sensitivity-related considerations for the detection of limited amounts of CA¹¹, further work is necessary to elucidate this apparent discrepancy between the distribution of CA-containing and TH-IR neurons in the human infant neurosecretory nuclei.

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- 1 Chard, T., Hudson, C.N., Edwards, C.R.W. and Boyd, N.R.H., Release of oxytocin and vasopressin by the human foetus during labor, *Nature*, 234 (1971) 352–354.
- 2 Goudsmit, E., Neijmeijer-Leloux, A. and Swaab, D.F., The human hypothalamoneurohypophyseal system in relation to development, aging and Alzheimer's disease. In D.F. Swaab, M.A. Hofman, M. Mirmiran, R. Ravid and F.W. van Leeuwen (Eds.), *The Human Hypothalamus in Health and Disease, Progress in Brain Research, Vol. 93*, Elsevier, Amsterdam, 1992, pp. 237–248.
- 3 Hökfelt, T., Martensson, R., Björklund, A., Kleinau, S. and Goldstein, M., Distributional maps of tyrosine-hydroxylase-immunocytochemical neurons in the rat brain. In A. Björklund and T. Hökfelt (Eds.), *Handbook of Chemical Neuroanatomy, Vol. 2*, Elsevier, Amsterdam, 1984, pp. 277–379.
- 4 Hoppenstein, J.M., Miltenberger, F.W. and Moran, W.H., The increase in blood levels of vasopressin in infants during birth and surgical procedures, *Surg. Gynecol. Obstet.*, 127 (1968) 966–974.
- 5 Hornby, P. and Piekut, D., Catecholamine distribution and relationship to magnocellular neurons in the paraventricular nucleus of the rat, *Cell Tissue Res.*, 248 (1987) 239–249.
- 6 Hyypää, M., Hypothalamic monoamines in human fetuses, *Neuroendocrinology*, 9 (1972) 257–266.
- 7 Kiss, J.Z. and Mezey, E., Tyrosine hydroxylase in magnocellular neurosecretory neurons, Response to physiological manipulations, *Neuroendocrinology*, 43 (1986) 519–525.
- 8 Kitahama, K., Luppi, R.H., Berod, A., Goldstein, M. and Jouvet, M., Localization of tyrosine-hydroxylase immunoreactive neurons in the cat hypothalamus, with special reference to fluorescence histochemistry, *J. Comp. Neurol.*, 262 (1987) 578–593.
- 9 Kloosterman, G.J., On intrauterine growth, *Int. J. Obstet. Gynecol.*, 8 (1970) 895–912.
- 10 Li, Y.W., Halliday, G.M., Joh, T.H., Geffen, L.B. and Blessing, W.W., Tyrosine hydroxylase-containing neurons in the supraoptic and paraventricular nuclei of the adult human, *Brain Res.*, 461 (1988) 75–86.
- 11 Manier, M., Feuerstein, C., Passagia, J.G., Mouchet, P., Mons, N., Geffard, M. and Thibault, J., Evidence for the existence of L-Dopa and dopamine-immunoreactive nerve cell bodies in the caudal part of the dorsal motor nucleus of the vagus nerve, *J. Chem. Neuroanat.*, 3 (1990) 193–205.
- 12 Nagatsu, T., Levitt, M. and Udenfriend, S., Tyrosine hydroxylase,

- the initial step in NE synthesis, *J. Biol. Chem.*, 239 (1964) 2910–2917.
- 13 Nobin, A. and Björklund, A., Topography of the monoamine neurons systems in the human brain as revealed in fetuses, *Acta Physiol. Scand.*, Suppl. 388 (1973) 1–40.
 - 14 Olson, L., Boreus, L.O. and Seiger, A., Histochemical demonstration and mapping of 5-hydroxytryptamine- and catecholamine-containing neurons systems in the human fetal brain, *Z. Anat. Entwickl. Gesch.*, 139 (1973) 259–282.
 - 15 Oosterbaan, H.P. and Swaab, D.F., Amniotic oxytocin and vasopressin in relation to human fetal development and labour, *Early Human Dev.*, 19 (1989) 253–262.
 - 16 Panayotacopoulou, M.T., Guntern, R., Bouras, C., Issidorides, M.R. and Constantinidis, J., Tyrosine hydroxylase-immunoreactive neurons in paraventricular and supraoptic nuclei of the human brain demonstrated by a method adapted to prolonged formalin fixation, *J. Neurosci. Methods*, 39 (1991) 39–44.
 - 17 Pearson, J., Brandeis, L. and Goldstein, M., Appearance of tyrosine hydroxylase immunoreactivity in the human embryo, *Dev. Neurosci.*, 3 (1980) 140–150.
 - 18 Pickel, V.M., Specht, L.A., Sumal, K.K., Joh, T.H., Reis, D.J. and Hervonen, A., Immunocytochemical localization of tyrosine hydroxylase in the human fetal nervous system, *J. Comp. Neurol.*, 194 (1980) 465–475.
 - 19 Pohjavuori, M. and Fyhrquist, F., Hemodynamic significance of vasopressin in the newborn infant, *J. Pediatr.*, 97 (1980) 462–465.
 - 20 Su, H.S., Peng, Z.C. and Li, Y.W., Distribution of catecholamine-containing cell bodies in the human diencephalon, *Brain Res.*, 409 (1987) 367–370.
 - 21 Swaab, D.F., Hofman, M.A. and Honnebier, M.B.O.H., Development of vasopressin neurons in the human suprachiasmatic nucleus in relation to birth, *Dev. Brain Res.*, 52 (1990) 289–293.
 - 22 Takahashi, H., Nakashima, S., Ohama, E., Takeda, S. and Ikuta, F., Topography of the catecholamine neurons in the brainstem of the human fetus: an immunohistochemical study using antibodies to tyrosine hydroxylase, *Brain Nerve*, 38 (1986) 87–97.
 - 23 Van den Pol, A.N., Herbst, R.S. and Powell, J.F., Tyrosine hydroxylase immunoreactive neurons of the hypothalamus: a light and electron microscopic study, *Neuroscience*, 13 (1984) 1117–1156.
 - 24 Verney, C., Zecevic, N., Nikolic, B., Alvarez, C. and Berger, B., Early evidence of catecholaminergic cell groups in 5- and 6-week-old human embryos using tyrosine hydroxylase and dopamine- β -hydroxylase immunocytochemistry, *Neurosci. Lett.*, 131 (1991) 121–124.