EFFECT OF BENACTYZINE AND ARECOLINE ON Na,K-ATPase ACTIVITY AND CONTENT OF Na^+ AND K^+ IONS IN THE RAT BRAIN

E. V. Semenov, A. N. Petrov, and S. S. Krylov

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Activity of Na,K-ATPase and the content of Na^+ and K^+ ions in the rat brain were studied after administration of arecoline and benactyzine. Both drugs increased Na,K-ATPase activity, possibly on account of changes in the redistribution of Na^+ and K^+ ions in the nerve cell. Arecoline was shown to cause changes in the distribution of electrolytes characteristic of depolarization, and benactyzine changes characteristic of hyperpolarization of the nerve cell membrane.

KEY WORDS: *Na,K-ATPase*; *electrolytes*; *arecoline*; *benactyzine*; *rat brain.*

The central cholinolytic benactyzine is known to block M-cholinergic receptors [1], to interfere with the storage of mediators [9], to inhibit conditioned-reflex activity [6, 10], to cause changes in the EEG [4], and to disturb memory [7]. The cholinomimetic arecoline excites central M-cholinergic receptors and induces a characteristic tremor [2, 3], and causes EEG changes of excitation type [8]. Meanwhile, there is very little information on the mechanism of action of these substances at the biochemical level.

In this investigation the effect of benactyzine and arecoline on activity of transport Na,K-ATPase and on the content of Na^+ and K^+ ions in the rat brain was studied.

EXPERIMENTAL

Experiments were carried out on albino rats weighing 150-200 g. Benactyzine, in doses of 5 and 40 mg/kg, and arecoline, in a dose of 2.5 mg/kg were injected intraperitoneally in a dose of 0.1 ml solution/100 g body weight. Control animals received water. In the experiments in vitro benactyzine and arecoline were added until a final concentration of 10^{-3}-10^{-5} M was obtained. After a certain time the animals were decapitated and the brain removed and freed from as much blood as possible.

The membrane fraction was isolated and ATPase activity determined by the method of Hayvashi and Auditore with certain modifications [5]. Protein was determined by Lowry's method [11]. The content of electrolytes in the serum and brain tissue was determined [12] on a Perkin–Elmer (Sweden) flame spectrophotometer. The intracellular space was calculated relative to chloride, determined by Rusznyak's conductometric titration method.

EXPERIMENTAL RESULTS AND DISCUSSION

Benactyzine (5 and 40 mg/kg) in experiments in vivo caused an increase in Na,K-ATPase activity (Table 1). The duration of the blocking action of benactyzine on cholinergic mediation is known to depend on the dose of the drug given [7]. In a dose of 1 mg/kg, for instance, benactyzine has a M-cholinolytic action for less than 30 min, whereas in a dose of 40 mg/kg the effect lasts 4 h. The results showed that the enzyme remained in an activated state for about the same length of time.

Arecoline, like benactyzine, also increased Na,K-ATPase activity (Table 1). Considering that benactyzine and arecoline had a similar action on Na,K-ATPase activity but had opposite effects on M-cholinergic receptors, it was postulated that the action of these compounds on Na,K-ATPase is indirect.

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TABLE 1. Changes in Na,K-ATPase Activity (in μg P_i/mg protein/30 min) of Rat Brain following Administration of Arecoline and Benactyzine (M ± m)

Substance and dose		Time after injection of drugs			
		10 min	30 min	1 h	4 h
Arecoline (2,5 mg/kg)	Control	17,4±1,4 (8)	16,0±1,0 (12)	18,5±1,2 (8)	—
	Experiment	22,6±0,8 (8)	20,2±1,6 (12)	17,5±0,9 (8)	
	% of changes	130	126	95	
	P	<0,05	<0,05	>0,5	
Benactyzine (5 mg/kg)	Control	10,0±0,7 (8)	12,5±0,5 (8)	14,6±1,1 (7)	—
	Experiment	15,1±1,4 (8)	14,8±0,6 (8)	15,5±1,2 (7)	
	% of changes	151	118	106	
	P	<0,05	<0,05	>0,5	
Benactyzine (40 mg/kg)	Control	12,6±1,2 (7)	16,0±1,3 (8)	19,4±1,1 (8)	17,5±1,5
	Experiment	13,6±1,8 (7)	20,4±1,0 (8)	22,6±1,0 (8)	19,1±1,6
	% of changes	108	128	116	109
	P	>0,5	<0,05	<0,05	<0,5

Note. Number of experiments in parentheses.

TABLE 2. Effect of Arecoline and Benactyzine on Na,K-ATPase Activity (in μg P_i/mg protein/30 min) in vitro (M ± m)

Control	Arecoline			Benactyzine		
	10^{-3} M	10^{-4} M	10^{-5} M	10^{-3} M	10^{-4} M	10^{-5} M
33,6±1,7	33,6±1,8	34,4±1,6	33,6±1,4	30,4±1,7	32,0±1,9	33,0±1,7

Note. 8 control and 4 experimental investigations were made.

A deoxycholate extract of the membrane fraction isolated from rat brain was preincubated for 5 min at 37°C in the medium used to determine Na,K-ATPase activity. Different concentrations of benactyzine and arecoline were then added and the mixture was incubated for 30 min. Arecoline was found to have no effect on ATPase activity (Table 2). Benactyzine, in a concentration of 10^{-3} M, reduced the activity of the enzyme but had no effect in lower concentrations. The concentrations of benactyzine and arecoline tested in the experiments in vitro were much higher than their possible concentrations in the brain after intraperitoneal injection; by rough calculation it was 40 μg/g for benactyzine and 2.5 μg/g for arecoline. These results emphasized that activation of Na,K-ATPase in vivo could not have been the result of the direct action of benactyzine or arecoline on this enzyme.

An increase in the intracellular concentration of Na^+ ions in the brain (without brain stem) tissue was observed 10 min after injection of arecoline, and the intracellular concentration of K^+ ions was reduced after 30 min (Table 3). This change in the concentrations of Na^+ and K^+ ions is characteristic of the state of depolarization of the nerve cell. Toward 1 h after injection of arecoline the distribution of the electrolytes was similar in the experimental and control animals.

During the first 30 min of the action of benactyzine in a dose of 5 mg/kg the concentration of Na^+ and K^+ ions in the extracellular and intracellular space changed in a manner similar to that during the action of arecoline, but the changes were less marked. By the end of the first hour the intracellular concentration of these ions increased, after which it returned to the control level. During the action of benactyzine in a dose of 40 mg/kg the intracellular concentration of K^+ ions fell and the concentration of Na^+ ions was unchanged. This change in the relative concentration of Na^+ and K^+ electrolytes is characteristic of hyperpolarization of the nerve cell membrane. The concentration of Na^+ and K^+ ions 30 min after administration of benactyzine did not differ significantly from their concentrations in the control animals, but after 1 h their intracellular concentration was increased, to return to the initial values after 4 h.

During the first few minites after administration of benactyzine (5 mg/kg) the changes in the concentration of Na^+ and K^+ ions were in the same direction as those produced by arecoline, and only later did changes characteristic of hyperpolarization of the nerve cell

TABLE 3. Concentration of Extracellular and Intracellular Electrolytes in Rat Brain after Administration of Arecoline and Benactyzine (M ± m)

Substance and dose	Electrolytes	Localization of electrolytes	Concentration of ions (in meq) after injection of drug				
			10 min	30 min	1 h	2 h	4 h
Arecoline (2,5 mg/kg)	Control Na+	Extracellular	31,4±0,58	37,7±0,35	33,7±0,8	—	—
		Intracellular	1,48±0,07	1,77±0,04	1,67±0,12		
	Experiment Na+	Extracellular	30,0±0,4	37,7±0,8	33,8±0,34		
		Intracellular	1,92±0,0*	1,63±0,03*	1,69±0,07		
	Control K+	Extracellular	2,54±0,22	2,0±0,18	2,58±0,11	—	—
		Intracellular	16,3±0,56	29,1±1,3	20,7±0,34		
	Experiment K+	Extracellular	2,38±0,15	2,3±0,05	2,79±0,22		
		Intracellular	16,8±0,45	26,0±0,5*	20,8±0,56		
Benactyzine (5 mg/kg)	Control Na+	Extracellular	31,4±0,58	37,7±0,35	33,7±0,8	27,7±0,6	—
		Intracellular	1,48±0,07	1,77±0,04	1,67±0,12	1,48±0,03	
	Experiment Na+	Extracellular	31,2±0,5	38,4±1,2	33,6±1,0	27,3±0,4	
		Intracellular	1,69±0,22*	1,76±0,07	1,8±0,16	1,35±0,03	
	Control K+	Extracellular	2,54±0,22	2,0±0,18	2,58±0,11	1,54±0,16	
		Intracellular	16,3±0,56	29,1±1,3	20,7±0,34	24,4±1,2	
	Experiment K+	Extracellular	2,54±0,16	2,1±0,15	2,48±0,16	1,43±0,08	
		Intracellur	16,3±0,7	27,7±0,9	21,5±1,4	24,7±0,8	
Benactyzine (40 mg/kg)	Control Na+	Extracellular	23,6±0,7	34,4±0,6	27,2±0,5	27,7±0,6	30,5±0,8
		Intracellular	0,94±0,0	1,4±0,04	1,64±0,1	1,48±0,08	1,4±0,04
	Experiment Na+	Extracellular	24,6±0,38	33,3±1,6	25,0±0,2*	27,5±0,9	31,9±0,9
		Intracellular	0,92±0,07	1,44±0,08	1,96±0,14*	1,66±0,04	1,42±0,02
	Control K+	Extracellular	1,08±0,04	2,04±0,11	1,79±0,12	1,5±0,1	2,0±0,1
		Intracellular	16,3±0,6	16,3±0,4	17,1±1,6	24,4±1,2	19,0±0,9
	Experiment K+	Extracellular	1,16±0,04	1,63±0,14	1,4±0,2	1,43±0,09	2,14±0,2
		Intracellular	14,7±0,4*	17,5±0,9	21,7±1,0*	26,8±0,5	18,3±0,4

Note. 1) $P < 0.05$, 2) Number of experiments = 8.

arise. After administration of benactyzine in a dose of 40 mg/kg the period of depolarization did not occur and the hyperpolarizing action of the compound was manifested immediately.

LITERATURE CITED

1. S. V. Anichkov, Selective Action of Mediator [in Russian], Leningrad (1974), p. 8.
2. S. N. Golikov, Farmakol. Toksikol., No. 2, 38 (1958).
3. N. V. Golyakhovskii, Farmakol. Toksikol., No. 1, 8 (1948).
4. P. P. Denisenko, Central Cholinolytics [in Russian], Leningrad (1965).
5. N. R. Elaev and E. V. Semenov, Biokhimiya, No. 3, 636 (1974).
6. S. S. Krylov, Fiziol. Zh. SSSR, No. 4, 575 (1955).
7. S. S. Krylov, V. V. Vinogradov, S. A. Kal'ning, et al., Zh. Vyssh. Nerv. Deyat., No. 3, 541 (1970).
8. S. S. Krylov, V. V. Vinogradov, and S. A. Kal'ning, Zh. Vyssh. Nerv. Deyat., No. 6, 1223 (1971).
9. S. S. Krylov and E. A. Snegirev, Byull. Éksp. Biol. Med., No. 9, 49 (1970).
10. A. T. Selivanova, The Action of Cholinergic Drugs on Higher Nervous Activity [in Russian], Leningrad (1969).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
12. E. Valcana and P. Timiras, J. Neurochem., 16, 935 (1969).