Na,K-, Mg- AND HCO_3 -ADENOSINE TRIPHOSPHATASES IN THE RABBIT BRAIN CHOROID PLEXUS

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Abstract—Properties of adenosine triphosphatases (ATPases) in the choroid plexus of rabbit cerebral ventricles were investigated using tissue homogenates and subcellular fractions. Na,K-ATPase or HCO_3 -ATPase activity of choroid plexus was significantly lower than that of the brain or the kidney, respectively. Among the homogenates of choroid plexus from the lateral, third and fourth ventricles, there were no differences in the activities of Na,K-, Mg- and HCO_3 -ATPases. In the choroid plexus, Na,K-ATPase activity was demonstrated to be highest in $10,000\times g$ fraction, while the highest Mg- and HCO_3 -ATPase activities were observed in $8,000\times g$ fraction, the so-called mitochondrial fraction. Ion requirement, pH optimum and ouabain sensitivity of Na,K-ATPase in $10,000\times g$ fraction of choroid plexus were similar to findings in erythrocytes, brain and kidney. However, properties of Mg- and HCO_3 -ATPases in $8,000\times g$ fraction were considerably different from those found in the brain and gastric mucosa. In the choroid plexus, thiocyanate ion inhibited Mg-ATPase as well as HCO_3 -ATPase. These results indicate that characteristics of ATPase systems in the choroid plexus are to some extent different from those in tissues such as brain, kidney and gastric mucosa.

The cerebrospinal fluid is formed mainly in the choroid plexus present in the lateral, third and fourth ventricles, largely by active secretion rather than merely by ultrafiltration (1). Vates et al. (2) demonstrated that the Na-K exchange system in the choroid plexus had a primary functional importance in the formation of cerebrospinal fluid in the cat. Furthermore, the choroid plexus has been shown to involve active transport systems for bicarbonate ion (3) and Mg ion (4). To clarify the biochemical characteristics of these transport systems, we examined adenosine triphosphatase (ATPase) systems of the choroid plexus with regard to the effects of ions and inhibitors.

MATERIALS AND METHODS

Preparation of tissues: Adult rabbits (2.0–3.0 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.) and perfused with physiological saline through the heart. The choroid plexus of the lateral, third and fourth ventricles were quickly removed, immediately cooled on ice and homogenized in 9 vol. of ice-cold 0.25 M sucrose containing 1 mM EDTA and 25 mM imidazole-HCl (pH 7.0). The homogenate was fractionated by centrifugation in $1,000\times g$ (15 min) pellet, $8,000\times g$ (15 min) pellet, $10,000\times g$ (15 min) pellet, $100,000\times g$ (60 min) pellet and remaining supernatant. The pellets were resuspended in 1 mM EDTA-Tris (pH 7.0). Except for the first experiment with homogenates, the tissue preparations

were treated with 0.1 % sodium deoxycholate at 0°C for 30 min, or at 37°C for 10 min, at the protein concentrations over the range of 0.3–3.3 mg/ml.

Protein determination: Protein concentrations were estimated by the method of Lowry et al. (5) with crystalline bovine serum albumin as a standard.

Assay of ATPase: The activity of Na,K-ATPase was assayed at 37°C in 0.2 ml medium containing 50 mM Tris-HCl (pH 7.4), 1 mM EDTA-Tris, 6 mM MgCl₂, 100 mM NaCl, 10 mM KCl, 6 mM ATP-Tris and an aliquot of the tissue preparation with or without 1 mM ouabain, and was calculated as an ouabain sensitive activity. The activity of Mg- or HCO₃-ATPase was assayed at 37°C in 0.2 ml medium consisting of 50 mM Tris-HCl (pH 7.4), 1 mM EDTA-Tris, 6 mM MgCl₂, 6 mM ATP-Tris, 1 mM ouabain and an aliquot of the tissue preparation with or without 20 mM NaHCO₃. The HCO₃-ATPase activity was calculated as a HCO₃-stimulated portion of the activity. Following a 5-min preincubation period at 37°C, the reaction was initiated by the addition of ATP-Tris. The reaction was allowed to continue for 10 min, and was terminated by adding 0.2 ml of 10% (W/V) trichloroacetic acid. After centrifugation of the mixture at 3,000×g for 5 min, the amount of inorganic phosphate liberated during the incubation was measured using 0.3 ml of the supernatant fluid, by the method of Chen et al. (6).

Statistical analysis: Student *t*-test was used for a statistical comparison between mean values. The significance of difference was expressed in terms of P values.

RESULTS

ATPase activities in homogenates of different tissues: ATPase activities in the homogenate (without deoxycholate treatment) of lateral ventricular choroid plexus were shown to be comparable with those in brain, kidney and gastric mucosa (Table 1). Among them, Na,K-ATPase activity in the choroid plexus was significantly lower than that in the brain, and HCO₃-ATPase activity in the choroid plexus was significantly low as compared with that in the kidney.

ATPase activities in choroid plexus of lateral, third and fourth ventricles: In order to determine whether or not the differences in the ATPase activities are present among the choroid plexus in the lateral, third and fourth ventricles, ATPase activities in the homogenates

TABLE 1. ATPase activities in homogenates of choroid plexus, brain, kidney and gastric mucosa

	(μmoles Pi/mg protein/hr)		
	Na,K-ATPase	Mg-ATPase	HCO ₃ -ATPase
Choroid plexus	1.7±0.6 ^a	8.3±1.2	2.0±0.3 ^b
Brain	4.2±0.7 ^a	5.2±0.8	3.4±0.5
Kidney	2.7±0.5	7.3±0.9	6.2±0.8 ^b
Gastric mucosa	0.9±0.3	5.7±1.7	4.0±1.4

Each value is mean±S.E. of three determinations. a: P(choroid plexus vs. brain) <0.05. b: P(choroid plexus vs. kidney) <0.05. The homogenates were not treated with deoxycholate.

of these three choroid plexus were measured. The homogenates were treated with 0.1% deoxycholate at 0°C for 30 min. With this deoxycholate treatment, Na,K-, Mg- and HCO₃-ATPase activities were activated by approx. 3.5, 1.2 and 7.2 fold respectively (data not shown). As shown in Table 2, there were no significant differences among these three choroid plexus concerning Na,K-, Mg- and HCO₃-ATPase activities. Therefore, the choroid plexus of the lateral ventricle was used for the following experiments.

ATPase activities in subcellular fractions of choroid plexus: Subcellular distribution of ATPase activities in the lateral ventricular choroid plexus is shown in Table 3. The highest activity of Na,K-ATPase was found in the 10,000×g (15 min) fraction. In contrast, the highest activities of Mg- and HCO₃-ATPases were observed in 8,000×g (15 min) fraction. In the so-called microsomal fraction (100,000×g, 60 min), relatively low activities of Na,K- and HCO₃-ATPases were found. The 10,000×g fraction and 8,000×g fraction were used in subsequent experiments for Na,K-, Mg- and HCO₃-ATPases.

Na,K-ATPase in 10,000×g fraction: The reaction rate of Na,K-ATPase was accelerated in proportion to the amount of NaCl at a fixed concentration of KCl (10 mM), reached a maximum rate at 50 mM NaCl and thereafter remained constant (Fig. 1A). When the concentration of KCl was varied at a fixed concentration of NaCl (100 mM), the reaction rate of Na,K-ATPase was accelerated to a maximum rate at 5 mM KCl and remained constant over higher KCl concentrations (Fig. 1B). Lineweaver-Burk double reciprocal plots of these data yielded K_m for sodium of 12 mM and K_m for potassium of 2 mM.

TABLE 2. ATPase activities in homogenates of choroid plexus obtained from lateral, third and fourth ventricles

	(μmoles Pi/mg protein/hr)		
	Na,K-ATPase	Mg-ATPase	HCO ₃ -ATPase
Lateral ventricle	6.0±1.0	9.7±0.9	14.3±2.7
Third ventricle	6.6±1.2	10.9±1.8	18.5±4.9
Fourth ventricle	6.1±1.6	12.2±1.0	16.1±4.8

Each value is mean±S.E. of six determinations. The enzyme solutions were treated with 0.1% sodium deoxycholate at 0°C for 30 min at 1.5–3.3 mg/ml of protein concentrations.

TABLE 3. ATPase activities in subcellular fractions of choroid plexus

	(μmoles Pi/mg protein/hr)		
	Na,K-ATPase	Mg-ATPase	HCO ₃ -ATPase
Homogenate	8.2	16.3	11.3
1,000×g pellet	13.5	19.0	16.4
8,000×g pellet	13.9	30.7	28.5
10,000×g pellet	31.7	12.3	8.4
100,000×g pellet	12.1	10.9	4.8
Supernatant	0	0.7	0.2

All preparations were treated with 0.1% sodium deoxycholate at 37°C for 10 min at 0.3–3.3 mg/ml of protein concentrations.

FIG. 1. Characteristics of Na,K-ATPase in the rabbit choroid plexus. (A) Effect of NaCl on Na,K-ATPase activity. The activity was assayed in a medium containing 50 mM Tris-HCl (pH 7.4), 1 mM EDTA-Tris, 6 mM MgCl_2 , varying concentrations of NaCl, 10 mM KCl, 6 mM ATP-Tris and an aliquot of tissue preparation ($10,000 \times g$ fraction) with or without 1 mM ouabain, and was calculated as an ouabain sensitive activity. (B) Effect of KCl on Na,K-ATPase activity. The incubation was carried out at a fixed NaCl concentration (100 mM). (C) Effect of pH on Na,K-ATPase activity. The experiment was performed as in (A) at 100 mM NaCl and 10 mM KCl. pH was adjusted with Tris-HCl. (D) Effect of ouabain on Na,K-ATPase activity. The experiment was performed at 100 mM NaCl and 10 mM KCl.

The effect of pH on the reaction rate of Na,K-ATPase is shown in Fig. 1C. The maximum rate of reaction of Na,K-ATPase was obtained around pH 7.6.

The effect of ouabain on the rate of Na,K-ATPase reaction is shown in Fig. 1D. The rate of reaction was reduced toward zero by the addition of ouabain at a concentration of 10^{-7} M or more. Half maximum inhibition was observed at about 2×10^{-6} M of ouabain.

Mg-ATPases in $8,000 \times g$ and $10,000 \times g$ fractions: The effect of magnesium ion on the rate of enzymic hydrolysis of ATP by $8,000 \times g$ fraction is shown in Fig. 2A. In the absence of MgCl_2 , the rate of hydrolysis of ATP at 37°C was negligible. The rate of ATP hydrolysis was accelerated by the addition of MgCl_2 and reached the maximum at 6 mM of MgCl_2 concentration, where ATP: Mg ratio was 1:1. At higher concentrations of MgCl_2 , the rate of reaction was somewhat reduced.

pH dependency of Mg-ATPase activity in $8,000 \times g$ fraction was examined using two buffer systems of Tris-HCl and glycine-NaOH (Fig. 2B). The pH optimum for Mg-ATPase

was around pH 9.6.

Magnesium ion requirement and pH optimum of Mg-ATPase in $10,000\times g$ fraction

FIG. 2. Magnesium and pH dependency of Mg-ATPase in the rabbit choroid plexus. (A) Mg ion dependency of Mg-ATPase in $8,000\times g$ fraction. The activity of Mg-ATPase was assayed in a medium containing 50 mM Tris-HCl (pH 7.4), 1 mM EDTA-Tris, varying concentrations of MgCl_2 , 6 mM ATP-Tris, 1 mM ouabain and an aliquot of tissue preparation. (B) pH dependency of Mg-ATPase in $8,000\times g$ fraction. The activity of Mg-ATPase was assayed in the same way as in (A) with MgCl_2 fixed at 6 mM using two different buffer systems of Tris-HCl (●—●) and glycine-NaOH (○—○).

FIG. 3. Bicarbonate, magnesium and pH dependency of HCO_3 -ATPase in the rabbit choroid plexus. (A) NaHCO_3 stimulation of Mg-ATPase in $8,000\times g$ fraction. NaHCO_3 was added to the incubation medium containing 50 mM Tris-HCl (pH 7.4), 1 mM EDTA-Tris, 6 mM MgCl_2 , 1 mM ouabain, 6 mM ATP-Tris and an aliquot of tissue preparation. HCO_3 -stimulated ATPase activity was designated as HCO_3 -ATPase activity. (B) Effect of MgCl_2 on HCO_3 -ATPase activity. (C) Effect of pH on HCO_3 -ATPase activity. The activity was assayed using three different buffer systems of Tris-maleate (■—■), Tris-HCl (●—●) and glycine-NaOH (○—○).

FIG. 4. Effects of thiocyanate ion on ATPase activities in the rabbit choroid plexus. (A) Effect of NaSCN on Mg-ATPase (●—●) and HCO_3^- -ATPase (○—○) in 8,000 $\times$ g fraction. (B) Effect of NaSCN on Mg-ATPase (●—●) and Na,K-ATPase (□—□) in 10,000 $\times$ g fraction.

were found to be similar to those of Mg-ATPase in the 8,000 $\times$ g fraction (data not shown).

HCO_3^- -ATPase in 8,000 $\times$ g fraction: ATPase activity stimulated by bicarbonate ion was designated as HCO_3^- -ATPase activity. The effect of increasing bicarbonate ion concentration on HCO_3^- -ATPase activity is shown in Fig. 3A. The maximum rate of HCO_3^- -stimulated ATP hydrolysis was reached at a bicarbonate ion concentration of 20 mM and remained virtually unchanged at bicarbonate ion concentrations up to 50 mM.

The influence of MgCl_2 on HCO_3^- -ATPase reaction rates was also examined (Fig. 3B). The maximum rate of reaction was obtained at 6 mM of MgCl_2 , where the molar ATP : Mg ratio was 1 : 1. The rate of reaction was decreased at higher concentrations of MgCl_2 .

pH dependency of HCO_3^- -ATPase activity was examined with buffer systems of Tris-maleate, Tris-HCl and glycine-NaOH (Fig. 3C). The pH optimum was at approx. pH 8.6.

Effect of thiocyanate ion on HCO_3^- -, Mg- and Na,K-ATPases: Thiocyanate ion, which is reportedly an inhibitor for HCO_3^- -ATPase in other tissues (7, 8), inhibited HCO_3^- -ATPase activity in the choroid plexus (Fig. 4A). Mg-ATPase activities in 8,000 $\times$ g and 10,000 $\times$ g fractions also were found to be inhibited, rather more markedly, by thiocyanate ion (Fig. 4A and 4B). Half maximum inhibition of HCO_3^- - and Mg-ATPase was observed at the thiocyanate concentration of 6 mM and 2 mM, respectively. Na,K-ATPase activity was not affected by thiocyanate ion up to 50 mM.

DISCUSSION

In mammals, the choroid plexus can be identified in each of two of the lateral ventricles, and the third and fourth ventricles. These tissues are richly vascularized and innervated both adrenergically and cholinergically, but are considerably different regarding density in supply (9, 10). Functional differences among the choroid plexus would thus be expected.

Nevertheless, we found no significant differences in the activities of Na,K-, Mg- and HCO_3 -ATPase.

The fractionation study of the choroid plexus indicated that Na,K-ATPase activity was highest in the $10,000 \times g$ fraction. Among the subcellular fractions prepared from renal outer medulla by the same method, the highest activity of Na,K-ATPase was observed in the $10,000 \times g$ fraction (data not shown). Na,K-ATPase in the choroid plexus is reportedly localized in the apical surface of plasma membrane (11), while the enzyme in the kidney is bound to the lateral and basal plasma membrane (12). Despite the different localizations, the enzyme activities in subcellular fractions of these tissues represented essentially the same pattern after the centrifugal fractionation, suggesting that membrane particles originating from the apical cell membrane also form relatively heavy microsomes. Mg- and HCO_3 -ATPase activities were highest in the $8,000 \times g$ fraction, the so-called mitochondrial fraction. Similar subcellular distribution of these enzymes has been reported in trout gill, rabbit kidney and rabbit gastric mucosa (13). However, in the dog gastric mucosa, Mg- and HCO_3 -ATPase activities are reported to be highest in the microsomal fraction (14) and, in the rat brain, the highest Mg-ATPase activity is reported to be in microsomal fractions while the highest HCO_3 -ATPase activity is in mitochondrial fractions (15). The discrepancy among these data may be due to species differences and structural difference in Mg- and HCO_3 -ATPase containing membranes.

It has been reported that the Na,K-ATPase system is present in the choroid plexus and intraventricular administration of ouabain results in an inhibition of net cerebrospinal fluid formation (2). Further characteristics of the choroid plexus Na,K-ATPase were examined in the present study. K_m for sodium (12 mM), K_m for potassium (2 mM), the pH optimum (pH 7.6) and half-inhibition concentration of ouabain ($2 \mu\text{M}$) were found to be similar to those reported with Na,K-ATPase prepared from erythrocytes (16, 17), brain (18, 19) and kidney (19, 20), suggesting that Na,K-ATPase in the choroid plexus functions in a similar manner as in the tissues described above.

Magnesium ion concentration in the cerebrospinal fluid is known to be significantly higher than that in a plasma ultrafiltrate (21) and the choroid plexus has recently been shown to involve active secretion mechanisms for magnesium ion (4). Optimal ATP : Mg ratio for Mg-ATPase activity in $8,000 \times g$ and $10,000 \times g$ fractions of choroid plexus was about 1, while a ratio more than 2 is reported for brain (15) and gastric mucosa (7, 13). Further, optimal pH for Mg-ATPase in the above-mentioned two fractions was over pH 9.0, while it was around pH 8.5 in other tissues (7, 14, 15, 22). Besides these differences between Mg-ATPase in the choroid plexus and in other tissues, the activity of Mg-ATPase was considerably high in the choroid plexus homogenate, among the tissues examined (choroid plexus, brain, kidney and gastric mucosa). The relationship between magnesium ion secretion mechanism and Mg-ATPase activity awaits clarification.

The buffer system of the cerebrospinal fluid consists almost exclusively of the bicarbonate-carbonic acid system, and the concentration of bicarbonate in the cerebrospinal fluid is actively regulated by the choroid plexus (3, 23). Correlation of bicarbonate transport

and $\text{HCO}_3\text{-ATPase}$ activity is still under discussion because of mitochondrial, but not microsomal, localization of $\text{HCO}_3\text{-ATPase}$ activity in several tissues (13, 15). However, in a recent report on renal cortical $\text{HCO}_3\text{-ATPase}$ it was suggested that $\text{HCO}_3\text{-ATPase}$ mediates a certain percentage of bicarbonate reabsorption in the nephron (24). Bicarbonate ion requirement of $\text{HCO}_3\text{-ATPase}$ in the choroid plexus was similar to that in frog gastric mucosa (7) and rat brain (15). The optimal ATP : Mg ratio for the maximum $\text{HCO}_3\text{-ATPase}$ activity in the choroid plexus was lower than the ratios in gastric mucosa (13) and brain (15). Optimal pH in the choroid plexus was similar to that in rat brain (15), but was higher than that in dog gastric mucosa (14). Participation of $\text{HCO}_3\text{-ATPase}$ in bicarbonate transport in the choroid plexus may be clarified with reference to some characteristics of this enzyme described herein.

Thiocyanate inhibits both $\text{HCO}_3\text{-ATPase}$ activity and hydrogen ion secretion, where hydrogen ion is considered to be formed from bicarbonate in the epithelial cells (8, 22). $\text{HCO}_3\text{-ATPase}$ activity in the choroid plexus was inhibited by thiocyanate to the same degree as in the gill of trout (8). Mg-ATPase activity also was reduced by thiocyanate in the choroid plexus. These findings may be of importance in consideration of roles of $\text{HCO}_3\text{-}$ and Mg-ATPases in the choroid plexus, because thiocyanate reduced the formation of cerebrospinal fluid in the ventriculo-cisternal perfusion of the rabbit (unpublished data).

The present study revealed the characteristics of Na,K- , Mg- and $\text{HCO}_3\text{-ATPases}$ in the choroid plexus, and the data suggested that these ATPases in the choroid plexus operated with parameters somewhat different from those in other tissues. In addition to $\text{HCO}_3\text{-ATPase}$, it is tempting to speculate that Mg-ATPase may be involved in the mechanisms of the formation of cerebrospinal fluid and related studies are ongoing in our laboratory.

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