INTERACTION BETWEEN Na^+ AND Ca^{++} IONS IN NERVE ENDINGS OF THE RAT BRAIN UNDER THE INFLUENCE OF BENACTYZINE AND ARECOLINE

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There are at least three types of exchange of Ca^{++} ions in the nerve endings of the brain: a) exchange of Ca^{++} connected with activation of Ca-activated, Mg-dependent ATPase or active transport of Ca^{++} ions through the membrane, b) Ca–Ca exchange, and c) Na–Ca exchange. The significance and role of these processes in the mechanism of action of pharmacological compounds are not yet clear. Investigations [1, 4, 5] have shown that cholinolytics can exert an action on cell membranes similar to the effects of Ca^{++} ions (the Ca-like action of cholinolytics). However, previous investigations [2] by the present writer showed that this action is not the result of replacement of Ca^{++} ions by the cholinolytic, but is connected with intensified mobilization of Ca^{++} from the tissue depots and with increased permeability of the nerve cell membranes for these ions. On the basis of the results of experiments in vitro with isolated synaptosomes from the rat brain it has been suggested that benactyzine activates the Ca-channels and inactivates the Na and K-channels of the membranes of nerve endings [3].

The object of the present investigation was to study the role of Na–Ca exchange in the mechanism of action of the central cholinolytic benactyzine and the cholinomimetic arecoline.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-250 g into which benactyzine was injected intraperitoneally in a dose of 40 mg/kg and arecoline was injected similarly in a dose of 2.5 mg/kg (equivalent to 0.1 ml of solution/100 g body weight). Water was injected into control animals. In all cases ^{45}Ca (10 μCi) or ^{22}Na (5 μCi) was injected into the rats 30 min before sacrifice. The animals were decapitated at known intervals, the brain was removed, and freed as far as possible from blood. The fraction of nerve endings (synaptosomes) was isolated in a sucrose density gradient [10]. The fraction of synaptosomes was separated and

TABLE 1. Incorporation of ^{45}Ca and ^{22}Na into Rat Brain Synaptosomes (in cpm/mg protein) after Administration of Benactyzine and Arecoline (M ± m; n = 6)

Drug	Experimental conditions	Incorporation of isotopes after injection of drug			
		10 min		60 min	
		^{45}Ca	^{22}Na	^{45}Ca	^{22}Na
Benactyzine	Control	93,2±5,4	123±2,1	51,1±4,8	137±4,3
	Experiment	103,8±7,0	98,4±6,5	70,9±4,3	148±8,5
	P	<0,25	<0,05	<0,05	<0,3
Arecoline	Control	98,2±5,2	142±3,5	54,2±4,1	105±4,0
	Experiment	67,1±1,5	264±15,4	46,0±2,1	136,5±7,8
	P	<0,05	<0,05	>0,1	<0,05

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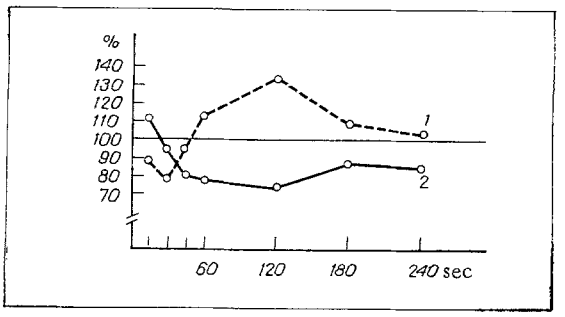

Fig. 1. Effect of benactyzine on uptake of ^{45}Ca (1) and ^{22}Na (2) by rat brain synaptosomes. Abscissa, incubation time of synaptosomes with isotopes (in sec); ordinate, deviation (in %) of indices from control, taken as 100%.

sedimented in 0.32 M sucrose (15 ml), after which it was centrifuged for 20 min at 10,000g. The residue was hydrolyzed in 1 N NaOH (0.7 ml) at 60°C for 30 min. The digest was neutralized with 1 ml 0.67 N HCl. Incorporation of ^{45}Ca and ^{22}Na into synaptosomes was determined by mixing 1 ml of the digest with 10 ml of scintillation solution (50 g naphthalene, 6 g 2,5-diphenyloxazole, 0.5 g 1,4-di-5-phenyl-2-oxazolyl, dioxane up to 1 liter) in a "Packard" scintillation counter. In the experiments in vitro the synaptosomes were prepared as described in [9]. Benactyzine or arecoline was added to 1 ml of the resulting suspension in a final concentration of $1.1 \cdot 10^{-6}$ and $1.6 \cdot 10^{-6}$ M respectively. The samples were preincubated for 15 min at 30°C, after which ^{45}Ca (0.5 μCi) or ^{22}Na (1 μCi) was added and the samples were incubated for different periods of time. Uptake of ^{45}Ca was stopped by addition of 0.3 ml of a solution containing 30 mM EGTA [ethylene-glycol-bis(3-β-aminoethyl ester)-N,N'-tetra-acetic acid] and 120 mM NaCl, pH 7.4, and 4 ml of a cooled solution containing 132 mM NaCl, 5 mM KCl, 1.2 mM NaH_2PO_4, 0.1 mM glucose, 20 mM Tris-HCl, pH 7.4, and 1.2 mM $CaCl_2$ (solution No. 1), to the incubation medium. After incubation with ^{22}Na the samples were placed in a dish with ice for 3 min, after which 4 ml of cold solution No. 1, not containing $CaCl_2$, was added, and the samples were then centrifuged at 4°C for 5 min at 11,000g. The residues were washed twice and then hydrolyzed as described above. The incorporation and degree of uptake of the isotopes were estimated as the number of counts per minute per milligram protein. Protein was determined by Lowry's method [6].

EXPERIMENTAL RESULTS

Incorporation of ^{45}Ca into rat brain nerve endings was increased 10 min after injection of benactyzine by 11% and 60 min after injection by 39% compared with the control (Table 1). Incorporation of ^{22}Na under these circumstances was reduced by 20% after 10 min, but after 60 min it was 8% higher than in the control (Table 1). Arecoline inhibited incorporation of ^{45}Ca into synaptosomes by 32 and 12% after 10 and 60 min respectively but activated incorporation of ^{22}Na by 85 and 30%. The results show that benactyzine and arecoline have opposite effects on the ability of Ca^{++} and Na^{+} ions to penetrate into synapses. Arecoline, a depolarizing agent, significantly increased permeability to Na^{+} and prevented incorporation of Ca^{++}. Benactyzine, on the other hand, led to accumulation of Ca^{++} ions in the nerve endings and significantly reduced (10 min after injection) the Na^{+} concentration, after which slight but not significant ($P > 0.05$) accumulation of these ions in the synaptosomes was observed.

The dynamics of the action of benactyzine and arecoline on uptake of ^{45}Ca and ^{22}Na by isolated synaptosomes was studied in experiments in vitro. During incubation of the synaptosomes with benactyzine an initial fall in ^{45}Ca uptake was observed, followed by accumulation of the isotope with maximal effect after incubation for 2 min, whereas ^{22}Na uptake was reduced at that time (Fig. 1). A relationship of reciprocity was observed between these processes with a coefficient of correlation of -0.68 ($P < 0.01$). Arecoline caused a significant increase in ^{22}Na uptake by synaptosomes (Fig. 2) and, in the first 30 sec, an increase also in the uptake of ^{45}Ca, the concentration of which subsequently was virtually indistinguishable from the control. The coefficient of correlation between these processes under the influence of arecoline was 0.83 ($P < 0.01$).

The results show that benactyzine inhibits uptake of ^{22}Na and promotes accumulation of ^{45}Ca in synaptosomes. Under the influence of arecoline, a brief increase in ^{45}Ca uptake is accompanied by a well-marked and prolonged increase in ^{22}Na uptake.

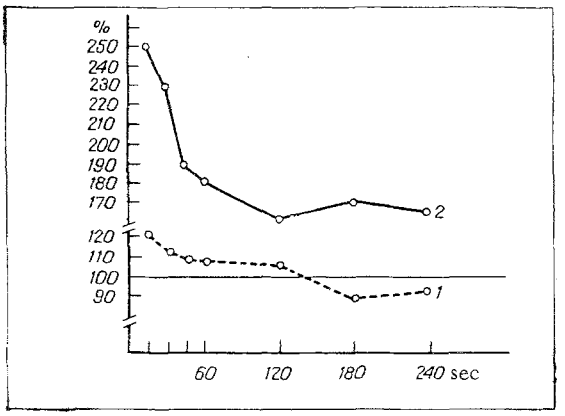

Fig. 2. Effect of arecoline on uptake of ^{45}Ca (1) and ^{22}Na (2) by rat brain synaptosomes. Legend as in Fig. 1.

The writer's earlier suggestion that benactyzine inactivates sodium channels in nerve ending membranes was thus confirmed by the results of the present investigation. It is considered [7, 8] that the ionophores for Na^+ and K^+ are three-dimensionally linked with acetylcholine receptors. It can accordingly be suggested that the cholinolytic benactyzine, sumultaneously with blocking acetylcholine receptors, inhibits sodium channels and reduces the flow of Na^+ ions into the neurons. Under these circumstances the calcium channels themselves begin to be activated and this leads to accumulation of Ca^{++} ions in the nerve endings. Arecoline, which excites acetylcholine receptors, increases membrane permeability for Na^+ ions and may also perhaps increase the permeability of the sodium channels very briefly for Ca^{++} ions, although in experiments in vivo a decrease in incorporation of the the latter into nerve endings was observed as early as after 10 min. During transmission of the nervous impulse, the possibility that Ca^{++} ions may penetrate into the neuron through sodium channels evidently cannot be ruled out, although this pathway for transport of Ca^{++} ions must lose its importance under the influence of pharmacological agents capable of interfering with mechanisms of permeability of cell membranes.

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