Maturation of the Hypothalamo-Neurohypophysial System

I. Localization of Neurophysin, Oxytocin and Vasopressin in the Hypothalamus and Neural Lobe of the Developing Rat Brain*

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Summary. Sections of the hypothalamus, median eminence and pituitary from fetal and neonatal rats were examined with the immunoperoxidase staining technique and light microscopy. Purified antisera raised against vasopressin and oxytocin, and antisera cross-reactive with rat neurophysin were used to localize these antigens in the hypothalamo-neurohypophysial system (HNS). Neurophysin was detected throughout the HNS of the 18-day fetal rat. Vasopressin was present in the hypothalamus and pituitary of the 19-day fetus, and in the median eminence of the 4-day neonate. Oxytocin was not detected in the pituitary until 1–2 days after birth, in the hypothalamus after 4 days, and in the median eminence after 8 days. During the first days after birth the supraoptic nucleus was more mature than the paraventricular nucleus. The HNS did not approach maturity until at least 7 days after birth. The relative maturity of the supraoptic nucleus compared with the paraventricular nucleus, and the detection of vasopressin before oxytocin are evidence for the one-neuron-one-hormone theory. The data do not exclude the possibility that the fetal hypothalamo-neurohypophysial system, and perhaps the fetal hormone, vasotocin, affect the initiation and course of parturition.

Key words: Rat hypothalamo-neurohypophysial system – Fetal development – Oxytocin – Vasopressin – Neurophysin

The posterior pituitary hormones oxytocin and vasopressin and the neurophysin proteins are synthesized in the supraoptic, paraventricular and accessory nuclei of the hypothalamus. These substances are transported within neurosecretory fibres that form the hypothalamo-neurohypophysial tract through the median eminence to the posterior lobe of the pituitary. Studies dealing with the timing and site of the

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appearance of neurosecretory material (NSM) during ontogenesis are few. The data have been summarized by Donev (1970) as follows: NSM appears after birth in those mammals with a short gestation and those whose young are born immature. The rat belongs to this group. In general the hypothalamo-neurohypophysial system (HNS) of mammals with a long gestation (e.g., guinea pig, sheep and man) is functional before birth. NSM is first detected at the site of storage in the pituitary and later at the site of synthesis in the hypothalamus. Its appearance is most retarded in the area of the median eminence.

The purpose of the present study was to determine the maturity of the fetal and neonatal rat HNS with an immunocytochemical approach in conjunction with the posterior pituitary lobe principles. It was considered that such an examination would contribute to an understanding of the functional maturity of the system at this time. The important question of whether the fetal HNS is able to contribute to the manifold interactions that control parturition was also considered.

Materials and Methods

Fetal and neonatal Wistar rats of either sex were killed by decapitation (age distribution in Table 1). The lower jaw and calvarium were removed and the heads were fixed in picric acid-formalin for 9–14 days at room temperature. The postnatal heads were decalcified in 7.5% (v/v) aqueous formic acid. Excess acid was washed out with 75% (v/v) aqueous ethanol for 2 days. Some neonate brains were dissected free from bony tissue and were not treated with formic acid before further processing. All specimens were dehydrated in alcohol, cleared in xylol and embedded in Paraplast (Sherwood Medical Industries, Missouri, U.S.A.). The tissue blocks were sectioned either transversely or sagitally at 8 μm.

Sections were stained with the three-layer immunoperoxidase technique (Graham and Karnovsky, 1966; Mason et al., 1969; Sternberger et al., 1970) but modified for neurophysin (Watkins, 1975) and the posterior pituitary hormones (Swaab and Pool, 1975; Choy and Watkins, 1977). In brief, sections were hydrated and incubated alternately with: (1) anti-neurophysin (1:1,000), anti-vasopressin (1:300), or anti-oxytocin (1:300) for 18 h at room temperature; (2) sheep anti-rabbit serum (1:10) for 60 min at room temperature and (3) peroxidase-antiperoxidase complex (1:50) for 60 min. The sections were washed after each step, and stained by immersion for five minutes in a solution of 0.025 % 3,3'-diaminobenzidine hydrochloride and 0.025 % H2O2 in Tris-HCl buffer pH 7.5). The stained sections were washed in water, dehydrated in alcohol, cleared in xylol and mounted under Eukitt (O. Kindler, Freiburg, West Germany). Control sections were prepared by omitting one of the immune reagents. Tests for specificity have been described (Choy and Watkins, 1977). Some sections were stained with standardized Harris's haematoxylin and eosin.

Table 1. Age distribution of rats

<table>
<thead>
<tr>
<th>Birthday</th>
<th>Fetal age (d)</th>
<th>Postnatal age (d)</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>18 19 20 21 0 1 1½ 2 2½ 3 4 5 6 7 8 9 10 11 12 20</td>
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| Number of Specimens | Hypothalamus | 4 4 6 0 6 3 2 2 2 3 5 2 4 5 2 3 5 0 3 3 64 |
|                     | Pituitary    | 4 4 6 0 7 3 2 2 2 3 5 2 4 4 2 4 3 0 0 0 57 |