NEURONAL DEVELOPMENT AND RELATED HISTOCHEMISTRY —ENZYMATIC POLARITY IN BRAIN CAPILLARIES—

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We performed ultracytochemical studies of Mg-ATPase and TPPase activities in circumventricular capillaries at the developmental stage of rats, from 17 gestational days to 8 postnatal weeks, comparing with hippocampal capillaries. During the course of perinatal development, the predominant localization of both enzymatic activities, particularly Mg-ATPase, in circumventricular capillaries was shifted from the luminal cell membrane to the antiluminal cell membrane. In all hippocampal capillaries observed, the predominant localization of both enzymatic activities was the antiluminal cell membrane. It was suggested that immaturity of circumventricular capillaries may be one of the causative factors inducing intraventricular and subependymal hemorrhages in neonates.

Intraventricular hemorrhage is often cited as a major cause of death in the neonate and generally recognized as a disease of the small premature (5, 11, 27). Nearly one-half of all prematurely born neonates with birth weights less than 1,500 g develop periventricular and intraventricular hemorrhages (7). Much work has been done as to the mechanism of such hemorrhages (1, 3, 4, 10, 19, 28, 29, 31), but little is understood about the pathogenesis. It is believed at present that the primary lesion involves bleeding into the germinal layer beneath the ependyma of the lateral ventricle over the head and body of the caudate nucleus, with subsequent rupture of the hematoma into the ventricular system.

To develop better understanding, we attempted to observe the ultrastructural localization of magnesium-dependent adenosine triphosphatase (Mg-ATPase) and thiamine pyrophosphatase (TPPase), membrane-bound enzymes, activities in the circumventricular tissue, comparing them with those of hippocampus in perinatal rats.

MATERIALS AND METHODS

Male white Wistar rats at 17–21 gestational days, 1, 3 and 18 postnatal days and 8 postnatal weeks were observed in the present study. Concerning the gestated fetus, virgin females at 8 or 9 weeks of age were mated with mature male rats of the same strain and fetuses were obtained by caesarean section of anesthetized animals.

The observed rats were anesthetized with pentobarbital sodium (Nembutal, 0.1 ml/100 g, intraperitoneally). Vascular perfusion through the heart was initiated

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with physiological saline solution, at room temperature, for approximately 30 s., followed by cold mixtures of 1% glutaraldehyde and 2% paraformaldehyde in 30 mM PIPES buffer, pH 7.3, 830 mOsm. After vascular perfusion for 15 min, the brain was removed and immersed in the same fixative for 15 min at 0–4°C. The materials were rinsed for several hours with several changes of cold 30 mM PIPES buffer, pH 7.3, containing 8% sucrose, 330 mOsm. Then, the circumventricular and hippocampal tissues were sectioned with a Microslicer (Dosaka EM, Kyoto) at approximately 40 μ m in thickness. These sections were incubated in the medium by Ogawa and Mayahara (21) for Mg-ATPase and for TPPase by Ogawa *et al.* (22) for 30 min at 37°C. Control sections were incubated without substrate added to the incubation medium. After incubation, the sections were osmicated in cold 1% osmium tetroxide in Caulfield's buffer, dehydrated in graded concentrations of ethanol, treated with propylene oxide and embedded in Spurr's medium (TAAB). Thin sections prepared using a Ultracut E (Reichert-Jung) were poststained with uranyl acetate and examined under a JEM 2000EX (JEOL) electron microscope at 100 kV.

RESULTS

1. Mg-ATPase activity in the vascular endothelial cells.

In the circumventricular tissue, the reaction products for Mg-ATPase activity were precipitated on the luminal and antiluminal sides of the plasma membrane from the 17th day of the gestational fetus until the full term (Fig. 1a). Generally, the activity associated with the luminal plasma membrane was stronger than that of the antiluminal plasma membrane. The Mg-ATPase-positive plasma membranes were intermingled with those of no activity. Reaction products were rarely present in pinocytotic vesicles of the vascular endothelial cells. However, no reaction products were observed on the cellular organelles. In the first day after birth, the activity was localized on the antiluminal plasma membrane (Fig. 1b). The pinocytotic vesicles showed more intense activity of Mg-ATPase. On the 3rd and 18th days after birth,

FIG. 1a–b. Mg-ATPase activity in the circumventricular capillary. a. At the 19th day of the gestational fetus, Mg-ATPase-positive plasma membranes in the vascular endothelial cells were intermingled with those of no activity. b. At the first day after birth, reaction products were predominantly precipitated on the antiluminal plasma membrane. The pinocytotic vesicles showed more intense activity of Mg-ATPase.

FIG. 2. Mg-ATPase in the hippocampal capillary. At the 19th day of gestational fetus, the activity was already localized on the antiluminal plasma membrane.

reaction products were observed on the antiluminal membrane. In the hippocampal tissue, the reaction products for Mg-ATPase activity were already precipitated on the antiluminal side of the plasma membrane from the 17th day of the gestational fetus (Fig. 2). That is to say, no difference between the fetus and adult rats was evident.

2. TPPase activity in the vascular endothelial cells.

In the circumventricular tissue, no reaction products for TPPase activity were precipitated on the luminal and antiluminal sides of the plasma membrane from the 17th day of the gestational fetus until the full term (Fig. 3a). In the hippocampal tissue, the intense TPPase activity was already observed on the antiluminal side of the plasma membrane at the 17th day of the gestational fetus, indicating the same activity in adult rats (Fig. 3b).

FIG. 3a-b. TPPase activity at the 20th day of the gestational fetus. a. In the circumventricular capillary, no reaction products were observed on the cell membranes of vascular endothelial cells. b. In the hippocampal capillary, the reaction products for TPPase activity were precipitated on the antiluminal side of the plasma membrane.

DISCUSSION

It was suggested by the present study that the developing capillaries in the circumventricular tissue indicate a remarkable characteristic around the perinatal period, in comparison with that of the hippocampus which was changes of the localization pattern in Mg-ATPase and TPPase activities between the gestational fetus and newborn rats. In 1984, Inomata *et al.* reported the enzyme-ultracytochemical localization of capillary endothelial cells in the adult rat central nervous system (15). It was suggested that differences in the intensity of the enzyme activities of the luminal and antiluminal plasma membranes may reflect the polarity in the capillary endothelial cells and relate to blood-brain barrier (BBB) mechanisms. As indicated by Rapoport (25), cerebral capillary endothelium is a metabolically active tissue. Hence, the capillary enzymes most probably play an active role in the maintenance of the BBB. Both enzymatic activities in the hippocampal tissue indicated the polar arrangement from the 17th day of the gestational fetus, in comparison with that of the circumventricular tissue. That is to say, a result was obtained that capillaries in the circumventricular tissue indicate poor development.

The factors predisposing to intraventricular hemorrhage and the concomitant aberrations of blood flow, blood pressure, and blood volume are becoming better defined (29). Numerous studies (6, 8, 9, 13, 14, 16-18, 23) have correlated events known to alter cerebral blood flow, such as hypercarbia, volume expansion, and pneumothoraces, with intraventricular hemorrhage. Cooke (2) and Milligan (20) have reported increases in the cerebral blood flow index in infants with hypercarbia and volume expansion who subsequently developed intraventricular hemorrhage. Similarly, others (13, 24) have noted increased cerebral blood flow velocity in neonates with seizures prior to the development of intraventricular hemorrhage.

It is more common that periventricular and intraventricular hemorrhages originate from the subependymal germinal matrix. Recent advances in neuroradiological techniques (position emission tomography and nuclear magnetic resonance) may make it possible not only to relate neurodevelopmental outcome to structural damage of the neonatal brain but also to functional and ischaemic lesions (26, 30). The vessels in the region of the subependymal germinal matrix are not structurally mature; collagenous support is lacking, they have less elastin, and capillaries seem to make direct anastomosis at right angles to venules in the subependymal germinal matrix (10, 12). In addition, our results showed that the polar arrangement of enzymatic activities in the capillary means a poor development.

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