DIFFERENTIATION OF THE DIFFERENT EFFECTS INDUCED BY ANTICHOLINESTERASE AGENTS

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Earlier, we discovered that proserine, which is regarded by most authors as an anticholinesterase agent only, affects a frog's isolated skeletal muscles in different ways, depending on the concentration: 1) the use of comparatively low concentrations ($1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$) causes repeated contractions; 2) the use of higher concentrations ($2 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$) is attended by the disappearance of these contractions; and 3) still higher concentrations induce contracture of the muscle [1, 2].

The first type of reaction has also been observed by N. A. Kozlov and M. Ya. Mikhel'son [3]; G. A. Panosyan [4] has reproduced all three variants of the proserine reaction (he discovered the first two variants, using the anticholinesterase agent phosphacol [diethyl p-nitrophenyl phosphate]).

The purpose of this work was to determine how the different variants of the skeletal muscle reaction would develop under the influence of the anticholinesterase agent Tensilon (edrophonium; 3-hydroxyphenyldimethylethylammonium chloride)[N-ethyl-N-(m-hydroxyphenyl)-N,N-dimethylammonium bromide].

We selected this preparation because it acts primarily on the skeletal muscles and has almost none of the muscarine effects characteristic of other anticholinesterase agents, proserine included [8, 9, et al.]. Tensilon was recommended for the treatment of myasthenia (myasthenia gravis) and has been found helpful in the diagnosis of this disease; it is inefficient in the treatment of this condition, however, because of the brevity of its effect. The brevity of Tensilon's effect can be explained by the instability of the complex formed, i.e., the enzyme cholinesterase + the inhibitor Tensilon. This proposition is in accord with Wilson's data [10]; this author, studying the association and dissociation rate of cholinesterase and Tensilon or proserine, established that the rate of cholinesterase association with the two substances depends on the concentration of the latter in the environment. In concentrations inducing 90% inhibition of cholinesterase activity, proserine causes 45% inhibition after one minute, Tensilon the same after only three seconds. The dissociation time of 50% of the cholinesterase + proserine complex is about seven minutes, that for the complex with Tensilon, less than 0.5 second.

EXPERIMENTAL METHOD

The experiments were performed on a frog's (Rana temporaria) isolated rectus abdominis muscle. The sartorius muscle was used in a few experiments. The experimental muscle was subjected to the action of the experimental substances one hour after isolation. The contractions which developed were recorded on a kymograph. Fifteen experiments were performed under these conditions at different intervals after denervation of the muscle (see method [2]). We used Roche Tensilon chloride, Schuhardt curare and proserine of Soviet manufacture in the experiments.

EXPERIMENTAL RESULTS

The first variant described above of the reaction which we observed earlier under the influence of proserine was reproduced in the experiments with Tensilon. In concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$, Tensilon induced repeated contractions characterized by rapid development and relaxation (Fig. 1). Analogous contractions were observed in the case of the sartorius muscle under the influence of the same concentrations of Tensilon. Tensilon's effect was preceded by a latent period lasting 2-25 minutes, but of shorter duration than that observed in the experiments with proserine. The duration of the latent period became longer as the concentration of substance decreased. The contractile reaction induced by Tensilon in our experiments lasted 15-40 minutes (the analogous proserine reaction lasted more than an hour).

Therefore, this variant of the contractile reaction is characterized by a more rapid development and a shorter duration when induced by Tensilon. This reaction could be intensified by proserine in low concentrations ($1 \cdot 10^{-6}$) and depressed by curare ($1 \cdot 10^{-5}$ to $5 \cdot 10^{-6}$). It gradually diminished with denervation and disappeared completely by about the 14th day after the nerves were transected (15-16 or more days after denervation, we observed a slowly developing contracture under the influence of Tensilon in concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$, instead of repeated, brief contractions).

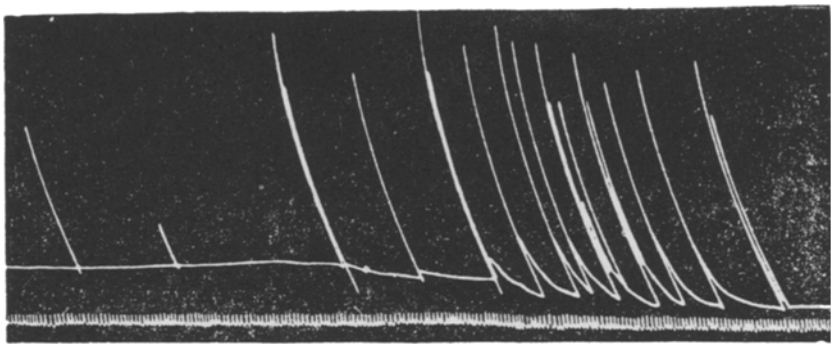

Fig. 1. Series of contractions of frog's rectus abdominis muscle induced by 1: 600,000 Tensilon. Time indicated in 5-second marks.

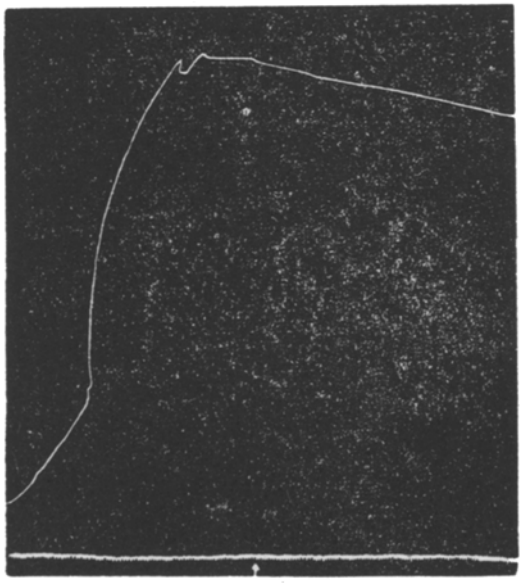

Fig. 2. Contracture of frog's rectus abdominis muscle induced by 1: 20,000 Tensilon (reduced $1\frac{1}{2}$ times). Arrow denotes addition of 1: 100,000 curare.

If the muscle was preliminarily treated with proserine in a concentration of $2 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$, it reacted to Tensilon by contracture. The repeated brief contractions did not appear in this case.

Higher concentrations of Tensilon ($1.6 \cdot 10^{-5}$ to $2 \cdot 10^{-5}$) caused a rapidly developing contracture type of contraction which was very slow to relax, although we observed on this background separate, low-amplitude contractions with rapid relaxation. Still higher concentrations of Tensilon ($3.3 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$) caused in all cases the rapid development of considerable contracture. This contracture was somewhat reduced by curare ($1 \cdot 10^{-5}$ to $2 \cdot 10^{-5}$), as can be seen from Fig. 2.

The data presented indicate that Tensilon reacts with the cholinergic system of a skeletal muscle to a greater degree than proserine, possibly with the actual contractile structure of the muscle as well. As well as the experiments using myographic recording, we also conducted experiments on the same muscles using electromyographic recording. According to the data obtained in these experiments, proserine and Tensilon,

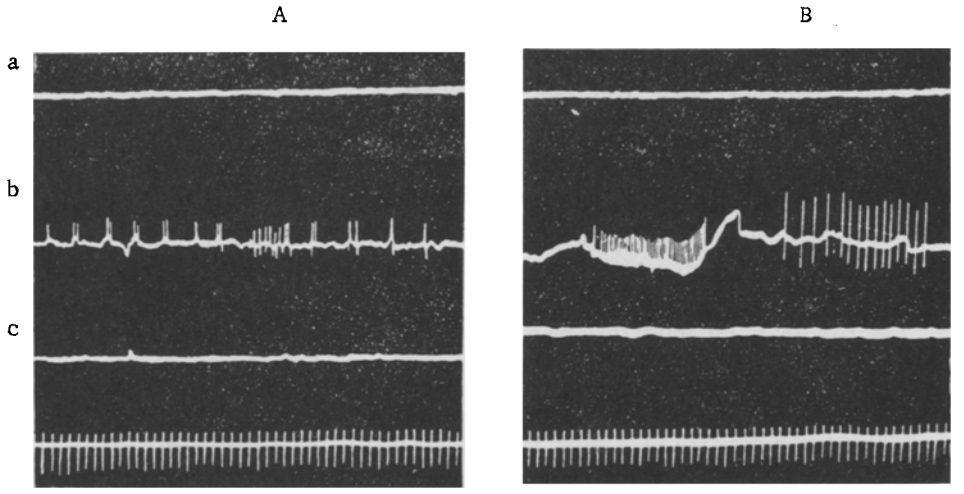

Fig. 3. Effect of proserine on electrical activity of frog's rectus abdominis (A) and sartorius (B) muscles. a) Absence of electrical activity before treatment of muscle with proserine; b) appearance of potentials under the influence of 1:200,000 proserine; c) disappearance of potentials under the influence of 1:25,000 proserine. Time shown in 0,1-second marks.

in concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$, promote the development of bioelectric potentials and increase the amplitude, frequency and duration of the spontaneously developing potentials; Tensilon's effect, however, is less lasting than that of proserine.

In higher concentrations ($2 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$), proserine reduced or completely inhibited the electrical activity of the muscles (Fig. 3). Tensilon was not tested in these concentrations.*

The results of the experiments using electromyography are in accord with the literature data. Fatt and Katz [6, 7] using frog's muscles, and Boyd and Martin [5], using muscles of warm-blooded animals, have shown that low concentrations of proserine (neostigmine – $5 \cdot 10^{-7}$) can be used to increase the amplitude and duration of the miniature potentials of end plates; Boyd and Martin further observed the reduction of these potentials with the use of higher concentrations of proserine (the authors do not state which concentrations or given any explanation for the results obtained).

Our earlier observations that the reactivity of frog's skeletal muscles to anticholinesterase agents varies considerably with the seasons was confirmed in this work. The greatest reactivity was observed during the winter season; the least, during the autumn and spring. The reaction is variable during the summer months.

SUMMARY

The effect of two anticholinesterasic substances – proserine and Tensilon – were compared in experiments on isolated frog muscles.

Three variants of reaction are provoked by eserine depending on its concentration: 1) repeated rapidly developing contractions of short duration; 2) disappearance of these contractions in using large concentrations; 3) contracture, when even higher concentrations are used.

Only two reactions were seen in testing Tensilon; they corresponded to the first and the third variants of proserine reaction. The first variant provoked by proserine and Tensilon is prevented by curare, denervation and sufficiently high concentrations of proserine (1:50 – 20,000). It is attributed to the anticholinesterasic properties of these preparations. The contracture variant in response to proserine and Tensilon application is explained by a combination of acetylcholine component (removed by curare) with a direct action of the mentioned anticholinesterasic substances on the muscle, which is not eliminated under the effect of curare. Proserine

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and Tensilon in the concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$ promote the appearance of muscle biopotentials. In higher concentrations (1 : 50 – 20,000) proserine depresses the electric activity provoked by its lower concentrations. The mechanism of this effect is still obscure.

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