spontaneous departures did not significantly exceed the control figures, whereas the vectorogram allowing for the significance of the shifts had the same configuration as after 25 mg/kg caffeine. The situation can be summed up by saying that the behavior of the rats appeared to be even more purposive than after administration of 0.5 mg/kg or, more especially, of 2 mg/kg amphetamine.

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EFFECT OF BENACTYZINE AND ARECOLINE ON Mg^{2+}-ATPase ACTIVITY AND CONTENT OF Ca^{2+} AND Mg^{2+} IONS IN THE RAT BRAIN

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

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The effect of the central cholinolytic benactyzine (40 mg/kg) and of the cholinomimetic arecoline (2.5 mg/kg) on activity of Mg^{2+}-dependent ATPase was studied and the content of Ca^{2+} and Mg^{2+} ions determined in rat brain. Benactyzine and arecoline caused biphasic changes in the activity of the enzyme and content of the electrolytes. It is concluded that inhibition of the enzyme is linked with the accumulation of Ca^{2+} ions and its activation with an increase in the concentration of Mg^{2+} ions in brain tissue. It is suggested that benactyzine and arecoline exert their influence on the liberation and retention of neuromediators in the tissue depots through these effects.

KEY WORDS: *brain; benactyzine; arecoline; Mg^{2+}-dependent ATPase; electrolytes.*

The action of the central cholinolytic benactyzine is accompanied by an increase in liberation of neuromediators from the brain of several animals [1, 3, 4]. The cholinomimetic arecoline causes acetylcholine to accumulate in the rat brain [9]. The mechanism of these effects remains unexplained. A leading role in the regulation of neuromediator liberation is ascribed to Ca^{2+} and Mg^{2+} ions [6, 11]. Both liberation and storage of neuromediators in the tissue depots are dependent on the presence of ATP, which is utilized in various ATPase reactions. A change in ATPase activity should lead to liberation or accumulation of neuromediators. In the investigation described below the effect of benactyzine and arecoline on the activity of Mg^{2+}-dependent ATPase was studied and the concentrations of Mg^{2+} and Ca^{2+} ions were determined in rat brain.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-250 g which were given intraperitoneal injections of benactyzine (40 mg/kg) and arecoline (2.5 mg/kg) (in solution,

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TABLE 1. Activity of Mg^{2+}-Dependent ATPase of Rat Brain after Administration of Benactyzine and Arecoline (in μg P_{in}/mg protein, in 30 min)

Preparation	Experimental conditi	Time after injection of preparation											
		10 min			30 min			1 h			4 h		
		n	M ± m	P	n	M ± m	P	n	M ± m	P	n	M ± m	P
Preparation (40 mg/kg)	Control	7	8,1±0,6	<0,25	8	6,4±0,3	<0,05	8	8,5±0,7	>0,7	6	10,7±0,35	<0,05
	Experiment	7	7,1±0,5		8	5,0±0,2		8	8,7±0,8		6	12,8±0,7	
Arecoline (2.5 mg/kg)	Control	8	13,3±0,7	>0,2	12	6,2±0,4	>0,7	8	13,7±0,8	>0,1			
	Experiment	8	14,5±0,6		12	6,1±0,3		8	12,3±0,5				

TABLE 2. Content of Mg^{2+} and Ca^{2+} Ions in Brain Tissue of Rats after Injection of Benactyzine and Arecoline (in meq/g wet weight), M ± m

Preparation	Electrolyte	Experimental conditions	Time after injection of preparation				
			10 min	30 min	1 h	2 h	4 h
Benactyzine (40 mg/kg)	Mg^{2+}	Control	4,45±0,18	4,38±0,25	3,5±0,18	4,14±0,3	4,56±0,24
		Experiment	4,44±0,13	4,21±0,47	3,85±0,13	4,54±0,09	5,3±0,28
		P	>0,7	>0,5	>0,1	>0,2	>0,05
	Ca^{2+}	Control	1,16±0,06	1,27±0,1	1,12±0,03	1,31±0,1	1,4±0,08
		Experiment	1,19±0,05	1,49±0,08	1,34±0,09	1,31±0,08	1,14±0,06
		P	>0,5	<0,1	<0,05	—	<0,05
Arecoline (2.5 mg/kg)	Mg^{2+}	Control	3,1±0,13	3,1±0,19	3,27±0,05		
		Experiment	3,54±0,1	3,44±0,12	3,34±0,2		
		P	<0,05	>0,1	>0,7		
	Ca^{2+}	Control	1,5±0,05	1,72±0,09	1,7±0,03		
		Experiment	1,57±0,08	1,85±0,16	1,85±0,07		
		P	>0,5	>0,5	<0,1		

Legend. Here and in Table 3, in experiments with benactyzine n = 12; with arecoline n = 8.

TABLE 3. Mg^{2+}/Ca^{2+} Ratio after Injection of Benactyzine and Arecoline (M ± m)

Preparation	Experimental conditions	Time after injection of preparation			
		10 min	30 min	1 h	4 h
Benactyzine (40 mg/kg)	Control	3,84±0,23	3,45±0,15	3,12±0,13	3,26±0,14
	Experiment	3,73±0,19	2,82±0,24	2,87±0,08	4,65±0,18
	P	>0,7	<0,05	>0,1	<0,05
Arecoline (2.5 mg/kg)	Control	2,07±0,07	1,8±0,09	1,92±0,07	
	Experiment	2,25±0,1	1,86±0,06	1,8±0,05	
	P	>0,1	>0,7	>0,1	

of which the dose given was 0.1 ml/100 g body weight). Control animals received water. The rats were decapitated and the brain removed and freed as much as possible from blood. The ATPase activity was determined in the membrane fraction [7]; the concentration of sodium deoxycholate was 0.2% and incubation continued for 30 min. The reaction was stopped by the addition of an equal volume of 10% TCA. Samples were centrifuged and inorganic phosphate (P_{in}) was determined in the supernatant by using ammonium molybdate and 1% ascorbic acid. The protein content was determined by the method of Lowry et al. [8]. Activity of Mg^{2+}-ATPase in all cases was determined in the presence of Na^+ (10^{-1} M), K^+ (10^{-2} M), and Mg^{2+} (10^{-3} M) with the addition of 10^{-4} M strophanthin K (ouabain). Control samples contained all the ingredients except Na^+, K^+, and Mg^{2+} ions. The electrolyte content also was determined in the brain tissue [10] by means of a Perkin-Elmer flame spectrophotometer.

EXPERIMENTAL RESULTS AND DISCUSSION

After injection of benactyzine the Mg^{2+}-ATPase activity was almost unchanged during the first 10 min, but after 30 min it had fallen by 27%. Later the activity of the enzyme returned to its original level, and exceeded it after 4 h (Table 1).

The content of Ca^{2+} and Mg^{2+} ions soon after the injection of benactyzine was the same as in the control (Table 2). After 30 min a clear tendency was observed for the Ca^{2+} concentration in the tissue to rise, whereas that of Mg^{2+} remained unchanged. Meanwhile Mg^{2+}-ATPase activity fell. After 1 h the Ca^{2+} concentration was higher than in the control, but after 4 h it was lower. At that time a tendency was observed for the Mg^{2+} level and the activity of the enzyme to rise.

Activity of Mg^{2+}-ATPase did not change significantly after injection of arecoline. However, judging from the mean values, the tendency toward activation of the enzyme (at the 10th minute) coincided in time with an increase in the Mg^{2+} content in the brain tissue, whereas inhibition of Mg^{2+}-ATPase (at the 60th minute) coincided with a small rise in the Ca^{2+} ion concentration.

The results are evidence that Mg^{2+}-ATPase activity depends directly on the concentration of Mg^{2+} ions but inversely on the concentration of Ca^{2+} ions. In experiments in vitro with a total rat brain mitochondrial fraction it was found, incidentally, that Ca^{2+} ions inhibit Mg^{2+}-ATPase activity [2].

The results of the present experiments also show that changes in the enzyme activity under the influence of benactyzine and arecoline are dependent to some extent on a redistribution of the ions; the predominant factor is evidently the action of the ion whose content in the tissue is highest at the given moment. The role of redistribution of the ions can be seen more clearly if the Mg^{2+}/Ca^{2+} ratio is calculated at different time intervals after injection of the drugs (Table 3). Benactyzine caused a decrease in the ratio between the ions after 30 min and an increase after 4 h. After the administration of arecoline the opposite pattern was observed, i.e., shortly after injection (10 min) there was a tendency for the Mg^{2+}/Ca^{2+} ratio to rise, but after 1 h a tendency to fall.

At the 30th minute after injection of benactyzine, when the Mg^{2+}/Ca^{2+} ratio was low, ATPase activity was inhibited. Toward the 4th hour this ratio gradually increased, and so also did the activity of the enzyme. After administration of arecoline the same trend was observed in the changes in enzyme activity and in the ratio between the ions. The fact that ATPase activity was dependent on redistribution of the ions is also confirmed by the high values of the coefficient of correlation (for benactyzine $r = 0.87$; for arecoline $r = 0.98$) between the indices studied.

It can be concluded from a comparison of the results of this investigation with those of a study of the effect of benactyzine on acetylcholine and noradrenalin liberation in the brain [3, 4] that benactyzine acts on the neuromediator depot, probably through a decrease in Mg^{2+}-ATPase activity, leading to accumulation of Ca^{2+} ions in the brain tissue. After injection of arecoline, on the other hand, initially the Mg^{2+} concentration rises and activity of the enzyme increases a little; this evidently could lead to the retention of, for example, acetylcholine [9] in the tissue depots. Previously, in the case of nicotine, correlation was found between a fall in the activity of Mg^{2+}-dependent ATPase and the liberation of noradrenalin from the tissue depots [5].

It can tentatively be suggested that these results reflect the physiological effects of the cholinolytic benactyzine and the cholinomimetic arecoline on the processes of liberation or retention of neuromediators in the brain.

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EFFECT OF PHENTOLAMINE ON THE CEREBRAL CIRCULATION

V. G. Mal'tsev and M. D. Gaevyi

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The effect of phentolamine on the volume velocity of the cerebral blood flow, tone of the cerebral vessels, and partial pressure of oxygen in the brain tissue was studied in acute experiments on anesthetized and unanesthetized cats. Intravenous injection of phentolamine led to a prolonged fall of blood pressure and of the tone of the intracranial and, to a lesser degree, the extracranial vessels. The volume velocity of the cerebral blood flow was reduced in animals with marked phentolamine hypotension. If the perfusion pressure was stabilized, the blood flow was increased. Changes in pO_2 in the brain tissue corresponded largely to the blood flow. Preliminary atropinization and denervation of the carotid zones did not alter the effect of phentolamine. Phentolamine reduced or abolished the constrictor action of noradrenalin and phenylephrine on the brain vessels.

KEY WORDS: *circulation; brain vessels; phentolamine.*

Data on the effect of phentolamine on the cerebral circulation are few in number and contradictory in nature [7, 9-11]. Since phentolamine lowers the systemic blood pressure considerably, it is difficult to judge the response of the cerebral vessels to it.

The object of this investigation was to study the action of phentolamine on the vessels of the brain under conditions excluding the effect of general hypotension on the cerebral circulation.

EXPERIMENTAL METHOD

Acute experiments were carried out on 70 cats of both sexes weighing 2-3 kg under general (1 μg/kg urethane, intravenously) or local (0.25% procaine solution) anesthesia in conjunction with muscle relaxants (diplacin* was injected intravenously at the rate of 0.05-0.1 mg/kg per minute). In the overwhelming majority of experiments controlled respiration was used. The effect of phentolamine was judged from the volume velocity of the cerebral blood flow under conditions of an unstabilized and stabilized perfusion pressure, resistographic data, pO_2 in the brain tissue (by a polarographic method), and the velocity of the blood flow (by the hydrogen clearance method) in the parietal cortex [1-4, 6]. Phentolamine was injected intravenously in a dose of 0.5 mg/kg or intraarterially in doses of 0.005-0.1 mg/kg.

*1,3-Di(β-playneciniumethoxy)benzene hydrochloride — Translator.

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