INHIBITORY EFFECT OF MICROTUBULE-DISRUPTING AGENTS ON POTASSIUM STIMULATED ^{45}Ca UPTAKE BY RAT BRAIN SLICES

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Calcium ions play crucial roles in nervous tissues. Previously, it was reported that the release of neurotransmitters from nerve terminals is regulated by microtubules- and microfilament-disrupting agents (1), as well as cyclic nucleotides (2–6). Since it is well known that release of a variety of neurotransmitters is dependent on Ca^{2+} influx (7, 8), this suggests that the regulation is related with membrane permeability to Ca^{2+} .

Recent studies of the cytoskeleton have shown that microtubules are part of the cytoskeletal network (9) and that brain membrane preparations have the ability to bind colchicine, suggesting that synaptic membranes contain firmly bound tubulin (10). In squid giant axon (11, 12), cytoskeletal proteins associated with the inner surface of the axon can be involved in the regulation of axonal excitability. When colchicine was applied internally to the squid giant axon, a decrease of membrane excitability was observed in the electrophysiological experiments. This implies that membrane proteins-associated cytoskeletal components such as microtubules may play a role in regulating the permeability of the membrane to ions.

In this work we examine the inhibitory actions of microtubule-disrupting agents on the potassium stimulated ^{45}Ca uptake by cortical slices of rat brains.

Male Sprague-Dawley rats weighing 150–250 g were killed by decapitation. The cerebrum was immediately removed from the skull and placed in a ice-cold oxygenated standard medium containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl_2 , 1 mM CaCl_2 , 1.5 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.4 at 37°C), 10 mM D-glucose, and Hepes-NaOH (pH 7.4 at 37°C). Cortical slices were cut from each hemisphere using a Stadie-Riggs slicer. Portions of the tissues (about 20–30 mg of tissue) were placed in the standard medium bubbled with oxygen and preincubated for 15 min at 37°C . Then, 200 μl of $^{45}\text{CaCl}_2$ (about 0.5 μCi) with NaCl (825 mM) or KCl (825 mM) was added. The mixture was incubated for 30 sec at 37°C as previously described (3). Potassium stimulated ^{45}Ca uptake was linear during this period and reached a plateau at about 2 min. The final volume of the reaction system was 3 ml. The reaction was stopped by separating the slices from the reaction medium. The slices were rinsed with 5 ml of icecold washing medium (standard medium, but with 1 mM LaCl_3 instead of 1 mM CaCl_2), and then placed in 10 ml of ice-cold washing medium for 20 min. Then they were rinsed twice with 5 ml of ice-cold washing medium and transferred to a tube. After adding 1 ml of

1 N NaOH, the mixture was disrupted in a sonicator (Kontes Co.) and then neutralized with 5 N HCl. A 100 μ l aliquot of the mixture was transferred to a counting vial and 5 ml of ACS II (Amersham Co.) was added. Radioactivity was determined in a liquid scintillation counter (Packard Co., model 3385). Potassium stimulated ^{45}Ca uptake was calculated as the difference between the uptakes in high potassium medium (60 mM) and low potassium medium (5 mM).

Figure 1 shows the effects of colchicine and vinblastine on ^{45}Ca uptake by slices in standard medium containing 5 or 60 mM potassium at 37°C. An incubation period of 30 sec was used in these experiments during which the initial velocity of Ca^{2+} influx was measured (3). Both colchicine and vinblastine inhibited the initial rate of potassium stimulated ^{45}Ca uptake in a dose dependent manner, but did not affect ^{45}Ca uptake in the low potassium medium. However, high concentrations of vinblastine inhibited ^{45}Ca uptake in the low potassium medium. After removal of colchicine, potassium stimulated ^{45}Ca uptake was restored in 15 min (data not shown). As shown in Table 1, the concentrations of colchicine and vinblastine inducing 50%

inhibition of potassium stimulated ^{45}Ca uptake were both about 50 μM , and the 50 μM cytochalasin B reduced potassium stimulated ^{45}Ca uptake by approximately 25%. Lumicolchicine (0.1 mM), a colchicine

Fig. 1. Effects of various concentrations of colchicine (○—○, ●—●) and vinblastine (△—△, ▲—▲) on ^{45}Ca uptake. Cortical slices of the brain were preincubated in a standard medium for 15 min at 37°C and then incubated for 30 sec. When the effects of the antitubulin alkaloids colchicine and vinblastine was examined, these drugs were added to the standard medium before preincubation. Degrees of inhibition were calculated by the ratio of potassium stimulated ^{45}Ca uptake (○—○, △—△) or ^{45}Ca uptake by the low potassium medium (5 mM-K) (●—●, ▲—▲) in the antitubulin alkaloid-untreated and treated tissues.

Table 1. Effect of various agents on ^{45}Ca uptake

Pretreatment (mM)	^{45}Ca uptake		K-stimulated ^{45}Ca uptake (a-b)	Inhibition %
	(a) 60 mM-K	(b) 5 mM-K		
Control	0.361±0.033	0.153±0.021	0.208±0.033	0
Colchicine (0.05)	0.280±0.031	0.163±0.027	0.117±0.031	43.8
Vinblastine (0.05)	0.236±0.018	0.126±0.001	0.110±0.018	43.1
Lumicolchicine (0.10)	0.349±0.034	0.156±0.001	0.193±0.034	7.2
Ethanol*	0.317±0.021	0.153±0.011	0.164±0.021	0
Cytochalasin B (0.05)	0.275±0.052	0.154±0.001	0.121±0.052	26.2

Ethanol was used as a control in the cytochalasin B experiment. The results of ^{45}Ca uptake (n mole/mg tissue/30 sec) are the mean±S.E.M. of four experiments. Degrees of inhibition were calculated by the ratio of K-stimulated ^{45}Ca uptake in the agent-untreated and treated tissues. *The final concentration of ethanol in the medium was 1% (vol./vol.).

analog which does not disrupt microtubules, had no such effect.

Recent studies on the regulation of microtubules showed that microtubule assembly is influenced by cyclic nucleotides and divalent cations (13, 14). Low concentrations of calcium and magnesium ions have an effect on microtubule polymerization. *In vitro* polymerization studies showed that micromolar concentrations of Ca^{2+} can be substituted for Mg^{2+} which is the major ion required for microtubule assembly (15). The cytoplasmic microtubules of sea urchin embryos disappear *in vivo* when the embryos are placed into calcium-free sea water. Thus, by changing the structural properties of the microtubules, the cytoskeleton may be involved in a number of the dynamic functions of the membrane.

Our present results indicate that the inhibitory effect of these drugs on potassium stimulated ^{45}Ca uptake by brain slices can be a result of disruption of cytoskeletal components. The possibility exists that the drugs that affect a cell's cytoskeleton may also have nonspecific or alternate actions (16). However, the reversible inhibitions of potassium stimulated ^{45}Ca uptake by colchicine and a structurally different microtubule disrupting agent, vinblastine, and the lack of an inhibitory effect with lumicolchicine provide strong evidence that intact microtubules are required for potassium stimulated ^{45}Ca uptake. This explanation implies that the cytoskeletal components that interacted with the Ca^{2+} "channel" may play a significant role in the Ca^{2+} permeability of the synaptic membrane in the depolarized state.

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