EFFECT OF SPECIFIC INHIBITORS OF ACETYLCHOLINE SYNTHESIS ON ACTION OF CHOLINOMIMETIC PHARMACOLOGICAL AGENTS*

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In experiments on an isolated segment of the upper part of the frog's stomach, hemicholinium and triethylcholine prevented the action of acetylcholine, choline, arecoline, neostigmine, and eserine, while in experiments on the frog rectus abdominis muscle, they prevented the action of nicotine, subecholine (diiodide of the dicholine ester of suberic acid), arecoline, neostigmine, and eserine. This action is reversible and depends on the concentration of the substances (both inhibitors of acetylcholine synthesis and cholinomimetic agents) given and the duration of their action on the tissues.

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The view is widely held that the action of cholinomimetics does not depend on liberation of acetylcholine mediators, and it is based on the concept that this mediator is liberated only by presynaptic nerve endings. However, the results of the latest investigations have shown that acetylcholine can be synthesized in the postsynaptic part of the synapse and also by Shwann cells [3, 7, 8, 16]. This raises once again the question of the mechanism of action of cholinomimetic drugs.

Reports have recently been published of a number of new chemical compounds capable of causing specific inhibition of acetylcholine synthesis in the tissues: hemicholinium (HC-3; α-α'-dimethylaminoethanol-4,4'-bisacetophenone) and triethylcholine (N-triethylaminoethanol; the triethyl analog of choline). These substances have been shown to produce specific inhibition of acetylcholine synthesis by competitive displacement of choline from the corresponding biochemical reactions [1, 4, 5, 10, 14, 17, 18].

We have investigated the action of hemicholinium and triethylcholine on excitation of the M (muscarine-sensitive) cholinergic receptors of the smooth muscles of the frog's stomach and the N (nicotine-sensitive) cholinergic receptors of the frog (Rana ridibunda) rectus abdominis muscle under the influence of acetylcholine chloride (1×10^{-4}, 1×10^{-5}), arecoline hydrobromide (1×10^{-5}), nicotine base, and subecholine (1×10^{-5}, 1×10^{-6} on the rectus muscle) and of the anticholinesterase substances neostigmine (5×10^{-4}, 2×10^{-4}) and eserine salicylate (5×10^{-4}).

Experiments on the isolated segment of the stomach were carried out from October to February. According to S. É. Belen'kaya [2], acetylcholine, arecoline, and pilocarpine excite the gastric musculature of the frog, the reaction to these cholinomimetics corresponding to that observed in mammals after denervation of the stomach. S. I. Matveeva [6] found that degenerative changes take place in the intramural plexuses and ganglia of the frog's digestive tract during the winter; this process of winter degeneration may proceed far enough to cause death of individual parts or all of the neuron.

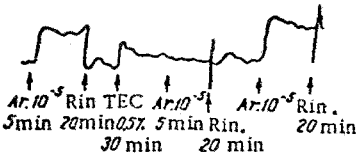

Fig. 1. Trace of contractions of segment of upper part of frog's stomach. Ar. 10^{-5} indicates action of arecoline in concentration of 1×10^{-5}; TEC 0.5%, action of triethylcholine in concentration of 5×10^{-3}; Rin.—rinsing.

EXPERIMENTAL METHOD

Isolated segments of the upper part of the stomach or the rectus abdominis muscle of the frog were placed in a jar of

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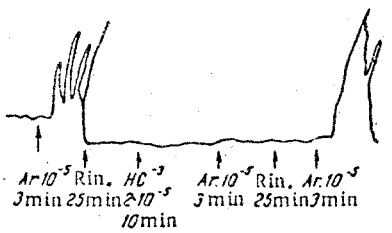

Fig. 2. Trace of contractions of segment of upper part of frog's stomach. HC-3 2×10^{-5} represents action of hemicholinium in concentration of 2×10^{-5}. Remainder of legend as in Fig. 1.

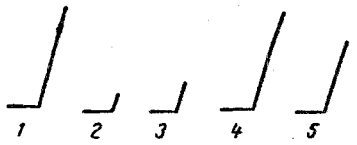

Fig. 3. Trace of contraction of isolated frog rectus abdominis muscle. 1) Action of neostigmine in concentration of 2×10^{-4} (1 min); 2) action of triethylcholine in concentration of 2×10^{-4} on the same muscle after rinsing out neostigmine (30 min); 3) combined action of triethylcholine (2×10^{-4}) and neostigmine (2×10^{-4}; 1 min) on the same muscle; 4) liquid bathing muscle replaced by solution of triethylcholine (2×10^{-4}) and choline (5×10^{-6}; 5 min); 5) action of neostigmine in concentration of 2×10^{-6} (1 min).

Schueller's (stomach) or Ringer's solution through which air was continuously bubbled. Contractions were recorded on a kymograph. Altogether 60 experiments were performed on organs taken from 60 different frogs of both sexes.

EXPERIMENTAL RESULTS

Preliminary administration of hemicholinium or triethylcholine prevented the effects of the cholinomimetic drugs used in these experiments. The sensitivity of the smooth muscle of the stomach and the striated rectus abdominis muscle to the cholinomimetic drugs used was restored after rinsing (Figs. 1-3). To prevent the action of cholinomimetics, high concentrations were required of hemicholinium (2×10^{-5} and higher) and, in particular, of triethylcholine (5×10^{-3} for the stomach muscles, 2×10^{-4} for the rectus abdominis muscle), and the higher the concentrations of cholinomimetics used in the experiment and the longer their action, the greater the concentrations and duration of action of these drugs on the tissues had to be. In some experiments (especially in the case of spontaneous rhythmic activity) hemicholinium and triethylcholine initially increased the tone of the gastric musculature, and this was followed by its relaxation. Triethylcholine prevented contracture of the striated muscle produced by neostigmine. The blocking action of triethylcholine was abolished by choline (Fig. 3). The blocking action of hemicholinium on the isolated striated muscle, as is reported in the literature, was abolished by neostigmine but not by choline [15].

Hence, these experiments clearly demonstrated the ability of specific inhibitors of acetylcholine synthesis (hemicholinium and triethylcholine) to prevent the action of cholinomimetics directly or indirectly exciting tissue cholinergic receptors of smooth (M receptors) or striated (N receptors) frog muscle.

Several workers have shown experimentally [4] that hemicholinium abolishes the effect of frequent stimulation of preganglionic fibers but does not prevent the action of extrinsic (pharmacological) acetylcholine on ganglion cells. The penetration of hemicholinium into cells has been shown to be directly proportional to the degree of their excitation. Naturally hemicholinium penetrates more rapidly into an electrically stimulated axon than into ganglion cells whose indirect excitation progressively fell with a decrease in the number of quanta of acetylcholine liberated by the stimulated axon in the presence of hemicholinium. The ability of hemicholinium and triethylcholine observed in our experiments to prevent excitation of the isolated frog rectus abdominis muscle under the influence of cholinomimetics is in full agreement with published data concerning the postsynaptic action of these inhibitors of acetylcholine synthesis on neuromuscular synapses of a recently isolated muscle [15, 17, 20].

There are no grounds for assuming that hemicholinium or triethylcholine participates in biochemical mechanisms of the postsynaptic membrane in any other way than in the biochemical mechanisms of the presynaptic membrane. Nachmansohn [16] considers that the biochemical processes taking place in the two membranes at a time of cholinergic excitation are identical. Finally, it will be noted that the results of the experiments described in this paper agree with previously published data on the analogous effect of substances inhibiting acetic acid formation in the tissues on the action of pharmacological cholinomimetics [19]; like choline, acetic acid is essential for acetylcholine synthesis.

LITERATURE CITED

1. R. I. Agranovich and E. S. Rozovskaya, in: Experimental and Clinical Basis for the Use of Neurotropic Drugs. Abstracts of Proceedings of a Scientific Conference [in Russian], Leningrad (1963), p. 3.
2. S. É. Belen'kaya, in: Material on Evolutionary Physiology [in Russian], Vol. 1, Moscow-Leningrad (1956), p. 41.
3. I. M. Glagoleva and E. A. Liberman, Uspekhi Sovr. Biol., 55, No. 1, 68 (1963).
4. S. N. Golikov and S. I. Loktionov, Vestn. Akad. Med. Nauk SSSR, No. 8, 77 (1963).
5. S. N. Golikov and S. I. Loktionov, Farmakol. i Toksokol., No. 6, 690 (1964).
6. S. I. Matveeva, Arkh. Anat., Gistol. i Émbriol., No. 1, 40 (1935).
7. É. M. Plisetskaya, Tsitologiya, No. 1, 20 (1961).
8. N. F. Skorobovichuk, Uspekhi Sovr. Biol., 57, No. 1, 115 (1964).
9. S. M. Tregubov, Material on the Physiology and Pharmacology of Cholinergic Processes [in Russia], Ordzhonikidze (1957).
10. W. C. Bowmen and M. J. Rand, Lancet, 1, 480 (1961).
11. G. B. Koelle, Nature, 190, 208 (1961).
12. G. B. Koelle, J. Pharm. (London), 14, 65 (1962).
13. J. P. Long, Fed. Proc., 20, 683 (1961).
14. F. C. McIntosh, Fed. Proc., 20, 562 (1961).
15. A. R. Martin and R. K. Orkand, Fed. Proc., 20, 579 (1961).
16. D. Nachmansohn, in: Molecular Biology, Problems and Perspectives [Russian translation], Moscow (1964), p. 282.
17. G. Schmidt, Dtsch. Med. Wschr., 87, 2540 (1962).
18. F. W. Schueller, J. Pharmacol. Exp. Ther., 115, 127 (1955).
19. F. G. Standaert and J. E. Adams, J. Pharmacol. Exp. Ther., 149, 113 (1965).
20. R. E. Thies and V. B. Brooks, Fed. Proc., 20, 569 (1961).