N-メチルニトロソウレアにより誘発された奇形性水小頭症ラット脈絡叢におけるウワバイン感受性 K+ 依存性p-ニトロフェニルフォスファターゼ(p-NPPase)活性

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Ouabain-Sensitive Potassium-Dependent p-Nitrophenylphosphatase Activity on Choroid Plexus in N-Methyl-Nitrosourea-Induced Dysgenetic Hydromicrocephalic Rat Offsprings

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ABSTRACT. Ultracytochemical localization of K^+-dependent p-nitrophenyl-phosphatase (p-NPPase) activity using Mayahara's method was investigated on the choroid plexus of N-methylnitrosourea (MNU)-induced dysgenetic hydromicrocephalic rat offsprings aged 12 weeks. The reaction sites were characteristically observed in lysosomes, multivesicular bodies, coated vesicles and on basal infoldings in the epithelial cells, in microvesicles in the interstitial cells, and in an increasing number of pinocytotic vesicles and along junctional complex in the capillary endothelial cells. These changes suggest that leaking blood components in choroidal edema might pass the choroidal cells.—KEY WORDS: choroid plexus, dysgenetic hydromicrocephaly, p-NPPase.

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In the previous report [1] the authors showed abnormal function of the choroid plexus in N-methylnitrosourea (MNU)-induced dysgenetic hydromicrocephalic rat offsprings, suggesting excessive leakage of blood plasma through the choroidal epithelium into the ventricular lumen. It is generally accepted that active transport linked to the activity of membrane-bound Na/k ATPase plays an important role for the production of cerebrospinal fluid (CSF) in the choroid plexus [2, 10, 11]. The purpose of this report is to investigate the role of choroid plexus in the present hydrocephalus, using ultracytochemical method of K^+-dependent p-nitrophenylphosphatase (p-NPPase) deviced by Mayahara *et al.* [8], for the p-PNPase was deeply associated with transport ATPase cytochemistry [5, 6].

The present experiment was conducted using several male wistar rats (CLEA Japan Inc.). Five dysgenetic hydromicrocephalic rat offsprings produced by similar method described previously [1] and 3 control rats were killed by bleeding from the abdominal aorta under ether anesthesia at the ages of 12 weeks. The choroid plexus in the lateral ventricle collected from these animals was immediately fixed in a mixture of 2% paraformaldehyde/0.5% glutaraldehyde/0.1 M cacodylate (pH 7.3) for 1 hr at 0°C–4°C. After fixation, tissues were overnight in 0.01 M cacodylate buffer (pH 7.3) with 8% sucrose. Frozen section of 20 μm cut with a cryostat was incubated in medium of Mayahara's method [8] (1.0 M glycine-KOH buffer, pH 9.0, 2.5 m*l*; 1% lead citrate dissolved in 50 mM KOH, 4.0 m*l*; 25%v/v dimethylsulphoxide, 2.5 m*l*; 0.1 M p-nitrophenylphosphate, 1 m*l*; Levamisole, 6.02 mg, final pH 8.8) for 10–20 min at room temperature. As the cytochemical control of the reaction, several specimens were preincubated with 10 mM ouabain in cacodylate buffer for 30 min at 0°C prior to the cytochemical incubation. Then, after washing with 0.1 M cacodylate buffer (pH 7.3 with 8% sucrose), the sections were

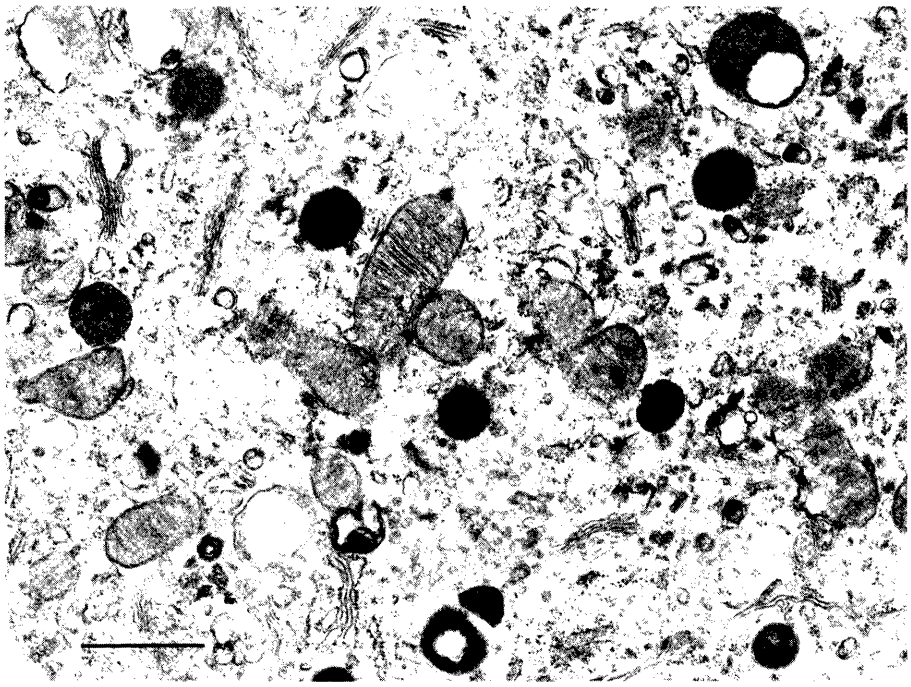

Fig. 1. Obvious reaction products in the lysosomes, multivesicular bodies and coated vesicles in the choroidal cell. p-NPPase activity, Bar=1 μm.

postfixed with 1% OsO_4 for 1 hr, dehydrated in graded ethanol series and embedded in Epoxy resin. Ultrathin sections were stained with uranylacetate and examined in an electron microscope (JEM 100C) at 80 kV.

In the choroid plexus of the control group the localization of K^+-dependent p-NPPase activity using Mayahara's method was well consistent with that in the report of Masuzawa *et al.* [7], except for the absence of the reaction products on the nuclear membrane. The reaction products in the control choroid plexus were observed in the microvilli, on the rough endoplasmic reticulum (RER) and along the basal plasmalemmas in the epithelial cells. The reaction products were also found along plasmalemmas and on the RER in the interstitial cells and capillary endothelium, and on the corpuscle of the red blood cells. In the choroid plexus of the MNU group, reaction precipitates were decreased in the altered microvilli and increased in other subcellular organelles of the epithelial cells. The reaction products were, in addition to the organelles noted in the control, localized in the lysosomes, multivesicular bodies and small coated vesicles, which appeared to increase various dense bodies in the epithelial cells (Fig. 1). Most of mitochondria had no reaction products, but was infrequently positive on the outer membrane of a few mitochondria involved in several large vacuoles formed in the epithelial cells. Fine reaction products were also localized on the plasma membrane of the basal infoldings (Fig. 2) and rarely at along the lateral plasma membrane but not at the junctional complex of epithelial cells (Fig. 3). Similar positive reaction was demonstrated, rather intensively, in the increased microvesicles in the interstitial cells and in an increasing number of pinocytotic vesicles (Fig. 4) and along the junctional complex (Fig. 5) in the capillary endothelium.

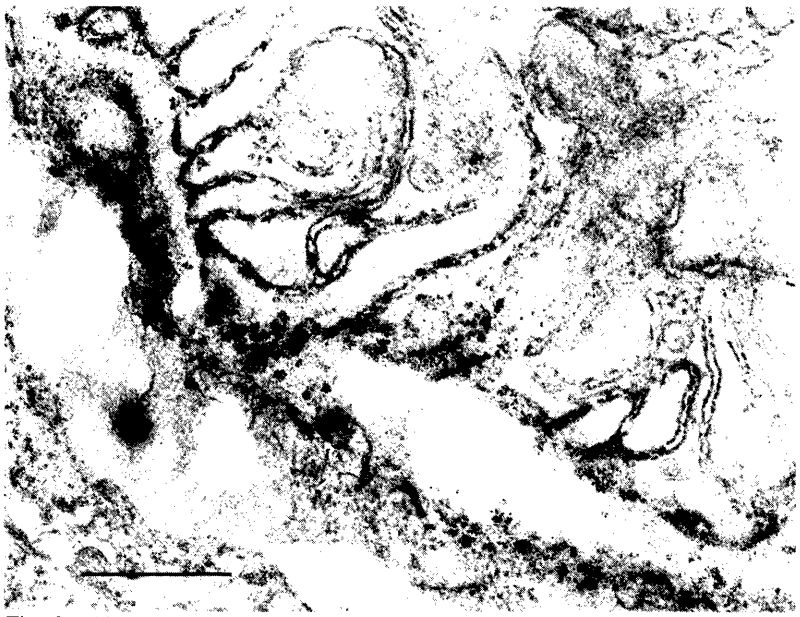

Fig. 2. Fine reaction products on the plasmalemmas of the basal infoldings in the choroidal cell. p-NPPase activity, Bar=500 nm.

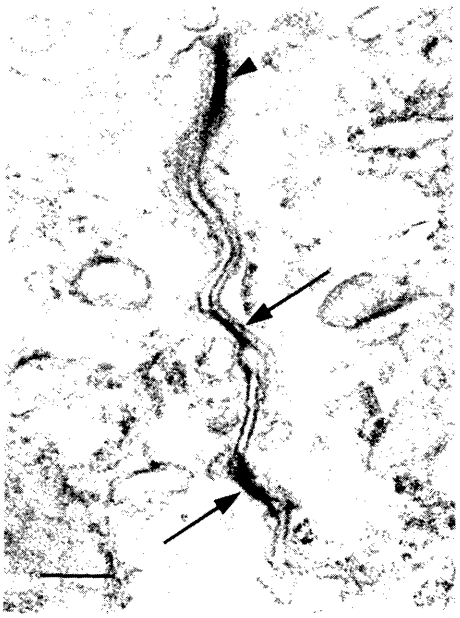

Fig. 3. Slight reaction products along the lateral plasmalemmas (arrows) in the choroidal cell. p-NPPase activity, Junctional complex (arrowhead), Bar=200 nm.

In the choroid plexus of the MNU group the localization of K^+-dependent P-NPPase activity using Mayahara's method was different in many ways from that in the choroid plexus of normal rats reported by Masuzawa *et al.* [7]. In the MNU group the localization of p-NPPase activity was exhibited in the lysosomes, multivesicular bodies and coated vesicles, on the basal infoldings in the epithelial cells, in the microvesicles in the interstitial cells, and in an increasing number of pinocytotic vesicles and along the junctional complex in the capillary endothelial cells different from that in the normal choroid plexus [7]. As described previously [1], the morphologic features in the choroid plexus of the MNU group were characterized by the age-related perivascular edema and alterations of the organelles in the epithelial cells with partial destruction of the cells. Although the present investigation was conducted on relatively intact choroidal cells exposed to MNU, the results of the previous and present experimemts suggested that the blood components leaking through the pinocytotic vesicles and junctional complex from the capillaries might be

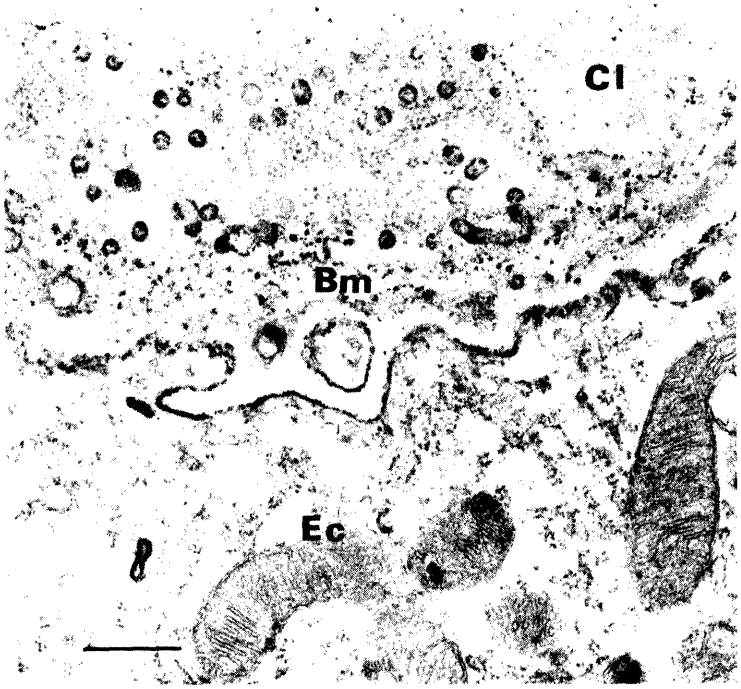

Fig. 4. Reaction product-containing increased pinocytotic vesicles in the capillary endothelium. p-NPPase activity, Cl; Capillary lumen, Bm; Basement membrane, Ec; Epithelial cell, Bar=500 nm.

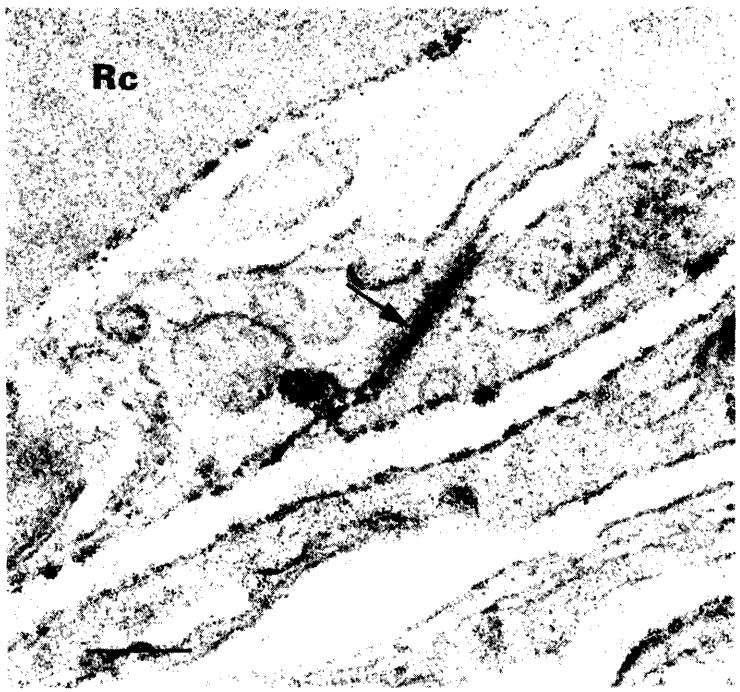

Fig. 5. Reaction precipitates along the junctional complex (arrow) in the capillary endothelium. p-NPPase activity, Rc; Red blood cell, Bar=200 nm.

released by either vesicular transport in the epithelial cells or destruction of the epithelial cells into the ventricular lumen. Such a movement of blood components as the vesicular transport was similarly observed in the choroid plexus when lanthanum in cats [4], protein in mice [3] or microperoxidase in rats [13, 14] was intravenously administered. In these experiments each tracer was well demonstrated in the choroidal intercellular space, however, in the present experiment the reaction products of p-NPPase were rarely seen along with the lateral plasma membrane of the epithelial cells. Regarding the localization of ATPase on the lateral plasma membrane of choroidal cells in normal animals, there are many controversial reports [7, 9, 12]. No reaction products of p-NPPase using Mayahara's method were found at long the lateral plasmalemmas of choroidal cells in normal rats [7]. Although the localization of p-NPPase on the membrane is noteworthy under abnormal states like the present model, further investigation at various stages of this hydrocephalus will be needed for elucidations on the localization of ATPase activity in the choroid plexus.

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要　約

N-メチルニトロソウレアにより誘発された奇形性水小頭症ラット脈絡叢におけるウワバイン感受性 K^+ 依存性 p-ニトロフェニルフォスファターゼ（p-NPPase）活性：相内聖峰，小林賢一，佐久間貞重（アップジョンファーマシュウティカルズ　リミテッド総合研究所）——12週齢ラットに N-メチルニトロソウレア（MNU）で誘発した奇形性水小頭症について，K^+ 依存性 p-NPPase の細胞化学的局在を検討した。馬屋原法による反応部位は毛細血管内皮の増生したのみこみ小胞と細胞間結合，間質細胞の小空胞および脈絡叢上皮の基底陥入，ライソゾーム，多胞体，または被覆小胞であった。これらの変化から，脈絡叢水腫においては，漏出血液成分が脈絡叢上皮細胞を通過することが示唆された。