Proliferation of Capillary Endothelial Cells in the Primary Plexus of the Hypophyseoportal System in Rats During Ontogeny

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A light-microscopy study of primary portal plexus formation in rats between day 14 of prenatal life and postnatal day 9, carried out using thymidine autoradiography, showed that the formation and growth of capillaries for this plexus occurs through proliferation of endothelial cells whose mitotic activity is highest on days 14-16 of prenatal development. Endothelial cell differentiation and capillary development are characterized by flattening of the endothelium, widening of capillary lumens, and the formation of capillary loops that penetrate into the median eminence. The findings of this study indicate that capillaries of the primary portal plexus mainly develop in rats during the perinatal period.

Key Words: *capillaries; hypothalamus; ontogeny; rats*

The primary portal plexus (PPP) of capillaries is an important component of the hypophyseoportal system. The vessels constituting the PPP are located on the surface of and within the median eminence and communicate, on the one hand, with vessels of the subependymal plexus in the arcuate nucleus of the hypothalamus and, on the other, with vessels of the adenohypophysis. Such architechtonics of this system allows for the transport of adenophyseotropic hormones from the hypothalamus to the adenohypophysis and of adenohypophyseal hormones in the reverse direction [9].

Studies of the hypophyseoportal system in rats during ontogeny using India ink to fill the vascular bed showed that the system begins to develop on day 13 of embryonal life [2,5,11]. A primary capillary plexus on the surface of the embryonal median eminence on prenatal days 15-16 was described [2,4]. Its formation is completed in the early postnatal ontogeny with the emergence of numerous capillary loops that penetrate into the median eminence [12]. The mechanisms responsible for such a rapid appearance of capillaries in large numbers in the PPP remain unknown. The aim of the present study was to examine one aspect of vascular genesis, namely the proliferative activity of capillary endothelium on the median eminence surface in rats during the formation of the primary plexus of the hypophyseoportal system.

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MATERIALS AND METHODS

In this study, Wistar rat fetuses (n=12) and pups (n=6) were examined, respectively, on days 14, 16, 18, and 20 of prenatal life and days 1-9 of postnatal life using thymidine autoradiography. For the examination, pups and pregnant females were injected with ^{3}H-thymidine intraperitoneally (5 μCi/g body weight) on the indicated days and 2.5 h later were perfused via the heart, under Nembutal

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anesthesia (40 mg/kg body weight), with physiological saline for 5 min and then with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer. Thereafter the brain was removed and the hypothalamus was dissected out and postfixed in the same fixative overnight at 4°C. The material was then washed in phosphate buffer, dehydrated through graded alcohols, and embedded in a mixture of Epon and Araldite. For light-microscopic examination, frontal and sagittal semithin (2 μ) sections prepared on an LKB-III ultratome were used. The radioactive label was detected with a liquid emulsion (Type M, manufactured at the Khimfotoproekt Institute, Moscow) applied to slides with the sections, which were then exposed in the dark for 1-2 months at 4°C. After development using an amidol developer, the sections were stained with toluidine blue and examined in a Zeiss light microscope at a magnification of 100 (objective)×15 (eyepiece). The proliferation of capillary cells was assessed by the nuclear labeling index (NLI) defined as the percentage ratio of the number of cells with radioactively labeled nuclei to the total number of endothelial cells.

RESULTS

Light-microscopic examination of the mediobasal hypothalamus demonstrated that the capillary plexus on the surface of the embryonal median eminence has, in the early ontogeny, a number of features previously described in the mouse [3]. This plexus is made up of capillaries with a wide lumen and a relatively thick endothelial wall.

The NLI of capillary cells was very high as early as on day 14 of prenatal development and attained its maximal value on day 16 (Fig. 1). Such a high mitotic activity is characteristic of protocapillary endothelium [1,15] and precedes the formation of the secondary organ-specific circulatory bed.

On day 18 of prenatal life, the PPP had a larger number of capillaries than before. Many of these had a wall which in section was formed by two endothelial cells. The lumen of such capillaries was narrow or closed by protruding endothelial cell perikarya [12]. A structure of this kind is typical of slightly differentiated continuous capillaries [3]. The plexus also contained more differentiated capillaries with fairly wide lumens and areas of flattened fenestrated endothelium oriented, for the most part, toward the median eminence [12]. A few capillary loops were seen to penetrate into the tissues of the median eminence. By that time (day 18), the NLI of capillary endothelial cells in the PPP had decreased to 18.7% (Fig. 1).

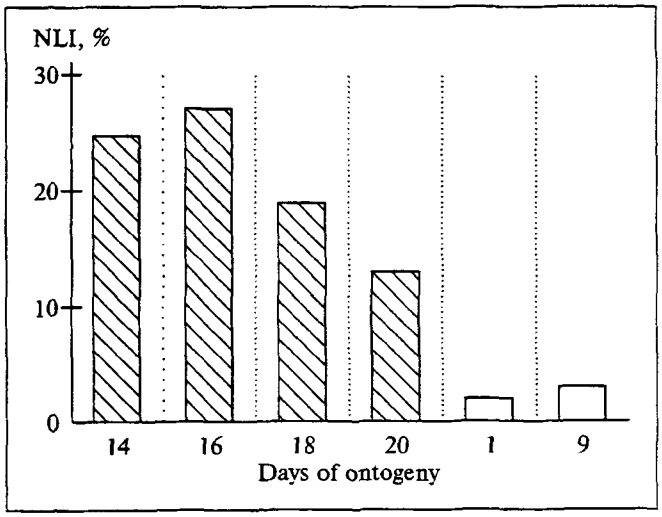

Fig. 1. Nuclear labeling index (NLI) of capillary cells in the primary portal plexus of rats at different times of prenatal (black bars) and postnatal (white bars) ontogeny.

By day 20 of prenatal development, the number of PPP capillaries had markedly increased. The capillaries had wide lumens and extended areas of flattened fenestrated endothelium. The NLI was still lower than on day 18 (Fig. 1).

After birth, the density of PPP capillaries on the median eminence surface considerably increased. In section, the capillary wall was made up of 2-5 endothelial cells with rather extensive areas of flat fenestrated endothelium [12]. Numerous capillary loops were seen penetrating into the outer zone of the median eminence, and some of them reached into the inner zone. These changes increased progressively from day 1 to 9 of postnatal life. The NLI of PPP capillary cells remained at low levels (3.35% on average) (Fig. 1).

Thus, as is evident from Fig. 1, the proliferative activity of PPP capillary cells was highest on days 14-16 of prenatal ontogeny. After birth it remained at a low level and by day 9 did not differ from that shown by mature capillaries of the brain [10].

The highest proliferative activity of capillary cells on the median eminence surface and, hence, the most rapid formation of new vessels thus coincide with the time when the axons of neurosecretory cells begin to grow into the median eminence [7,8,14]. The intensive axonal ingrowth in the prenatal period leads, in turn, to the formation of numerous axovasal contacts [13] at which neurohormones enter the portal bloodstream. The temporal correlation observed between the formation of new vessels for the PPP and the directional axonal growth into the median eminence suggests that these two events are interrelated. Possibly, the physiologically active substances released from neurosecretory

axons influence, at a particular stage of ontogeny, the regional proliferation of endothelial cells on the median eminence surface. The establishment of a functional hypophyseoportal system in prenatal ontogeny [2,6] is accompanied by differentiation of endothelial capillary cells in the PPP [3,12] and a decline of their proliferative activity.

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