EFFECT OF TRIETHYLCHOLINE AND PARAMYON ON THE ACTION OF GUANIDINE ON SORPTION OF VITAL DYE BY STRIATED MUSCLE

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UDC 612.744.2:612.388.014.46

Experiments carried out by Nasonov's method have shown that guanidine increases the sorptive properties of the isolated sartorius muscle of Rana temporaria relative to the vital dye methylene blue. This action is prevented by the cholinolytic (muscle relaxant) paramyon. Triethylcholine also increases sorption of vital dye by muscles. It does not prevent the increase in sorption of the dye by striated muscle produced by guanidine.

One of the authors [6] has previously shown that specific inhibitors of acetylcholine synthesis, namely hemicholinium and triethylcholine (N-triethylaminoethanol), inhibit the excitation of striated muscle in response to the action of acetylcholine, subechol (the dicholine ester of suberic acid), and nicotine. These drugs, in the modern view, excite specific chemoreceptors of the postsynaptic membrane in cholinergic synapses.

The object of this investigation was to study the effect of triethylcholine, an inhibitor of acetylcholine synthesis, on the action of guanidine.

The action of guanidine on the contractile activity of striated muscles gives rise to frequent twitches of varied amplitude, which are suppressed by curare and disappear after denervation [9]. The most recent electrophysiological investigations have shown that the action of guanidine is due to an increase in the liberation of acetylcholine by motor nerve endings evoked by a nervous impulse [7, 9, 10].

EXPERIMENTAL METHOD

Experiments were carried out on innervated and noninnervated areas of the isolated sartorius muscle of male frogs (Rana temporaria). The criterion of excitation of the muscles was an increase in their sorptive properties relative to the vital dye methylene blue. It has been shown by Nasonov's school [4] and also by Ginetsinskii and co-workers [1, 5] that the ability of acetylcholine to increase sorption of dye by striated muscle is a result of specific excitation of the cholinergic receptors of this muscle. The experiments were carried out by Nasonov's method [4]. The 254 experiments were divided into five series, to study the following problems: 1) the intensity of sorption of dye by a muscle immersed in a solution of the dye in Ringer's fluid (normal); 2) the same, in the presence of guanidine (guanidin); 3) the effect of preliminary immersion of the muscle in a solution of the cholinolytic drug paramyon in Ringer's solution on sorption of the dye in the presence of guanidine (paramyon + guanidine); 4) the effect of preliminary immersion of the muscle in a solution of triethylcholine in Ringer's solution on sorption of dye in the presence of guanidine (triethylcholine + guanidine); 5) the intensity of sorption of dye after preliminary immersion of the muscle in a solution of triethylcholine in Ringer's solution (triethylcholine).

Methylene blue was used in a concentration of $2 \cdot 10^{-4}$ and $4 \cdot 10^{-4}$, guanidine hydrochloride in a concentration of $1 \cdot 10^{-3}$, paramyon in a concentration of $4 \cdot 10^{-5}$, and triethylcholine in a concentration of $5 \cdot 10^{-3}$.

Department of Pharmacology, Orenburg Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 3, pp. 74-76, March, 1970. Original article submitted January 21, 1969.

The dye was extracted from the muscles with acidified 75° ethanol. The quantity of dye absorbed was determined with the FÉK-M photoelectric colorimeter and expressed in conventional units. The extinction (optical density of the solution) was calculated per gram dry weight of muscle and multiplied by 10^3 (to obtain whole numbers).

EXPERIMENTAL RESULTS

Guanidine caused an increase in sorption of dye by the innervated areas of the sartorius muscle (from 80±4.75 to 103±3.56 units; $P < 0.001$). This action was not exhibited after preliminary treatment of the muscle with the cholinolytic paramyon (sorption of the dye 87±5.27 units). Preliminary treatment of the muscle with triethylcholine did not prevent the ability of guanidine to increase sorption of the dye by the innervated part of the muscle, to a value of 109±9.53 units ($P < 0.01$). No reliable results were obtained for the noninnervated parts of the sartorius muscle, to indicate the effect or otherwise of guanidine or guanidine with paramyon on sorption of the dye by this part of the muscle. However, preliminary treatment of the muscle with triethylcholine increased sorption of the dye in the presence of guanidine by the noninnervated part of the muscle (experiment 93±8.45, control 74±3.66 units; $P < 0.05$). Triethylcholine itself caused an increased in sorption of dye both by the innervated (105±4.23 units; $P < 0.002$) and the noninnervated (119±8.37 units; $P < 0.002$) parts of the sartorius muscle.

Data concerning the presynaptic character of action of guanidine suggest that increased sorption of dye by the innervated part of the muscle was due to liberation of acetylcholine from the nerve endings under the influence of guanidine. Other mechanisms evidently lay at the base of the effects of triethylcholine in these experiments. To begin with, it is an interesting fact that triethylcholine itself significantly increased sorption of the dye by both innervated and noninnervated parts of the sartorius muscle. This effect was unchanged when guanidine acted on the muscle after preliminary treatment with triethylcholine. At the same time, as investigations in the author's laboratory have shown, preliminary treatment of the sartorius muscle with triethylcholine prevents the increase in sorption of dye by the innervated part of the sartorius muscle under the influence of acetylcholine. For instance, the innervated parts of the intact muscle absorbed 69±5.44 units of dye, compared with 93±7.34 units after the action of acetylcholine. After treatment of the muscle with triethylcholine and acetylcholine, sorption of the dye amounted to 81±6.84 units, i.e., it did not differ significantly from normal ($P > 0.05$).

The experiments also showed that the ability of guanidine to increase sorption of dye by the innervated part of the sartorius muscle is completely prevented by paramyon, which blocks the cholinergic receptors of the postsynaptic membrane, but is not prevented by triethylcholine, which effects synthesis of the acetylcholine mediator in the synaptic vesicles located in the axoplasm of the presynaptic nerve ending [3]. With these facts in mind, and remembering also the presynaptic character of action of guanidine, it can be postulated that guanidine promotes the liberation of acetylcholine, not from the synaptic vesicles, but from other tissue depots in the region of the presynaptic endings [8].

This hypothesis is confirmed by the results of electrophysiological investigations showing that guanidine causes a further increase in the end-plate potential after the action of calcium, which liberates acetylcholine from synaptic vesicles [10]. At the same time, guanidine has no effect on the frequency or amplitude of the miniature end-plate potentials, which are thought to be generated in connection with the spontaneous liberation of acetylcholine mediator from synaptic vesicles [2].

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