EFFECT OF DILTIAZEM AND TETRODOTOXIN ON DEVELOPMENT OF ANOXIC CONTRACTURE OF THE GUINEA PIG MYOCARDIUM

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It has been shown on the mammalian heart that contracture of myocardial fibers develops in anoxia or ischemia [4, 7-9]. One possible cause of anoxic contracture is accumulation of calcium ions in the cytoplasm of the myocardial cells [5, 8]. The main systems controlling the entry of calcium ions through the sarcolemma are the calcium channels and Na^+–Ca^{++} exchange, activity of which depends on the intracellular Na^+ concentration [1, 2].

To study the role of inward calcium and sodium currents in the development of anoxic contracture of the myocardial fibers, the action of diltiazem (a calcium channel blocker) and tetrodotoxin (a sodium channel blocker) on the development of anoxic contracture of the guinea pig myocardium was investigated during repetitive stimulation and under resting conditions (when the ionic channels of the sarcolemma are not activated).

EXPERIMENTAL METHODS

Experiments were carried out on papillary muscles of the right ventricle of male guinea pigs (350-500 g). The physiological saline used had the following composition: NaCl – 144 mM; KCl – 4.0 mM; $CaCl_2$ – 1.8 mM; $MgCl_2$ – 1 mM; Tris-HCl – 10 mM, pH 7.3-7.4, temperature 36°C. The preparation, placed in a perfusion chamber (1 ml), was stimulated by an electric field through two Ag-AgCl electrodes. All tests were carried out at a frequency of 1 Hz and the stimulus intensity was 3-4 times higher than the threshold. Mechanical activity of a papillary muscle was recorded under isometric conditions with a 6MKh2B mechanotron and N3021-4 automatic writer. Anoxic solutions were prepared by displacing oxygen with nitrogen. The value of pO_2, measured in the anoxic solution, was 13-15 mm Hg, compared with 560-580 mm Hg in oxygenated physiological saline. Measurements were made with an oxygen-sensitive electrode (Hellige). Diltiazem was obtained from Sigma (USA) and tetrodotoxin from Sankyo (Japan). The experimental investigations were conducted as follows: after perfusion of the papillary muscle for 1 h with initial physiological saline, the test substance (diltiazem or tetrodotoxin) was added to it and perfusion continued for another 20 min. Next, the preparation was perfused for 90 min with the anoxic solution together with the test substance. The magnitude of the anoxic contracture was expressed relative to the force of isometric contraction under normal conditions [7].

EXPERIMENTAL RESULTS

Perfusion of the repetitively stimulated muscle with anoxic solution, as the mean results of 7 experiments showed, caused the appearance of contracture after 7 ± 1.2 min; it reached its maximum after 29.2 ± 4.6 min (Fig. 1, curve 1).

Anoxic contracture of the resting muscle developed much more slowly: it appeared after 16.2 ± 1.6 min and reached a maximum after 58.3 ± 6.8 min (n = 6), measured from the beginning of perfusion with the anoxic solution. The maximal amplitude of anoxic contracture under these conditions, i.e., when excitation and contraction were ruled out, was on average 51% less than that of the stimulated muscle (Fig. 1, curve 2). Consequently, it can be tentatively suggested that about 50% of the developed anoxic contracture depends on the entry

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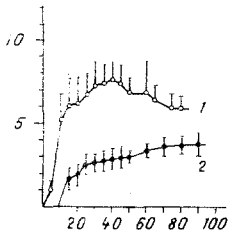

Fig. 1

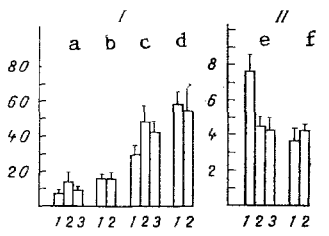

Fig. 2

Fig. 1. Effect of anoxia on development of contracture of guinea pig papillary muscle. 1) Repetitive stimulation; 2) at rest. Abscissa) time of perfusion with anoxic solution, min; ordinate) magnitude of contracture, expressed relative to force of isometric contraction under normal conditions ($p = 0.95$).

Fig. 2. Effect of diltiazem and tetrodotoxin on development and magnitude of anoxic contracture of guinea pig papillary muscle. I) Time of beginning of anoxic contracture and of reaching its maximum; II) magnitude of anoxic contracture; a) time of beginning of contracture during stimulation, b) the same, at rest, c) time when contracture reached maximum during stimulation, d) the same, at rest, e) maximal contracture during repetitive stimulation, f) the same, at rest. Abscissa: 1) anoxic solution, 2) anoxic solution with 10^{-6} M diltiazem, 3) anoxic solution with $3 \cdot 10^{-6}$ M tetrodotoxin; ordinate: in I) time of beginning of anoxic contracture and of reaching its maximum, min, in II) magnitude of contracture, relative to force of isometric contraction normally.

of Na^+ and Ca^{++} during depolarization of the myocardial cells and on the contraction process utilizing energy of ATP hydrolysis.

During repetitive stimulation of the myocardium diltiazen (10^{-6} M) reduced the amplitude of anoxic contracture (on average by 41%) and delayed its development: contracture began after 14.2 ± 4.7 min and reached its maximum after 48 ± 9.4 min (mean results of five experiments; Fig. 2, I, II).

In the presence of stimulation, diltiazem had no significant effect on the development of anoxic contracture or on its peak value (Fig. 2, I, II).

Thus diltiazem reduces anoxic contracture and delays its development only in the presence of repetitive myocardial stimulation. Its action may perhaps be due to blocking of the inward calcium current, and not to its membrane-stabilizing effect [6].

One of the systems which removes calcium from cells is the Na^+–Ca^{++} exchange system, operating by the Na^+ concentration gradient in the cytoplasm and external solution. The intracellular Na^+ concentration depends on the energy-dependent Na^+–K^+ pump of the sarcolemma and the inflow of Na^+ along sodium channels. Under anoxic conditions, due to exhaustion of the energy reserves of the myocardium, activity of the Na^+–K^+ pump is reduced and the Na^+ concentration in the cytoplasm increased [1-3]; this leads to a fall of the Na^+ concentration gradient and to accumulation of Ca^{++} in the cytoplasm.

It is to be expected that reduction of the Na^+ inflow by blocking of the sodium channels of the sarcolemma should reduce the amplitude of anoxic contracture of the myocardial fibers.

According to the average results of five experiments, tetrodotoxin ($3 \cdot 10^{-6}$ M) administered during repetitive stimulation reduces the maximal amplitude of the contracture by 43% and re-

duces the rate of its development: contracture began after 10 ± 1 min and reached its maximum after 42 ± 4.6 min (Fig. 2, I, II).

The results showed that not only Ca^{++} ions, entering along calcium channels, but also Na^{+} ions, entering along the fast sodium channels of the sarcolemma during membrane depolarization, play an essential role in the development of anoxic contracture of the guinea pig myocardium. Consequently, anoxic damage to the myocardium may be reduced by the use not only of calcium antagonists, but also of agents reducing the inward sodium current, such as Class 1 antiarrhythmics.

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