Short Communication

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 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 fetuses17,18,22.

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) nuclei10,16. However, in the fetal human brain neither TH-IR17,18,24 nor CA-fluorescent neurons6,13,14,20 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 hospital 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 μm paraffin frontal sections25 of each subject were alternately stained for TH or for vasopressin in order to determine the boundaries of the neurosecretory nuclei. After dehydration 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 (2×10 min); (d) incubated for 30 min in goat anti-rabbit serum (Betsie) 1:50 in incubation buffer; (e) rewarshed in TBS (2×10 min); (f) incubated for 1 h in peroxidase-antiperoxidase (PAP) 1:1000 in incubation buffer; (g) washed in TBS (2×10 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% \( \text{H}_2\text{O}_2 \); (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 perikarya 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, ovaly 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

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age</th>
<th>Sex</th>
<th>Cause of death</th>
<th>Body weight (g)</th>
<th>Brain weight (g)</th>
<th>*</th>
<th>Postmortem delay (hrs)</th>
<th>Fixation (days)</th>
<th>TH-IR neurons PVN</th>
<th>TH-IR neurons SON</th>
</tr>
</thead>
<tbody>
<tr>
<td>88.321</td>
<td>28w</td>
<td>M</td>
<td>Immaturity, E. coli sepsis, meningitis, convulsions, hyperbilirubinemia, open duct of Botalli</td>
<td>1060</td>
<td>125</td>
<td>25–50</td>
<td>24</td>
<td>31</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>90.263.3c</td>
<td>29w</td>
<td>M</td>
<td>Dysmaturity, hyaline membrane</td>
<td>760</td>
<td>160</td>
<td>2.3–5</td>
<td>24</td>
<td>28</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>89.188.5</td>
<td>29w</td>
<td>M</td>
<td>Prematurity, dysmaturity, pneumonia, open duct of Botalli, hyaline membrane</td>
<td>650</td>
<td>110</td>
<td>&lt; 2.3</td>
<td>24</td>
<td>30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>82.009</td>
<td>30w</td>
<td>M</td>
<td>Prematurity, pneumothorax, acidosis, ventricular haemorrhage</td>
<td>1690</td>
<td>210</td>
<td>50–75</td>
<td>24</td>
<td>33</td>
<td>±</td>
<td>–</td>
</tr>
<tr>
<td>90.253.3cd</td>
<td>31w</td>
<td>M</td>
<td>Convulsions due to ischaemia</td>
<td>1300</td>
<td>206</td>
<td>10–25</td>
<td>6.5</td>
<td>28</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>86.335.4</td>
<td>31w</td>
<td>F</td>
<td>Prematurity, hyaline membrane, hypoxia</td>
<td>1730</td>
<td>200</td>
<td>50–25</td>
<td>24</td>
<td>33</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>89.153.7</td>
<td>37w</td>
<td>M</td>
<td>Pneumothorax, streptococcus sepsis, bronchopneumonia, hypoxia</td>
<td>2930</td>
<td>360</td>
<td>25–50</td>
<td>48</td>
<td>30</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>87.361.3</td>
<td>39w</td>
<td>F</td>
<td>Perinatal asphyxia, brain oedema</td>
<td>1630</td>
<td>300</td>
<td>&lt; 2.3</td>
<td>5.5</td>
<td>24</td>
<td>+ + + +</td>
<td>+ + +</td>
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<tr>
<td>88.381.4d</td>
<td>40w</td>
<td>M</td>
<td>Asphyxia, acidosis</td>
<td>3450</td>
<td>430</td>
<td>25–50</td>
<td>12</td>
<td>32</td>
<td>–</td>
<td>–</td>
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<tr>
<td>88.353.2d</td>
<td>40w</td>
<td>M</td>
<td>Asphyxia, meconium aspiration</td>
<td>2530</td>
<td>360</td>
<td>&lt; 2.3</td>
<td>24</td>
<td>33</td>
<td>+ + + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>86.350.6</td>
<td>40w</td>
<td>F</td>
<td>Cerebral hypoxia</td>
<td>3720</td>
<td>375</td>
<td>50–75</td>
<td>24</td>
<td>19</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>87.280.3</td>
<td>40w</td>
<td>M</td>
<td>Hypoplastic left heart syndrome, anoxia</td>
<td>2950</td>
<td>400</td>
<td>2.3–5</td>
<td>24</td>
<td>35</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>90.259.1</td>
<td>42w</td>
<td>M</td>
<td>Pneumothorax, hyaline membrane</td>
<td>3400</td>
<td>390</td>
<td>25–50</td>
<td>24</td>
<td>28</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>87.152.4d</td>
<td>42w</td>
<td>M</td>
<td>Unknown, small bleeding in pons and nucleus dentatus</td>
<td>2790</td>
<td>350</td>
<td>2.5–5</td>
<td>24</td>
<td>35</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>85.002.3</td>
<td>3m</td>
<td>F</td>
<td>Bronchopneumonia, myocarditis, small sub-arachnoidal haemorrhage</td>
<td>ND</td>
<td>610</td>
<td>–</td>
<td>24</td>
<td>33</td>
<td>+ + + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>86.411.0</td>
<td>6m</td>
<td>M</td>
<td>Sudden infant death syndrome, brain oedema</td>
<td>ND</td>
<td>800</td>
<td>–</td>
<td>14</td>
<td>14</td>
<td>+ +</td>
<td>±</td>
</tr>
<tr>
<td>88.209.2</td>
<td>14m</td>
<td>M</td>
<td>Pneumococcen meningitis, pericarditis, endocarditis, brain oedema</td>
<td>ND</td>
<td>990</td>
<td>–</td>
<td>14</td>
<td>28</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>
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 μm. 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 μm. 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 μm.
perikarya has been observed in the ventral hypothalamic anlage as early as 5 weeks of gestation\textsuperscript{24}. TH-IR perikarya have also been demonstrated in the hypothalamus of fetuses of 17–21 weeks\textsuperscript{18}, 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

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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 $\mu$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 $\mu$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 trimester. In 5- to 6-week-old human fetuses, the locus coeruleus nuclei also contain TH. 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 gestation. 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 inputs. In the rat brain under normal conditions only very few TH-IR neurons are detectable in the magnocellular subnuclei of the PVN while in the SON TH-IR perikarya are found only occassionally. 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 production. After the expulsion phase of labor in humans, vasopressin levels reach values that are in the highest range ever found in physiology. Vasopressin levels in cord blood after normal delivery are much higher than after elective caesarian section not in labor, 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. It is considered to be an adaptive mechanism intended to redistribute cardiac output to vital organs such as the brain, heart and adrenals. 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 gestation 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. In view of the sensitivity-related considerations for the detection of limited amounts of CA, 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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