THE ROLE OF IMIDAZOLE AND ITS NATURAL COMPOUNDS IN THE FUNCTIONAL ACTIVITY OF THE MUSCLES

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Imidazole is widely represented in the organism in the form of the amino acid histidine, free and bound in the protein molecule, and in the form of dipeptides: carnosine and anserine, which are characteristic components of the skeletal muscles of vertebrate animals. It is known that carnosine increases the ability of the muscle to work under a single indirect stimulus [1, 3, 4] and restores the ability to work of a nerve-muscle preparation that has worked to exhaustion in a system of short tetanic contractions under indirect stimulation [4, 6] and after blockage of the transmission of a stimulus from nerve to muscle, caused by diplacin [6].

This communication is a continuation of these investigations and was devoted to elucidating the role of imidazole in the functional activity of the muscles.

PROCEDURE

In the work we used a procedure described earlier [6]. To compare the influence of imidazole or histadine with carnosine, these substances were introduced into the solutions surrounding the muscles, in equimolar concentrations

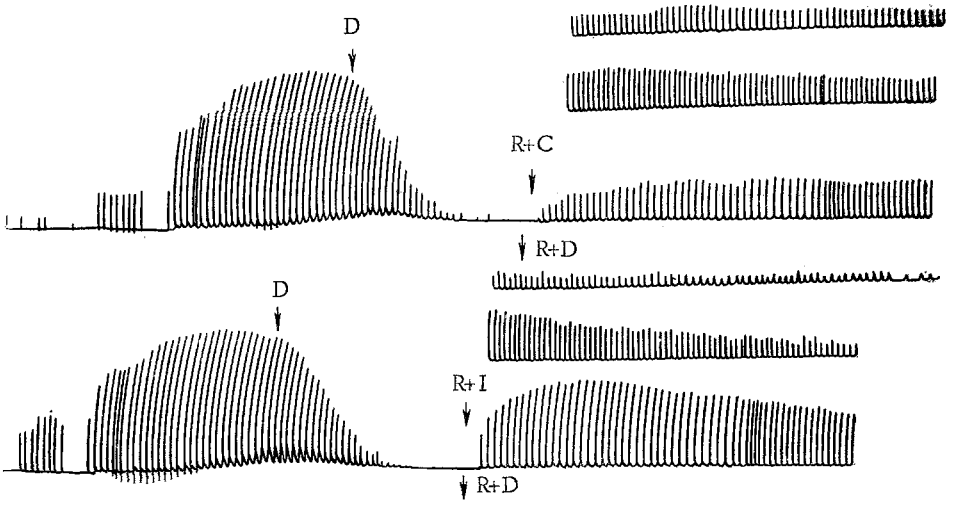

Fig. 1. Restoration of the ability to work of nerve-muscle preparations by carnosine and imidazole after a block induced by diplacin under conditions of indirect stimulation by short tetanic contractions. ↓ D) Moment of addition of diplacin to the solution surrounding the muscle; ↓ R + D below the curve—moment when Ringer's solution with diplacin was poured in; ↓R + C and ↓R + I—moment when solution containing carnosine or imidazole, respectively, was poured in.

TABLE 1. Characteristics of Indirect Excitability of Frog Nerve-Muscle Preparations Before and After Work in a System of Short Tetanic Contractions

Expt. no.	Composition of solution surrounding the working muscle	Before work		After work	
		pH	threshold (in mV)	pH	threshold (in mV)
1	Ringer's solution + carnosine	7,8	30	7,75	40
	" " +imidazole	7,8	30	7,7	35
2	" " + carnosine	7,8	15	7,7	20
	" " + histidine	7,8	15	7,7	35
3	" " + carnosine	7,8	40	7,75	40
	" " + imidazole	7,8	30	7,7	20
4	" "	7,8	10	7,78	Above 1000
	" " + imidazole	7,8	10	7,7	35

TABLE 2. Characteristics of Indirect Excitability of Frog Nerve-Muscle Preparations Before and After Work in a System of Short Tetanic Contractions During Removal of Diplacin Block

Expt. no.	Composition of solution surrounding the working muscle	Before work		After work	
		pH	threshold (in mV)	pH	threshold (in mV)
1	Ringer's solution+carnosine	7,8	20	7,8	40
	" " +imidazole	7,8	20	7,78	35
2	" " +histidine	7,8	30	7,8	70
	" " +imidazole	7,8	30	7,75	40
3	" " +histidine	7,8	35	7,8	90
	" "	7,8	35	7,5	250
4	" " +imidazole	7,8	40	7,75	40
		7,8	40	7,75	200

of 9 mM. The hydrogen ion concentration of the Ringer's solution was monitored potentiometrically. In both variations of experimental series I, symmetrical frog sartorius muscles (from Rana temporaria) worked in a system of short tetanic contractions. In the first variation, the solutions surrounding the muscles were replaced at the bottom of the exhaustion curve. One of the muscles was placed in Ringer's solution with carnosine, the other in Ringer's solution with imidazole or histidine. The muscles continued to work for another 20-40 min. In the second variation, symmetrical sartorius muscles functioned for 1.5-2.5 min, after which diplacin was added to the solution in a concentration of $9 \cdot 10^{-5}$ M. The amplitude of the muscle contraction began to drop immediately after the addition of diplacin; a complete block developed after 2.5-3 min. Then the Ringer's solution containing diplacin was decanted, and a Ringer's solution with carnosine was poured over one of the muscles, a solution with imidazole or histadine over the other.

The experiments of series II were aimed at restoring the ability of the muscles to work under an indirect stimulation by single pulses (one pulse per sec). Symmetrical frog sartorius muscles worked for 1.5-2 min. Then diplacin was added to the solutions surrounding the muscles in a final concentration of $5 \cdot 10^{-6}$ - $1 \cdot 10^{-5}$ M. After the development of a total block, fresh Ringer's solution containing diplacin in the same concentration required for the development of a complete block was poured over one of the muscles; fresh Ringer's solution with diplacin and imidazole (carnosine or histadine) was added to the other. The muscles were subjected to stimulation for another 30-40 min. Certain experiments of this series ended in a biochemical analysis according to the procedures described earlier [6].

RESULTS AND DISCUSSION

The experiments of series I showed that imidazole and histidine, just like carnosine, are capable of restoring the ability of the neuromuscular preparation to work after its exhaustion and after a block produced by diplacin (Fig. 1). However, immediately after addition to the solution, imidazole gave a more substantial increase in the amplitude of muscle contraction than carnosine or histidine, the increase then being replaced by a gradual decrease in the amplitude of tetanic contractions in the worst possible form. In Ringer's solution containing carnosine and histidine, the muscles worked uniformly, and only in some experiments did the tetanus have a tendency to pass from the plateau shape to the clonic form. Nonetheless, the indirect excitability was preserved in the work of muscles both in Ringer's solutions containing carnosine and histidine and in solutions with imidazole (Tables 1 and 2), while prolonged work in Ringer's solution was accompanied by a sharp increase in the threshold of excitability [6].

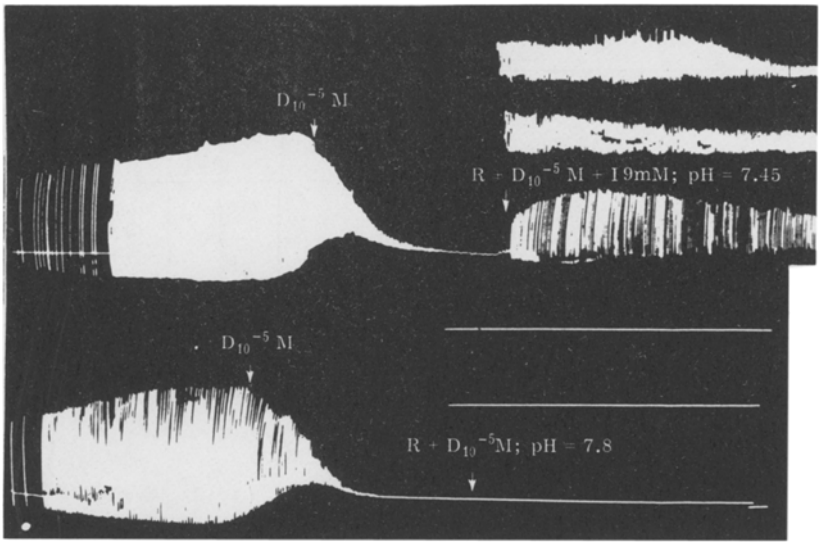

Fig. 2. Removal by imidazole of a block induced by diplacin under conditions of a single indirect stimulus. ↓ D_{10}^{-5} M—moment of addition of diplacin to the solution surrounding the muscle in a final concentration of 10^{-5} M; ↓ R + D_{10}^{-5} + I 9 mM above the curve—moment of addition of Ringer's solution containing diplacin and imidazole to the muscle.

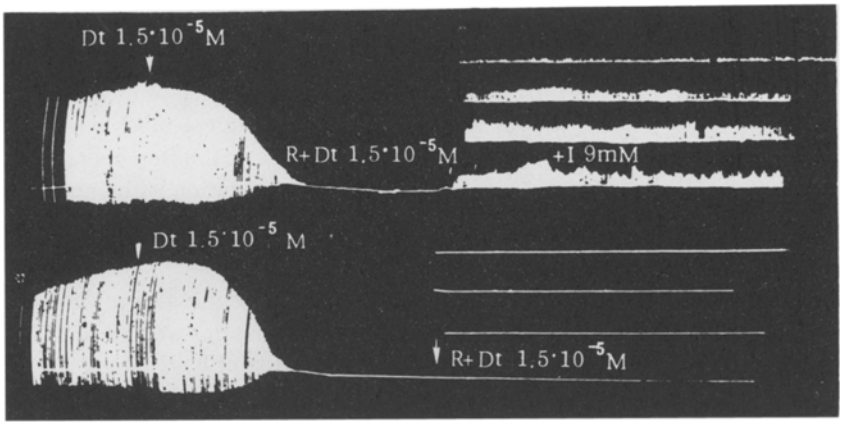

Fig. 3. Removal by imidazole of a block induced by ditilin under conditions of a single indirect stimulus. ↓ Dt 1.5 · 10^{-5} M—moment of addition of ditilin to the solution surrounding the muscle in a concentration of 1.5 · 10^{-5} M; ↓ R + Dt 1.5 · 10^{-5} M + I 9 mM above curve—moment of addition of Ringer's solution containing ditilin and imidazole to the muscle.

The appearance of the worst possible form of tetanus may be explained by the accumulation of AC * in the postsynaptic membrane of the myoneural region of the muscle [2]. Probably imidazole compounds promote this (imidazole to a greater degree, carnosine and histidine to a lesser degree) by blocking ACE or by more active liberation of AC by the nerve endings. The latter circumstance is extremely probable, since it is known that imidazole, and perhaps carnosine as well may stimulate AC biosynthesis under definite conditions [5]. Diplacin as a competitive

* AC—acetylcholine; ACE—acetylcholinesterase; ATP—adenosine triphosphate; P_{pc}—phosphocreatine phosphorus; P_{inorg}—inorganic phosphate.

TABLE 3. Content of Lactic Acid, ATP, P_{pc}, and P_{inorg} in the Muscles and Lactic Acid and P_{inorg} in the Surrounding Solutions After Work Under a Single Indirect Stimulus Under Conditions of Removal of Diplacin Block by Imidazole

Conditions of experiment	Diplacin conc. (in M)	Lactic acid (in mg%)				ATP	P_{pc}	P_{inorg}			
		Muscle	1st solution	2nd solution	Total			muscle	1st solution	2nd solution	Total
Ringer's solution+imidazole	5×10^{-6}	259	178	177	614	65,8	12,5	58,3	31,3	20,2	109,8
Ringer's solution	5×10^{-6}	209	148	98	455	71,4	17,4	49,5	30,8	19,2	99,5
Ringer's solution+imidazole	1×10^{-5}	224	181	157	562	77,3	13,4	51,1	40,2	21,2	112,5
Ringer's solution	1×10^{-5}	166	160	96	422	85,2	15,5	36,5	38,4	19,2	94,1

Note. The column "first solution" gives the amount of lactic acid that passed into the solution surrounding the muscle during work before replacement of the Ringer's solutions; the column "second solution" gives the amount of lactic acid that passed into the solution surrounding the muscle after the replacement of the solutions, during work of the control muscle in Ringer's solution and the experimental muscle in Ringer's solution with imidazole.

inhibitor is displaced by excess AC. The flareup of ability to work, which we observed when imidazole was added to the solution surrounding a muscle blocked by diplacin, may be explained on the basis of these hypotheses. However, in the presence of excess AC, the tetanus is converted to the clonic form, and this phenomenon obscures the process of restoration of ability to work in the tetanic form of stimulation, but it should not be present in a single stimulation.

In the experiments of series II (Fig. 2), immediately after the solution containing imidazole was poured in, there was a restoration of the functional ability of the nerve-muscle preparation. For 30-40 min, the amplitude of the contraction was even and reached approximately 40% of the original amplitude. In a control muscle, the block of the transmission of excitation was entirely retained. Carnosine and histidine also restored the ability of the muscles to work, but to a lesser degree in comparison with imidazole; the latter pertains especially to histidine. The effect of imidazole compounds in this form of the experiment may be quite justifiably called the removal of a competitive block, since the restoration of the ability of the muscles to work occurred against a background of the same diplacin concentration that produced the total block of neuromuscular conduction.

The data of a biochemical analysis showed (Table 3) that restoration of the ability of the muscles to work was accompanied by a great accumulation of lactic acid and P_{inorg} and a sharper reduction of the ATP and P_{pc} content in the muscle working in Ringer's solution with imidazole than in the control. However, the somewhat lowered level of high-energy compounds did not permit normal work of the muscles in Ringer's solution with imidazole. These data agree with the data of a biochemical analysis of muscles that have worked after exhaustion induced by frequent single stimuli, in a solution with carnosine [4], and once again confirm that the intensification of glycolysis in a working muscle cannot be considered the cause of the effect both of carnosine and of imidazole.

To verify the hypothesis of an increase in the AC concentration in the postsynaptic membrane under the influence of imidazole, we conducted experiments according to the scheme used in series II, the only difference being that the block was created by an equally effective concentration of ditilin —a depolarizing substance which acts similarly to an excess of AC. The concentrations of diplacin and ditilin that produced a total block of myoneural conduction on symmetrical frog sartorius muscles in the same time from the moment of addition to the surrounding solution were considered equally effective. For various muscles, such ditilin concentrations were $9 \cdot 10^{-6}$ - $1.5 \cdot 10^{-5}$M. As can be seen from Fig. 3, imidazole removed the block induced by ditilin . This fact casts doubts upon the hypothesis of the influence of imidazole on the increase in the AC concentration in the post-synaptic membrane. However, imidazole evidently is more capable of removing a competitive block.

Further investigations will be needed for a comparison of the ability of imidazole compounds to remove competitive and depolarization blocks and for a disclosure of the localization of their influence on the nerve-muscle preparation.

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