EFFECT OF BENACTYZINE AND ARECOLINE ON THE ^{45}Ca UPTAKE BY RAT BRAIN NERVE ENDINGS

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UDC 615.217.3-015.45:612.826.2

The effect of the central cholinolytic benactyzine and the cholinomimetic arecoline on the uptake of ^{45}Ca by rat brain synaptosomes was studied in vitro. Benactyzine was shown to cause biphasic changes (a decrease followed by an increase) in the intensity of uptake of the isotope, whereas arecoline led to a rapid initial increase in ^{45}Ca uptake. Benactyzine was shown to depress the effect of arecoline and depolarization on uptake of the isotope. It is concluded that the increase in ^{45}Ca uptake through the action of arecoline is connected with activation of Na-channels. Benactyzine, on the other hand, reduces the permeability of the these channels for ^{45}Ca and activates the Ca-channels proper.

KEY WORDS: synaptosomes, benactyzine, arecoline, depolarization, ^{45}Ca uptake.

Synaptic transmission in both the central and the peripheral nervous system depends on controlled secretion of neurotransmitters by nerve endings. A regulatory role in this process is played by Ca^{++} ions, which reduce the charge on the membrane of the synaptic vesicles and facilitate their adhesion to membranes of the terminals in the course of exocytosis [10, 12].

The central cholinolytic benactyzine increases the liberation of acetylcholine (ACh) and noradrenalin (NA) from the presynaptic formations of the brain [1, 3, 4]. During the action of the cholinomimetic arecoline, the opposite effect is observed, i.e., accumulation of ACh [14]. The participation of Ca^{++} in the liberation or accumulation of neuromediators in response to the action of these compounds is an interesting subject for research.

In the investigation described below the action of benactyzine and arecoline on the uptake of ^{45}Ca by isolated nerve endings of the rat brain was studied.

EXPERIMENTAL METHOD

Nerve endings (synaptosomes) were isolated from the rat brain in a sucrose density gradient [15]. The synaptosome-rich fraction was separated and washed to remove sucrose by adding 30 ml of a cold solution containing 132 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 0.1 mM glucose, and 20 mM Tris-HCl buffer, pH 7.4 (standard salt solution) slowly in the course of 30 min. The suspension was centrifuged at 11,000 g for 10 min at 4°C. The residue was resuspended in 10 ml standard salt solution, to which $CaCl_2$ was added in a concentration of 1.2 mM, and then incubated for 15 min at 30°C until ionic equilibrium was established. Benactyzine or arecoline was added to 1 ml of the resulting suspension in a final concentration of 1.1×10^{-6} and 1.6×10^{-6}M respectively. The samples were preincubated for 15 min at 30°C, after which ^{45}Ca (0.5 μCi) was added, and incubation then continued for different time intervals. The uptake of ^{45}Ca was stopped by adding to the incubation medium 0.3 ml of a solution (pH 7.4) containing 30 mM EGTA [ethyleneglycol-bis-(β-aminoethyl ester)-N_1N-tetra-acetic acid], 120 mM NaCl, and 4 ml of the cold standard solution containing $CaCl_2$. The samples were centrifuged for 5 min at 11,000 g and at 4°C. The residue was washed twice with the cold standard solution containing $CaCl_2$ and then hydrolyzed in 1N NaOH (0.7 ml) at 60°C for 30 min. The digest was neutralized with 1 ml of 0.67N HCl. The uptake of ^{45}Ca was determined by mixing 1 ml of the digest with 10 ml of scintillation fluid (50 g naphthalene, 6 g 2,5-diphenyloxazole, 0.5 g 1,4-di-5-phenyl-2-oxazolylbenzene, dioxane to a 1 liter) in a Packard liquid scintillation counter.

Protein was determined by Lowry's method [11].

(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Golikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 9, pp. 301-304, September, 1978. Original article submitted December 8, 1977.

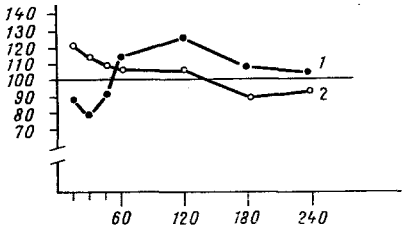

Fig. 1

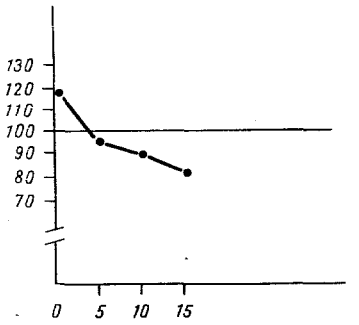

Fig. 2

Fig. 1. Action of benactyzine (1) and arecoline (2) on binding of Ca by synaptosomes. Abscissa, time of incubation of synaptosomes with isotope (in sec); ordinate, ^{45}Ca uptake (in % of control). Here and in Fig. 2, uptake of isotope without addition of drug taken as 100.

Fig. 2. Reduction in ^{45}Ca uptake by benactyzine after preincubation of synaptosomes with arecoline. Abscissa, time of incubation of synaptosomes with benactyzine (in min); ordinate, ^{45}Ca uptake (in % of control).

TABLE 1. Uptake of ^{45}Ca (in cpm/mg protein) by Synaptosomes Previously Depolarized by KCl (M ± m)

Synaptosomes	Time of incubation with ^{45}Ca, sec		
	15	30	45
Without depolarization (n = 6)†	206,0±17,1	208,4±15,9	216,0±10,0
Previously depolarized (n = 9)†	479,9±38,5*	518,5±52,7*	593,5±45,8*

*$P < 0.05$.

†n) Number of experiments.

EXPERIMENTAL RESULTS

During incubation of synaptosomes with benactyzine the ^{45}Ca uptake fell in the first 45 sec, but later it rose considerably and returned to the control level after 4 min (Fig. 1). Arecoline, on the other hand, increased uptake of the isotope for 45 sec but left it practically unchanged during the subsequent period of observation. Antagonism was thus observed between the action of benactyzine and arecoline during the first 45 sec, and biphasic changes in ^{45}Ca uptake took place under the influence of benactyzine. In the experiments to study the effect of benactyzine on uptake of the isotope after preliminary incubation of synaptosomes with arecoline, benactyzine was found to inhibit the action of arecoline on this process (Fig. 2). In this case the synaptosomes were preincubated with arecoline for 15 min and benactyzine was added at different times after the beginning of incubation. After addition of the isotope to the samples, incubation in all cases continued for 30 sec, i.e., the period of time during which arecoline increased the ^{45}Ca uptake but benactyzine inhibited it.

The results show that the cholinolytic benactyzine and the cholinomimetic arecoline both increased the uptake of ^{45}Ca. However, these processes differed significantly in time, evidently because benactyzine and arecoline interact with different components of the membranes. Ca^{++} ions are known to be able to pass through the rapidly activated channels of the early current or, in other words, through Na-channels [8], and also through the channels of the late current, or the Ca-channels proper [7]. Consequently the results of the present investigation can probably be interpreted as activation of Na-channels following administration of arecoline and their blocking, with subsequent activation of the Ca-channels proper, during the action of benactyzine.

To test this hypothesis experiments were carried out with preliminary depolarization of the nerve endings by replacement of a considerable proportion of the Na^+ in the incubation medium by K^+ (final K^+ concentration 69 mM). Under these circumstances the ^{45}Ca uptake by the synaptosomes was more than doubled (Table 1). If benactyzine was added against this background, it ultimately reduced the uptake of the isotope compared with the ^{45}Ca uptake by previously depolarized synaptosomes (Fig. 3), whereas arecoline had virtually no action on this process. These results confirmed the view that channels of the early current are inhibited by benactyzine.

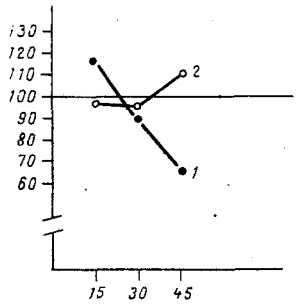

Fig. 3. Binding of ^{45}Ca during action of benactyzine (1) and arecoline (2) after preliminary depolarization of synaptosomes. Abscissa, time of incubation of synaptosomes with isotope (in sec); ordinate, ^{45}Ca uptake (in % of control). Uptake of isotope by depolarized synaptosomes taken as 100.

Evidently not all the Ca^{++} ions are involved in the process of liberation of neuromediators. Katz [9], for instance, considers that the trigger role in relation to exocytosis is played only by ions which penetrate into nerve endings through the slowly activated Ca-channels; their concentration, moreover, must increase under these circumstances from 10^{-7} to 10^{-5}M. It can therefore be tentatively suggested that the brief increase in ^{45}Ca uptake observed initially after the addition of arecoline does not lead to any significant change in the concentration of ions within the nerve endings, and in that case conditions sufficient for liberation of neuromediators are not created. Horeover, as Muscholl and co-workers [13] consider, for reasons which are still unknown, activation of muscarinic (M-) cholinergic receptors reduces the ability of Ca^{++} to influence the control of liberation of mediators, especially NA, on the tissue depots. Meanwhile the increase in ^{45}Ca uptake observed under the influence of benactyzine, which probably takes place through the Ca-channels proper, is sufficient to lead to the liberation of ACh and NA from the corresponding nerve endings.

The results of this investigation suggest that the similarity between the action of muscarinic cholinolytics of the benactyzine type and of Ca^{++} (i.e., the calcium-like action of M-cholinolytics), which several investigators have described [2, 5, 6], is probably due not to direct replacement of Ca^{++} ions by the M-cholinolytic, but to a change in the redistribution of ions, as a result of which neuromediators are secreted by the nerve endings.

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