SITE OF APPLICATION OF THE INFLUENCE OF IMIDAZOLE ON A NEUROMUSCULAR PREPARATION

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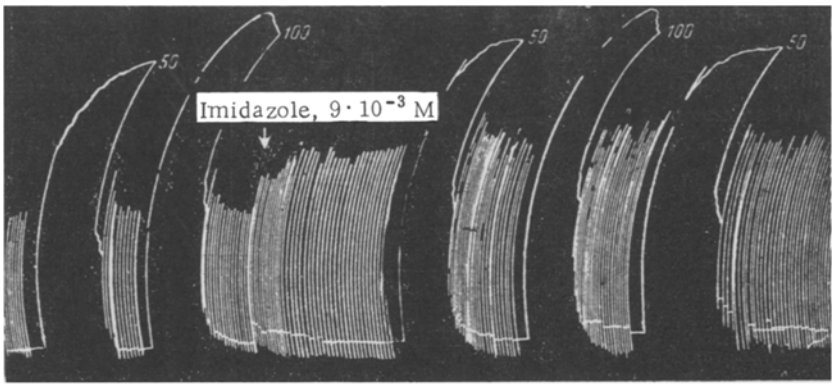
Imidazole and its natural compound—the muscle dipeptide carnosine—increase the ability of a neuromuscular preparation to work during fatigue; they restore the ability of muscles to work under conditions of indirect stimulus by short tetanic contractions and remove a diplacin block during work in a system of indirect single stimuli [1, 5, 6, 7]. It has been hypothesized that imidazole and carnosine play a role in the mediator transmission of impulses [5]. The action of imadazole compounds may be based upon an anticholinesterase effect. Their significance may also be explained by an increase in the biosynthesis or yield of acetylcholine (AC) in the postsynaptic membrane or by an increase in the sensitivity of the receptor to AC. This work was devoted to a verification of these hypotheses and contains data on the question of the site of application of the influence of imidazole on a neuromuscular preparation.

EXPERIMENTAL RESULTS

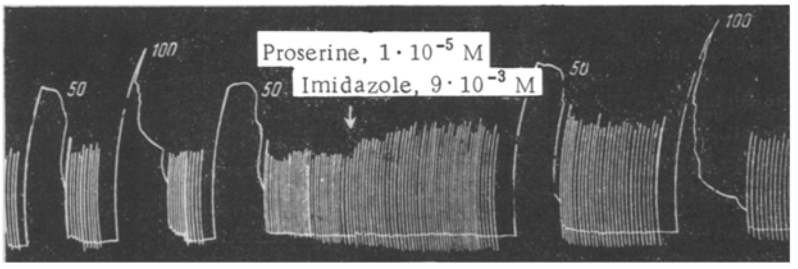
The experiments were conducted on a neuromuscular preparation of the sartorius muscle and on a preparation of the frog rectus abdominis muscle (Rana temporaria). The muscles worked in vessels containing Ringer solution, which was frequently changed and continuously aerated. The investigated substances were introduced into the Ringer solution surrounding the muscle. Equality of the pH of the solutions was established potentiometrically. In all the experiments, imidazole was used in a concentration of 9 mM. The stimulus was delivered from an IG-6 stimulator.

In series I, the frog sartorius muscle was subjected to single indirect stimuli at 8 sec intervals. The muscles worked for 30-40 min. Then, against a background of single stimuli with 3-5 min intervals, the muscles were subjected to indirect tetanic stimulation with a frequency of 50 and 100 cps and duration 10 sec. A recording was made (control). The Ringer solution was decanted and replaced with Ringer solution containing imidazole. Single and tetanic contractions of the muscle were again recorded. Imidazole intensified the single contractions of the sartorius muscle by 20-25%. The tetanic contractions of the sartorius muscle also were intensified by imidazole, but their shape was unchanged (Fig. 1a). After the recording, the muscles were thoroughly washed free of imidazole. A control recording was taken, after which proserine in a concentration of $1 \cdot 10^{-5}$ M was added to Ringer solution surrounding the muscle. In such a concentration, proserine entirely blocked acetylcholinesterase. The shape of the tetanus assumed a clonic form 15 min after the addition of proserine, especially at a frequency of 100 cps. The single contraction of the muscle was unchanged. Imidazole, added against a background of the action of proserine, exerted the same effect as without proserine: the height of the single contraction became greater, the shape of the tetanus was unchanged, but its height was even somewhat increased (Fig. 1b).

On the basis of the data cited, it would be difficult to think that the action of imidazole is based on an anticholinesterase effect. This is also confirmed by the results of series II, in which experiments were conducted on the influence of imidazole on the sensitivity of action of the frog rectus abdominis muscle; the method of construction of cumulative concentration-versus-action curves was used to evaluate the cholinomimetic action [4]. This method, proposed for pharmacological investigations by Clark [9], was subsequently developed theoretically by Schild [10] and Ariens [8].



a



b

Fig. 1. Increase in the amplitude of single and tetanic contractions of the frog sartorius muscle under the influence of imidazole (a) and under the influence of the same preparation, but against a background of proserine (b).

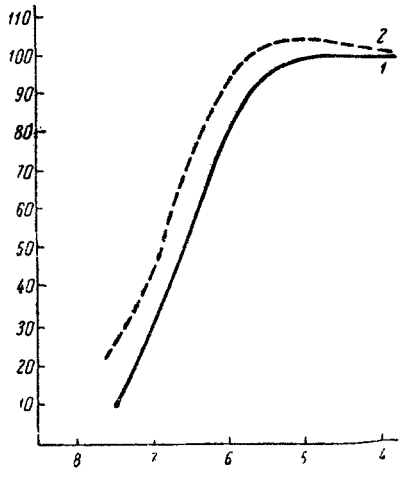


Fig. 2. Influence of imidazole on the sensitivity of the frog rectus abdominis muscle to acetylcholine. Ordinate) contraction (in % of acetylcholine maximum); abscissa) negative logarithm of the acetylcholine concentration. 1) acetylcholine curve; 2) acetylcholine curve against a back ground of imidazole influence.

Unseparated pairs of frog rectus abdominis muscles (10 preparations) were suspended in a bath and left for an hour in Ringer solution with proserine ($1 \cdot 10^{-6}$ M) with periodic replacement of this solution and continuous aeration. During this time, acetylcholinesterase was entirely inhibited—the effect of proserine has time to reach a maximum. AC solutions with increasing concentrations were prepared in Ringer solution containing proserine ($1 \cdot 10^{-6}$, M). At first a Ringer solution containing AC in a concentration of $3.2 \cdot 10^{-8}$ M was poured into the bath. The muscles began to contract immediately. After 15 min, the contraction of the muscles reached a maximum for the given AC concentration, and it was recorded, as usual, according to the height of the rise of the recording lever along a millimeter scale. Then, without washing, higher concentrations of AC were successively added to the bath: $1 \cdot 10^{-7}$, $3,2 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, $3,2 \cdot 10^{-6}$, $1 \cdot 10^{-5}$, $3,2 \cdot 10^{-5}$, and $1 \cdot 10^{-4}$ M, and the shortening of the muscle was recorded. After recording of the effect of the highest AC concentration, a 1.2% solution of KCl was added to the bath, and the maximum possible muscle contraction was registered.

The data obtained for each muscle (in millimeters) were converted to percent of this muscle, induced by AC. The potassium contracture was also expressed in percent of the maximum contracture induced by AC. Then averages were taken (in percent). The potassium maximum comprised 123% of the acetylcholine maximum. The data obtained were used to construct a concentration-versus-action curve, possessing an S-shape (Fig. 2). This curve served as a control.

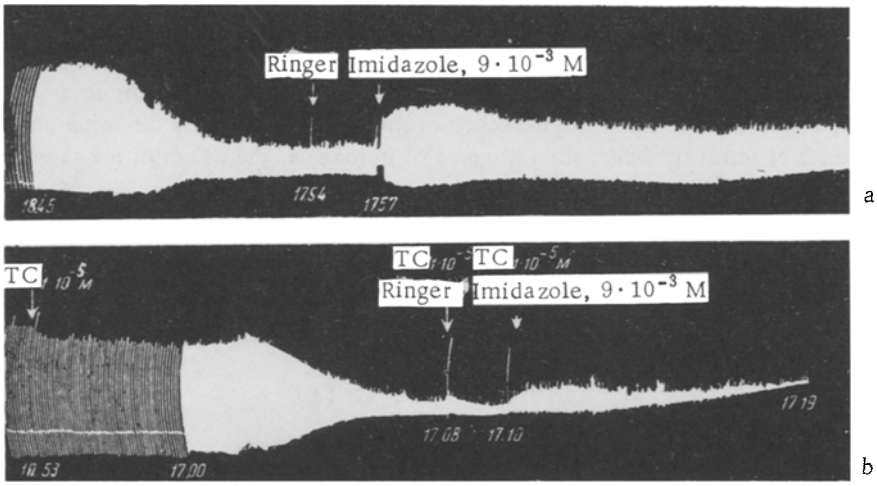


Fig. 3. Influence of imidazole on the ability to work of the frog sartorius muscle during fatigue under conditions of indirect (a) and direct (b) single stimulation.

Another ten muscles were used to obtain data reflecting the contracture of frog rectus abdominis muscles in response to the action of AC in the presence of imidazole. For the most part, the experiment was conducted just like the control. The only difference lay in the fact that the Ringer solutions contained not only proserine ($1 \cdot 10^{-}$ M) and AC in the same increasing concentrations, but also imidazole (9 mM).

The results obtained were converted to percent of the acetylcholine maximum, on the basis of the potassium maximum equal to 123%, and plotted on a graph to obtain a curve (see Fig. 2). As can be seen from the data cited in Fig. 2, the curve reflecting the influence of imidazole is shifted toward lower AC concentrations. This means that in the presence of imidazole, smaller concentrations of AC are required for a response contraction of the rectus abdominis muscle of the same height. These data definitively confirm the fact that the effect of imidazole cannot be explained by an anticholinesterase action. Moreover, it is difficult to believe that imidazole acts presynaptically, i.e., increases the yield of AC. It is quite obvious that imidazole exerts a postsynaptic action. However, this does not exclude its direct influence on the membrane or contractile structures of the muscle.

From the stand point of the idea of activation of the contractile structures, it might have been expected that imidazole would exert an influence on the muscle during direct stimulation. In series III, we compared the influence of imidazole on the fatigue curve of sartorius muscles during frequent (one impulse per second) single indirect and direct stimulation. Symmetrical muscles were used in the experiments. One muscle was first subjected to an infrequent (one impulse per 8 sec) indirect stimulation, then to frequent stimulation, and the fatigue curve was obtained. The Ringer solution was decanted, and a solution containing imidazole was poured in. A stable increase in the ability to work of the muscle was observed (Fig. 3a). Another muscle was subjected to direct infrequent stimulation, then D-tubocurarin added in a concentration of $1 \cdot 10^{-5}$ M to block the receptors. This concentration was established in special experiments conducted before hand; it was at least twice as great as the concentration of D-tubocurarin, the blocking effect of which was removed by 9 mM imidazole during indirect stimulation; hence, the influence of imidazole on the receptor was excluded in this series of experiments in the case of direct stimulation.

D-Tubocurarin, added to the muscle in the case of direct stimulation, somewhat reduced the amplitude of the contraction. This indicated that the receptors were blocked. Stimulation was switched to frequent; the fatigue curve was obtained, and imidazole was added against this background. Under the influence of imidazole, in 3 out of 5 experiments we obtained a small increase in the amplitude of the muscle contraction during direct stimulation (Fig. 3b). In the case of indirect stimulation, in all experiments a significant increase in the ability to work of the muscle was noted. These data indicate that in spite of the fact that imidazole has a direct effect upon the muscle tissue, it evidently acts chiefly through the synaptic region.

In supplement to these experiments, we conducted the experiments of series IV. The contractile activity of the frog sartorius muscle and rectus abdominis muscle was induced by guanidine. Guanidine is believed to disturb the relationship of AC with the protein to which it is bound [2, 3], as a result of which AC passes out into the

postsynaptic region in large quantities. As a result of this, indirect stimulation is simulated. The muscles were placed in a bath with aerated Ringer solution, and after an hour subjected to the influence of guanidine in a 1:5,000-10,000 dilution. The contractions were recorded for a period of 30 min, and then imidazole was added, which intensified the contractile activity of the muscles, and moreover a small contracture arose in all the experiments on the rectus abdominis muscle under the influence of imidazole. Evidently the same phenomena occurred here as in the experiments of series II: under the influence of imidazole, the effect of the same AC concentration was intensified.

D-Tubocurarin blocked the contractile activity induced by guanidine, while imidazole entirely removed this block. Thus, the data obtained once again confirm the fact that the influence of imidazole consists of the creation of favorable conditions for the transmission of impulses from nerve to muscle and partially of activation of the contractile elements of the muscles.

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