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ATP-DEPENDENT Cl^- UPTAKE BY PLASMA MEMBRANE VESICLES FROM THE RAT BRAIN

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SUMMARY: Uptake of Cl^- by plasma membrane vesicles from the rat brain was stimulated by ATP at 37°C, but not by β, γ-methylene ATP or at 0°C. The addition of Triton X-100 or sucrose to the incubation medium diminished the ATP-stimulated Cl^- uptake, suggesting that Cl^- was transported across the membranes into the intravesicular space. This ATP-stimulated Cl^- uptake was not affected by 1 mM ouabain, 1 μM oligomycin, 0.1 mM γ-aminobutyric acid or 0.1 mM picrotoxin. Thus, non-mitochondrial ATP-driven Cl^- transport through a system other than Na,K-ATPase or Cl^- channels occurs in neuronal plasma membrane vesicles.

Inhibitory regulation of a variety of neuronal activities is known to be mediated by hyperpolarizing postsynaptic potentials generated by Cl^- (1-3), whose intracellular concentration is reportedly lower than that expected from passive distribution (4), suggesting the presence of a Cl^- transport mechanism. We previously reported that Cl^--stimulated Mg^{2+}-ATPase (Cl^--ATPase) is a candidate for a Cl^- translocating system in the plasma membrane fractions of the brain (5-10). This report describes the ATP-dependent Cl^- transport in the Cl^--ATPase rich plasma membrane vesicles from the rat brain.

EXPERIMENTAL PROCEDURES

Membrane preparations : Cl^--ATPase rich plasma membrane vesicles were prepared by the treatment of rat brain microsomes with ethylenediaminetetraacetic acid (EDTA) as described previously (7).

Abbreviations: Mes; 2-(N-morpholino)ethanesulfonic acid, PMSF; phenylmethylsulfonylfluoride.

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Briefly, brains obtained from ether-anesthetized male Wistar rats (180-200 g) were homogenized in 8 vol. of ice-cold buffer solution containing 0.25 M sucrose, 1 mM EDTA, 1 mM PMSF and 12.5 mM Tris-Mes (pH 7.4), and centrifuged (1000 g, 10 min; 10,000 g, 20 min; 100,000 g, 30 min). Translucent layers of the final pellets were suspended in 5 mM EDTA (pH 7.4 with Tris), stirred for 30 min, and then centrifuged at 10,000g for 5 min. The supernatant fraction was saturated 30 % with ammonium sulfate, stirred for 20 min, and centrifuged at 10,000 g for 15 min. The precipitate was suspended in 5 mM EDTA and dialyzed overnight against the same solution. The resulting preparation was stored at -70°C and used as Cl^--ATPase rich plasma membrane vesicles. The protein concentration was measured by the method of Lowry et al. (11).

Assay of Cl^- uptake: The plasma membrane vesicles (0.4 mg protein) were preincubated at 37°C for 4 min in 200 μl of reaction mixture usually containing 50 mM Tris-Mes (pH 7.4), 1 mM EDTA, 1 mM ouabain, 0.1 mM picrotoxin and 6 mM Cl-Tris with 0.1 μCi ^{36}Cl. The reaction was started by the addition of 3mM each of ATP-Tris (pH 7.4) and Mg acetate, and at the indicated times the samples were filtered on a membrane filter (Whatman GF/B glass filter) and washed with 15 ml of 100 mM potassium gluconatete (pH 7.4). The radioactivity retained on the filters was measured in Scintisol EX-H (Dojin) in a liquid scintillation counter (Packard 2000 CA TRI-CARB). Non-specific binding of $^{36}Cl^-$ was assayed in parallel in the presence of 200 mM Cl-Tris and subtracted from all the values.

Materials : [^{36}Cl]HCl (15.2 mCi/g) was obtained from De Pont/NEN. ATP-Tris was purchased from Sigma.

RESULTS AND DISCUSSION

As shown in Fig. 1, the addition of 3 mM ATP:Mg (ATP-Tris + Mg acetate) increased Cl^- uptake at 37 ℃ by the membrane vesicles, with the maximal level being two-fold higher than that in the

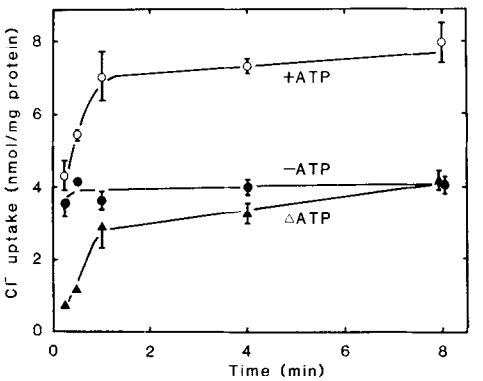

Fig. 1. Time course of Cl^- uptake in the presence or absence of ATP(3 mM) by plasma membrane vesicles from the rat brain. Δ = (+ATP) - (-ATP). Values are means ± S.E. from three experiments.

absence of ATP:Mg. After the addition of ATP:Mg, the ATP-stimulated Cl^- uptake (Δ ATP in Fig. 1) increased time-dependently for 1 min and then reached a plateau level. As shown in Fig. 2, however, such increased Cl^- uptake was not observed with a non-hydrolyzing ATP analogue; β, γ-methylene-ATP at 37℃, or with ATP at 0℃, suggesting that ATP-stimulated Cl^- uptake was mediated by hydrolysis of ATP or an energy requiring process. When 0.2 % Triton X-100 was added to the incubation medium at 4 min of incubation, both ATP-dependent and ATP-independent Cl^- uptake markedly decreased (Fig. 2). This occurred probably through the damage of the membranes by this detergent. Further, 0.4 M sucrose decreased both ATP-dependent and ATP-independent Cl^- uptake probably due the reduction in intravesicular space by a hyperbaric solution. These findings suggest that Cl^- uptake involves translocation of Cl^- into the intravesicular space. Table I shows the effects of a Na, K-ATPase inhibitor;ouabain (1 mM), a mitochondrial ATPase inhibitor; oligomycin (1 μ M), a transmitter that facilitates chloride channels; γ-aminobutyric acid (1 mM) and a chloride channel

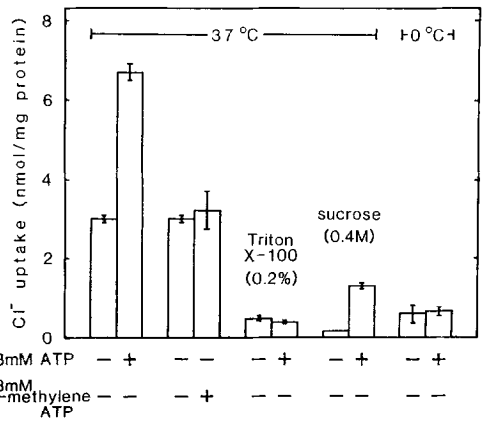

Fig. 2. Effects of ATP(3 mM), β, γ-methylene ATP(3 mM), Triton X-100(0.2 %), sucrose(0.4 M) and low temperature(0 ℃) on Cl^- uptake in plasma membrane vesicles. Cl^- uptake was measured 4 min after the addition of ATP or an ATP analogue. Triton X-100 was added at 4 min of incubation and $^{36}Cl^-$ uptake was measured 2 min later. Values are means ± S.E. from four experiments.

Table I. Effects of compounds affecting ATPases and Cl^- channels on Cl^- uptake in plasma membrane vesicles

Compounds	Cl^- uptake (nmol/mg protein)		
	-ATP	+ATP	Δ ATP
None (n=16) *	3.02±0.09	6.72±0.24	3.70±0.24
1.0 mM ouabain (n=4)	2.86±0.09	6.11±0.10	3.24±0.10
1.0 μM oligomycin (n=4)	4.21±0.52	7.86±0.46	3.66±0.46
0.1 mM GABA (n=4)	2.85±0.06	6.34±0.31	3.49±0.31
0.1 mM picrotoxin (n=4)	3.26±0.10	6.70±0.27	3.44±0.27

Values are means ± S.E. Cl^- uptake was measured 4 min after the addition of 3 mM ATP:Mg. *The control assay (None in the table) was done in the absence of any coumpond tested. Significance of the difference between the values (test compound vs None) was tested by the Student's t -test.

blocking agent;picrotoxin (0.1 mM) on the ATP-stimulated Cl^- uptake. None of the reagents affected the ATP-stimulated Cl^- uptake, suggesting that the process is not mediated by Na,K-ATPase, mitochondrial ATPase or chloride channels. Since this ATP-dependent Cl^- uptake occurred without the addition of Na^+, K^+ or HCO_3^- to the incubation medium, Na^+/Cl^- symport (12), K^+/Cl^- symport (13), $Na^+/K^+/Cl^-$ cotransport (14) and Cl^-/HCO_3^- antiport (15) do not appear to be involved in this Cl^- transport. In animal tissues, ATP-depedndent Cl^- transport has been most recently reported only in Aplysia intestine (16,17).

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