The results of this investigation indicate that the heat loss center in different animals contains mechanisms which incorporate cholinergic neurons whose activity is not significantly inhibited by PG. There is also reason to suppose that the development of febrile reactions is modulated to a considerable degree by the functional activity of central cholinergic and monoaminergic systems.

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LITERATURE CITED

1. L. Kh. Allikmets, V. A. Vakhing, and I. P. Lapin, Zh. Vyssh. Nerv. Deyat., No. 6, 1044 (1968).
2. Yu. S. Borodkin, N. A. Losev, and V. A. Krauz, Farmakol. Toksikol., No. 3, 259 (1970).
3. V. N. Gurin, Yu. I. Bogritsevich, A. I. Kubarko, et al., in: The Pharmacology and Toxicology of New Products of Chemical Synthesis [in Russian], Minsk (1975), p. 84.
4. V. N. Gurin, A. I. Kubarko, Yu. I. Bogritsevich, et al., in: Proceedings of the 12th Congress of the I. P. Pavlov All-Union Physiological Society [in Russian], Tbilisi (1975), p. 171.
5. V. N. Gurin, A. I. Kubarko, Yu. I. Bogritsevich, et al., in: Proceedings of the 4th All-Union Congress of Pharmacologists [in Russian], Leningrad (1976), p. 56.
6. W. Feldberg and P. N. Saxena, J. Physiol. (London), 219, 547 (1971).
7. W. Feldberg and P. N. Saxena, J. Physiol. (London), 219, 739 (1971).
8. W. Feldberg and A. S. Milton, in: Pharmacology of Thermoregulation. Symposium, Basel (1973), p. 302.
9. R. F. Hellon, Pharmacol. Rev., 26, 283 (1975).
10. B. Kandasamy and J. Girault, in: Temperature Regulation and Drug Action (Proceedings of Symposium), Basel (1975), p. 124.
11. H. Laburn, C. J. Woolf, G. H. Willies, et al., Neuropharmacology, 14, 405 (1975).
12. J. M. Lipton and G. P. Trzcinka, Am. J. Physiol., 231, 1638 (1976).
13. A. S. Milton and S. Wendlandt, J. Physiol. (London), 218, 325 (1971).
14. R. D. Myers and T. L. Yaksh, J. Physiol. (London), 218, 609 (1971).
15. J. T. Stitt, J. Physiol. (London), 232, 163 (1973).

ABOLITION OF THE PRESYNAPTIC ACTION OF CARBACHOL BY TUBOCURARINE

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The presynaptic action of carbachol (C) was studied in experiments on a neuromuscular preparation of the sartorius muscle of Rana ridibunda. C was shown to significantly reduce the quantum composition of the endplate potentials (mEPP) as a result of the direct action of C on motor nerve endings. D-Tubocurarine caused a marked decrease in the sensitivity of motor nerve endings to C. Relations between C and tubocurarine as regards their action on the quantum composition of mEPP were of the competitive antagonism type. Atropine, in low concentrations, had no effect on the presynaptic action of C. The results of these experiments indicate that the decrease in mEPP under the influence of C is mediated through specific nicotinic cholinergic structures of motor nerve endings.

KEY WORDS: nerve ending; carbachol; presynaptic action; tubocurarine; competitive relations.

Many cholinomimetic drugs are known to possess a presynaptic action, manifested as changes in the process of mediator liberation in response to motor nerve stimulation [2, 9, 10, 12]. In particular, acetylcholine

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TABLE 1. Effect of C ($5 \cdot 10^{-6}$ M) on mEPP of Neuromuscular Preparations in Ringer's Solution without (A) and with (B) Tubocurarine

Expt. No.	A			B		
	before expt. (M_1)	during action of C (M_2)	$\frac{M_2}{M_1} \cdot 100\%$	before action C (M_2)	during action of C (M_2)	$\frac{M_2}{M_1} \cdot 100\%$
1	50,7	36,8	27,4	17,7	18,1	2,3
2	30,8	21,3	30,9	26,2	33,2	26,7
3	21,3	13,0	39,0	65,3	67,2	2,9
4	21,7	20,1	7,4	26,2	29,3	11,8
5	42,2	28,9	31,5	10,5	13,4	27,6
6	16,1	9,0	44,1	0,9	1,1	22,7
$\bar{x}$	30,5	21,5	30,1	24,5	27,1	15,6
$s\bar{\Delta}$	1,84			1,01		
$t\bar{\Delta}$	4,86			2,55		
P	<0.01			$>0,05$		

(ACh), succinyl choline (SCh), and carbachol (C) reduced the quantum composition of end-plate potentials (mEPP) in neuromuscular preparations on rats [12] and frogs [2, 9, 10]. The fact that these substances, in low concentrations, acted on mediator liberation suggested that their presynaptic effect is mediated through specific cholinergic nerve endings [12, 15].

The present investigation was undertaken to test this hypothesis. Since establishment of competitive relations between cholinomimetic drugs and their antagonists is important evidence of the receptor nature of their action, it was decided to study the effect of C on mEPP in the presence of the nicotinic cholinolytic D-tubocurarine and the muscarinic cholinolytic atropine.

EXPERIMENTAL METHOD

Experiments were carried out on a neuromuscular preparation of the sartorius muscle of Rana ridibunda in the fall and winter. A standard microelectrode technique was used to record synaptic potentials. During the experiment the neuromuscular preparation was kept in a bath with a capacity of 7 cm^3, containing running Ringer's solution, which could easily be replaced by a similar solution containing the test drugs. The rate of flow of the solutions through the bath was 8 ml/min. The experiments were carried out at room temperature and the pH of the solutions was kept at 7.3. Action potentials of the muscle fiber were blocked either by reducing the Ca^{++} ion concentration in the Ringer's solution to 0.9 mM and addition of Mg^{++} in a concentration of 5 mM [8] or by dividing the muscle fibers transversely [1, 6]. Depending on the method of blocking of myoneural transmission the mEPPs were calculated either by the direct method — division of the mean amplitude of the EPP by the mean amplitude of the miniature EPP, or by calculation of the coefficient of variation of EPP amplitudes during stimulation of the nerve with a frequency of 0.5 Hz [8, 11]. The experimental results were subjected to statistical analysis by Student's t-test with a 0.95 level of significance. The effect of C on synapses of intact neuromuscular preparations and on preparations previously treated with atropine was compared by Wilcoxon's criterion.

EXPERIMENTAL RESULTS

Experiments on neuromuscular preparations (Ca^{++} concentration 0.9 mM, Mg^{++} concentration 5 mM in the Ringer's solution) showed that C, in a concentration of $5 \cdot 10^{-6}$ M, acting for 15 min, led to a decrease in the amplitude of mEPP on average by 30.1 ± 5.2% of its initial value (Table 1). This decrease could be explained either by the direct action of the agonist on motor nerve endings or by ionic changes accompanying activation of the postsynaptic membrane [14]. To study the importance of depolarization of the end plate of the muscle fiber in the decrease in mEPP, the effect of C was studied in a concentration which did not act on the postsynaptic membrane. The experiments showed that C, in a concentration of $1 \cdot 10^{-6}$ M, reduced mEPP by 14.3 ± 5.0% ($P < 0.05$) but caused virtually no change in the membrane potential of the muscle fiber. These results indicate a direct action of C on motor nerve endings. To study the connection between the presynaptic effect of C and activation of nicotinic cholinergic structures of nerve endings, the presynaptic action of the mimetic was studied in the presence of D-tubocurarine. These experiments were started after preliminary incubation of the neuromuscular preparation for 1 h in Ringer's solution with D-tubocurarine ($1 \cdot 10^{-7}$ g/ml),

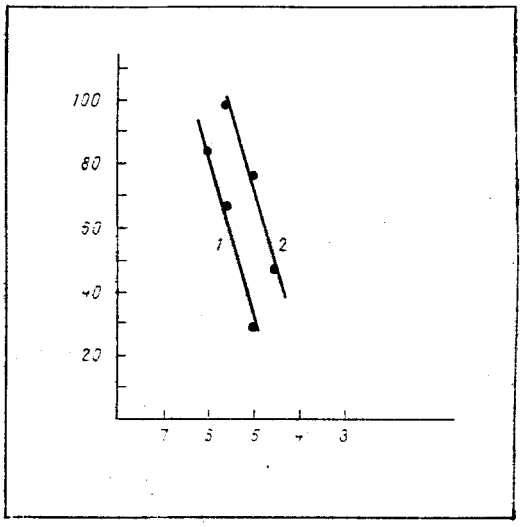

Fig. 1. Presynaptic action of C as a function of its concentration. Abscissa, negative logarithm of carbachol concentration (M); ordinate, quantum composition of EPP (in % of initial value). 1) Effect of C on quantum composition of EPP in Ringer's solution without tubocurarine; 2) effect of C on quantum composition of EPP in Ringer's solution with tubocurarine. Each point on curves represents results of six experiments.

when the mEPP, somewhat reduced as a result of the presynaptic action of the D-tubocurarine itself [5,13], had become stabilized. The experiments showed that curarization considerably reduced the sensitivity of the motor nerve endings to C (Table 1). These results indicate that nicotinic cholinergic receptors play an important role in the mechanism of the presynaptic action of C. To determine the character of relations between C and D-tubocurarine as regards their effect on mEPP, concentration—effect curves for intact and curarized ($5 \cdot 10^{-7}$ g/ml) muscles were plotted. Experiments were carried out on a "divided" neuromuscular preparation, for the permanent disturbance of action potential generation in the divided muscle fibers enabled the action of high doses of the cholinomimetic to be studied. The results of these experiments are illustrated in Fig. 1 and they show that under the influence of tubocurarine the concentration—effect curve for C was shifted toward higher concentrations but remained parallel to the first curve obtained for preparations not treated with D-tubocurarine. This arrangement of the concentration—effect curves is evidence of competitive—antagonistic relations [3] between C and D-tubocurarine on nerve endings.

To study the existence of muscarinic cholinergic receptors on the presynaptic membrane similar experiments were carried out with atropine. In low concentrations atropine is known to have no presynaptic action [7], but to keep the experimental conditions the same as were used to study the effect of D-tubocurarine, the action of C on mEPP of the atropinized preparation was studied after preliminary incubation of the neuromuscular preparation for 1 h in Ringer's solution with atropine. These experiments showed that atropine, in a concentration of $4 \cdot 10^{-8}$ M, did not change the intensity of presynaptic action of C ($P > 0.05$) and reduced it slightly in a concentration of $1 \cdot 10^{-6}$ M. The specificity of atropine as a muscarinic cholinolytic is known to be exhibited in concentrations of the order of 10^{-8} M, and in higher concentrations it may have a nicotinic cholinolytic action [4]. It can be concluded from the results that the decrease in mEPP under the influence of C is effected through nicotinic cholinergic structures of the motor nerve endings. The fact that C gave a presynaptic effect in concentrations lower than those required to depolarize the end plate suggests the existence of differences in the organization of cholinergic receptors of the motor nerve endings and presynaptic membrane of amphibian muscle fibers.

LITERATURE CITED

1. I. N. Volkova, E. E. Nikol'skii, and G. I. Poletaev, Fiziol. Zh. SSSR, 61, 1433 (1975).
2. I. N. Volkova, E. E. Nikol'skii, G. I. Poletaev, et al., in: Abstracts of Scientific Proceedings of the 12th Congress of the All-Union Physiological Society [in Russian], Tbilisi (1975), p. 6.
3. I. V. Komissarov, Elements of the Theory of Receptors in Molecular Pharmacology [in Russian], Moscow (1969).

4. M. Ya. Mikhel'son and É. V. Zeimal', Acetylcholine [in Russian], Leningrad (1970).
5. A. Auerbach and W. Betz, J. Physiol. (London), 213, 691 (1971).
6. J. A. B. Barsted and L. Lillehei, Arch. Int. Pharmacodyn., 175, 379 (1968).
7. R. Beranek and F. Vyskocil, J. Physiol. (London), 195, 493 (1968).
8. J. del Castillo and B. Katz, J. Physiol. (London), 124, 560 (1954).
9. S. Ciani and C. Edwards, J. Pharmacol. Exp. Ther., 142, 21 (1963).
10. C. Edwards and K. Ikeda, J. Pharmacol. Exp. Ther., 138, 332 (1962).
11. D. Elmqvist and J. Quastel, J. Physiol. (London), 177, 463 (1965).
12. J. I. Hubbard, R. F. Schmidt, and T. Yokota, J. Physiol. (London), 181, 810 (1965).
13. J. I. Hubbard, D. F. Wilson, and M. Miyamoto, Nature, 223, 531 (1969).
14. B. Katz, Proc. Roy. Soc. London B, 155, 455 (1962).
15. W. F. Riker, Arch. Neurol. (Chicago). 3, 488 (1960).