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Synaptosomal sodium pump activity depends on microfilament cytoskeleton integrity

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INTRODUCTION AND METHODS. The involvement of cytoskeleton rearrangements in volume-dependent regulation of ion-transport has been demonstrated in several types of cells and tissues (1). Hypotonic swelling of rat brain isolated nerve endings (synaptosomes) leads to activation of sodium pump and increase of ^{3}H-ouabain specific binding (2,3). Cytochalasin B which is known to depolimerize actin microfilaments modified a dependence of ^{3}H-ouabain binding on incubation medium osmolarity (2). In the present study we investigated an influence of disruption of cytoskeleton integrity on synaptosomal sodium pump activity. Synaptosomes were prepared from rat forebrains according the method of Hajos (4). Stock suspensions of synaptosomes (10 mg protein/ml) were preincubated for 20 min with drugs disrupting cytoskeleton integrity in medium A containing (in mM): 132 NaCl, 5 KCl, 1.3 $MgCl_2$, 1 $CaCl_2$, 1.2 NaH_2PO_4, 10 glucose, 20 HEPES-Tris (pH 7.4, 37°C). Colchicine and cytochalasin B (CB) were obtained from Sigma Chemicals (St. Louis, MO) and dissolved in ethanol and DMSO, respectively. Final concentrations of vehicles did not exceed 0.2%. Vinblastine and vincristine were purchased from Gedeon Richter (Budapest, Hungary) and dissolved in H_2O. Aliquots of synaptosomes (final protein concentration - 1 mg/ml) were added to medium A containing 0.5 μCi ^{86}RbCl as radiotracer and cytoskeleton poisons. Sodium pump activity was measured as ouabain-sensitive K^+ ($^{86}Rb^+$) uptake as described earlier (3).

RESULTS AND DISCUSSION. Figure 1A, shows that 40 μM CB inhibited sodium pump by 35-40%. By contrast, agents depolimerizing microtubules (colchicine, vinblastine and vincristine) were less potent (Fig. 1A). Effect of CB was dose-dependent and saturated at 10-20 μM of the drug (Fig. 1B). Unlike sodium pump (ouabain-sensitive K^+ uptake) ouabain-resistant K^+ transport was not significantly affected by CB (Fig. 1B). We did not find any influence of 10-100 μM CB on Na^+,K^+-ATPase activity in isolated synaptosomal plasma membranes (data not shown) that indicates CB action through F-actin depolimerization. Although *in vitro* IC_{50} for CB-induced micrifilament depolimerization is 10^{-7}-10^{-6} M, in living cells the IC_{50} close to 10^{-5} M (5). Several studies have shown that CB in the similar concentration range affects volume-dependent activation of cation and anion channels as well as cell volume normalization in anisosmotic media (1,6,7). In polarized cells Na^+,K^+-ATPase is associated with cytoskeleton through ankyrin binding (8). Our results suggest that changes in microfilament cytoskeleton integrity may influence the sodium pump activity in brain synaptosomes.

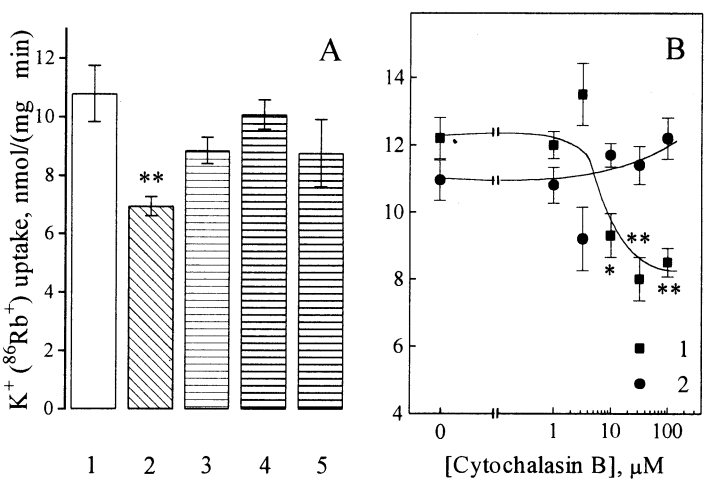

Fig. 1. A, influence of 40 μM cytochalasin B (2), 1 mM colchicine (3), 50 μM vinblastine (4) and 50 μM vincristine (5) on sodium pump activity in brain synaptosomes vs. control (1). B, dose-dependence of the effect of cytochalasin B on ouabain-sensitive (1) and ouabain-resistant (2) K^+ influx. Data are means of 2-3 experiments performed in quadruplicate ± SEM. *P<0.05, **P<0.01.

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