7. L. L. Iversen, Nature, 285, 285 (1980).
8. P. J. Roberts, H. K. Gupta, and M. S. Shargill, Brain Res., 155, 209 (1978).
9. V. V. Zakusov, R. U. Ostrovskaya, S. N. Kozheshkin, et al., Arch. Int. Pharmacodyn., 229, 313 (1977).

EFFECT OF ARECOLINE AND MUSCARINIC AND NICOTINIC CHOLINOLYTICS ON ^{22}Na INCORPORATION INTO RAT BRAIN NEURONS

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Among the numerous effects of atropine-like substances in experimental animals, the appearance of slow-amplitude potentials similar to the waves found during natural sleep has been found on the EEG, and has been described as an EEG of "sleep" type [8, 9, 12]. However, the animals' behavior is characterized by wakefulness and increased motor activity. The reason for these effects may be that muscarinic cholinolytics disturb relations between excitation and inhibition among neurons, with the result that brain functions are disorganized, possibly because of a disturbance of ionic permeability of the neuron membranes.

The writer showed previously [4] that muscarinic cholinolytics and cholinomimetics modify the permeability of nerve cell membranes for monovalent cations.

In the present investigation the action of the cholinomimetic arecoline and of muscarinic and nicotinic cholinolytics on nerve cell membrane permeability was studied in different parts of the rat brain.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-250 g, which received intraperitoneal injections of benactyzine (40 mg/kg), glypin (10 mg/kg), tropazine (40 mg/kg), adiphenine (40 mg/kg), and arecoline (2.5 mg/kg) made up in a volume of 0.1 ml solution/100 g body weight. Control animals received injections of water. An injection of ^{22}Na (5 μCi) was given to the rats 30 min before sacrifice. The animals were decapitated after definite time intervals and the brain was removed and placed in a dish with ice. Separate parts of the brain were taken (hypothalamus, medulla and midbrain, basal ganglia, cortex) and hydrolyzed in 1N NaOH (0.7 ml) at 60°C for 30 min. The digest was neutralized with 1 ml of 0.67 N HCl. Incorporation of ^{22}Na was determined by mixing 1 ml of the digest with 10 ml SM-7 scintillation solution in a liquid counter (from Packard, England) with a counting efficiency of 80%. The degree of incorporation of the isotope was estimated in cpm/mg protein. Protein was determined by the method of Lowry et al. [10].

EXPERIMENTAL RESULTS

After injection of benactyzine incorporation of ^{22}Na into hypothalamic neurons was increased (Table 1), but into neurons of the medulla—midbrain and cortex it was reduced. Incorporation of ^{22}NA into nerve cells of the basal ganglia was increased a little after 1 h. Glypin increased ^{22}Na incorporation into nerve cells of the basal ganglia but considerably reduced neuron membrane permeability in the cortex (by 39%) and in the medulla and midbrain (by 35% after 10 min).

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TABLE 1. Incorporation of ^{22}Na into Neurons of Various Rat Brain Structures (in cpm/mg protein) after Administration of Cholinolytics and Cholinomimetics (M ± m)

Drug	Experimental conditions	Incorporation of isotope after injection of solution							
		hypothalamus		medulla – midbrain		basal ganglia		cortex	
		10 min	60 min	10 min	60 min	10 min	60 min	10 min	60 min
Benactyzine	Control	529,1±43,8 (5)	403,8±39,4 (6)	443,6±38,3 (5)	293,8±22,3 (6)	649,0±58,1 (5)	458,2±33,4 (6)	962,8±47,9 (5)	1008,4±73,8 (6)
	Experiment P	798,7±28,1 (5) <0,05	549,0±41,8 (6) <0,05	310,9±34,5 (5) <0,05	252,2±20,8 (6) <0,25	625,6±63,0 (5) >0,5	521,0±45,3 (6) <0,3	787,9±44,5 (5) <0,05	694,5±58,0 (5) <0,05
Glypin	Control	405,0±26,4 (8)	420,2±37,4 (6)	245,5±11,6 (8)	276,9±29,2 (6)	317,9±31,2 (7)	413,0±36,0 (6)	1155,4±116,5 (8)	1120,1±96,0 (6)
	Experiment	475,4±46,0 (8) <0,2	441,4±40,0 (6) >0,5	159,0±17,8 (8) <0,05	279,5±23,5 (6) >0,5	417,2±33,0 (7) <0,05	552,2±42,5 (6) <0,05	707,0±65,7 (8) <0,05	688,4±64,0 (6) <0,05
Adiphenine	Control	543,6±41,4 (5)	403,8±39,4 (6)	273,8±30,7 (5)	130,3±12,1 (6)	440,0±39,3 (5)	434,4±32,4 (6)	891,0±71,5 (5)	948,4±75,6 (6)
	Experiment	574,7±20,2 (5) >0,5	318,5±23,5 (6) >0,05	353,4±25,4 (5) <0,1	146,2±11,4 (6) <0,4	383,4±34,8 (5) >0,3	389,0±37,5 (6) >0,3	753,0±63,2 (5) <0,2	481,2±45,4 (6) <0,05
Tropazine	Control	405,2±26,4 (8)	420,2±37,4 (6)	245,5±11,6 (8)	256,1±23,8 (8)	317,9±31,2 (7)	413,0±36,0 (6)	1155,4±116,5 (8)	1120,1±96,0 (6)
	Experiment	350,9±37,5 (8) <0,3	467,6±43,4 (6) <0,5	274,5±20,4 (8) <0,25	269,6±22,7 (8) >0,5	323,6±29,6 (7) >0,5	466,4±36,6 (6) >0,3	947,6±108,3 (8) <0,2	686,8±65,3 (6) <0,05
Arecoline	Control	537,4±30,7 (7)	354,6±16,3 (7)	358,7±26,4 (8)	224,7±19,4 (8)	544,5±42,9 (8)	358,3±36,7 (8)	904,7±58,3 (8)	1043,0±84,5 (7)
	Experiment	468,3±47,0 (7) <0,25	318,1±14,0 (7) <0,2	497,6±32,3 (8) <0,05	308,2±26,4 (8) <0,05	636,9±44,6 (8) <0,2	356,3±32,0 (8) >0,5	2083,8±227,9 (8) <0,05	1267,1±62,8 (7) >0,05

Legend. Number of tests shown in parentheses.

The changes in neuron membrane function in response to benactyzine and glypin, noted above, were to some extent similar. Both these drugs increased ^{22}Na incorporation into neurons of the hypothalamus and basal ganglia, i.e., structures in which mainly catecholaminergic neurons are distributed [1, 2, 13], and they reduced neuron membrane permeability in the cortex. Meanwhile, there were differences between the action of benactyzine and glycin: The activating action of benactyzine was shifted toward structures containing predominantly noradrenergic neruons, whereas that of glypin was shifted toward structures with predominantly dopaminergic neurons.

Under the influence of the nicotinic cyolinolytics adiphenine and tropazine no significant differences were found in the changes in neuron membrane permeability for ^{22}Na in the brain regions tested during the first 10 min. Incorporation of ^{22}Na into cortical neurons was inhibited 1 h after injection of these drugs, and in hypothalamic neurons also after injection of adiphenine. The results indicate that the action of nicotinic cholinolytics is delayed compared with that of muscarinic, and is manifested 1 h after injection. The fact must be emphasized that whereas injection of muscarinic cholinolytics had opposite effects on ^{22}Na incorporation into neurons of the cortex, hypothalamus, and basal ganglia, the nicotinic cholinolytics had no such effect. Nicotinic cholinolytics inhibited permeability to ^{22}Na in cortical neurons but had virtually no effect on neuron membrane permeability in other structures, except for a decrease in ^{22}Na incorporation into hypothalamic neurons following injection of adiphenine.

The cholinomimetic arecoline stimulated ^{22}Na incorporation into neurons of the cortex (by 130%) and medulla and midbrain (by 38%) and by a lesser degree in the basal ganglia (by 17%) during the first 10 min after injection, but ^{22}Na incorporation into the hypothalamus was reduced by 13%. The effect of arecoline, 60 min after injection, on neuron membrane permeability was significantly reduced in the various brain structures. Arecoline activates permeability in structures with predominantly cholinergic mediation but has virtually no effect on structures containing predominantly biogenic amines. Muscarinic cholinolytics affect structures of both types, and under these circumstances neuron membrane permeability is inhibited in structures with cholinergic mediation but activated in structures containing biogenic amines. After injection of nicotinic cholinolytics neuron membrane permeability was inhibited in the cortex and hypothalamus but unaffected in other structures.

Changes in neuron membrane permeability following injection of muscarinic cholinolytics and the cholinomimetic arecoline are largely the result of activation of Na^+, K^+-ATPase, as previous investigations showed [4]. However, the possible exchange of ^{22}Na for intracellular Na^+ cannot be completely ruled out, especially in structures in which incorporation of ^{22}Na is increased.

The writer suggests that the results indicate changes in membrane permeability of brain neurons but not of glial cells, for the neuroglia is known to be selectively permeable to K^+ but not Na^+ ions [6, 7]. In this case the glial cells play the role of regulator of the K^+ concentration in the extracellular space and synaptic chain [3].

LITERATURE CITED

1. B. V. Aleshin, Usp. Sovrem. Biol., 74, 142 (1972).
2. A. Yu. Budantsev, Monoaminergic Systems of the Brain [in Russian], Moscow (1976), p. 67.
3. R. N. Glebov and G. N. Kryzhanovskii, Functional Biochemistry of Synapses [in Russian], Moscow (1978), p. 190.
4. E. V. Semenov, A. N. Petrov, and S. S. Krylov, Byull. Eksp. Biol. Med., No. 2, 180 (1977).
5. P. Bradly and J. Elkes, J. Pharmacol., 120, 14 (1953).
6. H. Bradford and S. Rose, J. Neurochem., 14, 373 (1967).
7. H. Haljamae and A. Hamberger, J. Neurochem., 18, 1903 (1971).
8. F. Irmis, Activ. Nerv. Sup., 13, 217 (1971).
9. A. Kotev, A. Atsev, and E. Stefanova, Voen.-Med. Delo (Sofia), 28, 8 (1973).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. A. Rougeul and M. Agathon, Int. J. Neuropharm., 3, 353 (1964).
12. M. Vogt, J. Physiol. (London), 123, 451 (1954).
13. A. Wikler, Proc. Soc. Exp. Biol. (New York), 79, 261 (1952).