EFFECT OF STRETCHING ON VENTRICULAR MYOCARDIAL ACTION POTENTIALS IN MAN AND RABBIT

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It was shown previously [1] that the action potentials (AP) of cardiomyocytes behave differently during cyclic stretching of the isolated atrial myocardium of patients with heart defects at a constant rate, depending on the initial characteristics. If the AP had the character of slow responses, their configuration and amplitude changed sharply even as a result of slight stretching. Conversely, no changes in AP were observed during stretching of the myocardium if they had a rapid depolarization phase.

The aim of this investigation was to study the initial sensitivity of the ventricular myocardial AP of patients with heart defects to stretching and changes in their sensitivity under the influence of ethmozine* and ethacizine†. For this purpose the electrical response was first investigated to stretching of the patients' ventricular myocardium in which normal AP were recorded, and also the ventricular myocardium in which the AP were recorded, and also the ventricular myocardium in which the AP were of the slow response type. Second, correlation between stretching and AP was studied in the myocardium of patients with outwardly normal AP before and after treatment with ethmozine or E-DAA, which delay the depolarization phase of AP [2, 3]. Third, the response to stretching of AP in the rabbit papillary muscle was studied before and after the action of these same antiarrhythmics.

EXPERIMENTAL METHOD

Preparations obtained from human papillary muscles excised during operations on the heart were used. These preparations consisted of a strip of endocardial tissue 8-10 mm long and with a cross section of 1-1.2 mm^2. Investigations also were conducted on rabbit papillary muscles excised from the right ventricle. The preparations were fixed in a 5-ml Plexiglas bath so that one end was rigidly connected to a mechanotron force transducer, the other to the lever of the stretching system. The bath was perfused with carbogen-aerated solution of the following composition (in mM): NaCl — 137, $NaHCO_3$ — 10, NaH_2PO_4 — 0.5, Na_2HPO_4 — 1.5, $MgCl_2$ — 1.25, KCl — 4.5, $CaCl_2$ — 5, glucose — 10 (pH 7.2-7.3), temperature 33-35°C. Ethmozine and E-DAA were added to the solution in amounts required to create a final acting concentration of $2 \cdot 10^{-5}$-$2 \cdot 10^{-6}$ M. The preparations were stimulated by above-threshold pulses through massive platinum electrodes (field stimulation) with a frequency of 0.3 Hz. The muscle was stretched continuously at a speed of 0.03 mm/sec from L_o to L_{max}, where L_o denotes the length at which the passive and actively developed tension were close to zero, and L_{max} the length at which the actively developed tension of the muscle was maximal. Under cyclic deformation conditions the muscle was stretched to L_{max} and its length returned at the same rate to L_o. Electrical activity was recorded by means of glass microelectrodes, filled with 3 M KCl solution. The method of fixation of the floating microelectrode ensured reliable recording during cyclic deformation of the muscle. Altogether 10 human and 9 rabbit papillary muscles were tested.

*2-carbethoxyamino-10-(3-morpholylpropionyl)-phenothiazine HCl
†ethmozine diethylamine analog (E-DAA)

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TABLE 1. Effect of Stretching on Amplitude of AP (A), Time Taken to Reach Maximum (TRM), and Repolarization Time at the 50% of Maximal Amplitude Level (RT_{50}) in Myocardial Preparations from Patients with Heart Defects and in Rabbit Papillary Muscle (M ± m)

AP	Ethmozine, $2 \cdot 10^{-5}$ M, E-DAA, $2 \cdot 10^{-6}$ M	$\frac{A_{L_{max}}}{A_{L_0}}$	$TRM_{L_{max}} / TRM_{L_0}$	$RT_{L_{max}} / RT_{L_0}$
Slow AP from patients with heart defects (n = 4)	–	0,74 ± 0,11	1,50 ± 0,27	0,83 ± 0,08
Normal AP of patients with heart defects (n = 8)	–	1,00	1,00	1,00
Normal AP of patients with heart defects (n = 8)	+	0,75 ± 0,08	1,78 ± 0,20	0,57 ± 0,09
AR of rabbit papillary muscle (n = 3)	+	0,80 ± 0,09	1,69 ± 0,35	0,57 ± 0,09

Legend. A_{L_0}, TRM_{L_0}, RT_{L_0}) parameters of AP recorded before beginning of deformation at L_0, length at which passive and actively developed tension are minimal; $A_{L_{max}}$, $TRM_{L_{max}}$, $RT_{L_{max}}$) parameters of AP recorded during stretching of myocardial preparation at time of reaching L_{max}, length at which active force is maximal. $P < 0.05$

EXPERIMENTAL RESULTS

In some myocardial preparations from patients, besides normal AP, it was also possible to record slow AP, which were sensitive to stretching from L_0 to L_{max}: their amplitude and duration decreased with an increase in length, and the time taken to reach a maximum increased. The relative values of the parameters of these slow AP of the patients' mycardium at different lengths are given in Table 1.

In most papillary muscle preparations from patients AP had parameters characteristic of the ventricular myocardium of warm-blooded animals. As the writers showed previously [1], normal atrial AP of patients are insensitive to deformation within the physiological range of stretching. It was also found that normal AP in the human ventricular myocardium change neither in magnitude nor in shape during stretching of the muscle from L_0 to 150% L_0. An AP recorded in the human papillary muscle, which remained unchanged during cyclic deformation of the myocardial preparation, is shown in Fig. 1a. The relative magnitudes of the parameters of these AP at different lengths are given in Table 1.

It can be seen in Fig. 1b, which shows AP from the same myocardial preparation after addition of ethmozine to the solution in a concentration of $2 \cdot 10^{-5}$ M, that the amplitude of AP fell and the rate of the depolarization phase was reduced. The duration of the AP, measured at the 50% of maximal level, was virtually constant. The fall in amplitude of AP and slowing of the depolarization phase are evidence of inhibition of activity of Na channels in the cardiomyocyte membrane [2, 3]. Ethmozine and E-DAA caused qualitatively identical changes in these parameters of AP, the only noteworthy feature being that when E-DAA was used this effect was reached at significantly lower concentrations of the drug. For instance, to obtain AP of the type illustrated in Fig. 1b, it was sufficient to use E-DAA in the concentration of $2 \cdot 10^{-6}$-10^{-6} M.

The behavior of AP after administration of the antiarrhythmics in response to stretching of the preparation differed sharply from the behavior of the normal AP. Superposition of AP recorded in one cell during stretching from L_0 to 150% L_0 (Fig. 1c) and during the return

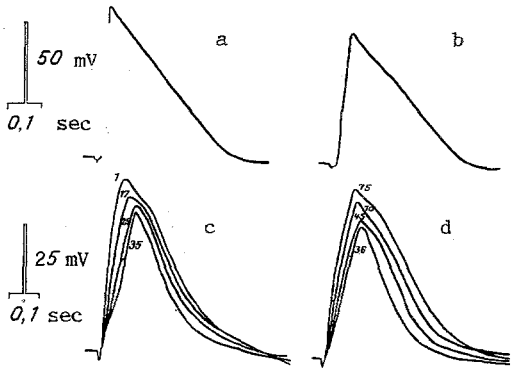

Fig. 1. AP in human papillary muscle. a) In original solution; b) under influence of ethmozine ($2 \cdot 10^{-5}$); c) superposition of AP recorded in one cell during stretching of myocardial preparation from L_o to L_{max} at the rate of 0.03 mm/sec; d) the same during return to initial length. Numbers indicate serial numbers of AP from beginning of deformation from L_o (1) to L_{max} (35) and return to L_0 (70). Explanation in text.

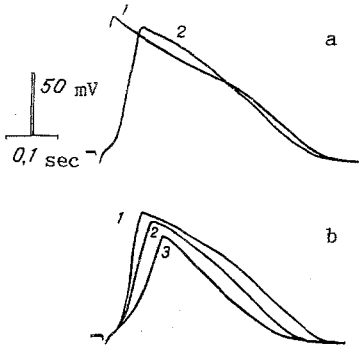

Fig. 2. AP in rabbit papillary muscle. a) In original solution (1) and during action of ethmozine in a dose of $2 \cdot 10^{-5}$ M (2); b) AP recorded at initial length of muscle L_o (1), at length of muscle equal to 135% L_o (2) and 150% L_o (3).

to the initial length (Fig. 1d), is shown graphically in Fig. 1, where the numbers denote the serial number of the AP after the beginning of deformation. These data show that with an increase in the degree of the deforming action, disturbances of electrogenesis became more marked. AP corresponding to L_{max} (No. 35) had low amplitude and a very slow depolarization phase. When the length of the preparation returned to L_o, the AP gradually recovered its shape (Fig. 1d). AP No. 70 differed a little from AP No. 1, although they both corresponded to the length L_o of the preparation. After the initial length L_o had been reached the deforming action was stopped, but evolution of the shape of AP still continued during several cycles of excitation. The 5th AP after removal of the deforming force (No. 75) had almost completed the recovery process: AP returned to its original shape, i.e., the return to normal AP generation was marked by some degree of hysteresis.

AP of the rabbit papillary muscle in the original solution and after addition of ethmozine ($2 \cdot 10^{-5}$ M) are illustrated in Fig. 2a. Changes arising in the magnitude and shape of AP under the influence of ethmozine were similar to those observed in the human papillary muscle.

Normal AP were insensitive to stretching of the preparation from L_o to L_{max}. However, electrical activity in the solution with ethmozine was disturbed and depended on the length of the muscle. Three AP recorded at the initial length of the muscle L_o and at lengths 135%

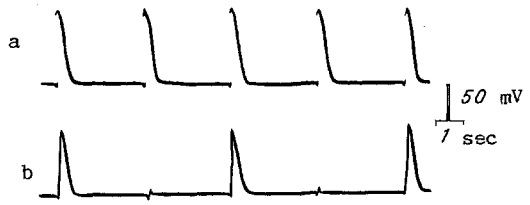

Fig. 3. Continuous recording of AP of rabbit papillary muscle during stretching, with length of preparation approximately L_{max}. a) In original solution; b) in solution containing ethmozine ($2 \cdot 10^{-5}$ M).

L_o and 150% L_o are given in Fig. 2b. The trend of recovery of AP was qualitatively indistinguishable from that in the human myocardium. Data on changes in AP parameters during stretching of the myocardial preparation from L_o to L_{max} in solutions with ethmozine ($2 \cdot 10^{-5}$ M) are given in Table 1.

Changes in AP shown in Fig. 2 were observed in experiments on 3 rabbit papillary muscles. In the remaining 6 preparations a change in length affected AP generation differently. Cuts of a continuous trace of AP in the rabbit myocardium, corresponding to the final stage of stretching, when the length of the muscle reached its maximal value, are shown in Fib. 3a, b. AP of the rabbit papillary muscle in the original solution, which were normal in shape, were insensitive to deformation of this kind (Fig. 3a). However, in solution containing one of the antiarrhythmics, the picture changes. Cardiomyocytes responded by AP generation not to every stimulating impulse. In cases when AP were formed, uniform changes could be observed in the parameters of AP, characteristic of the action of these antiarrhythmics (Fig. 3b).

Since ethmozine and E-DAA reduce the excitability of myocardial tissue a little, we increased the strength of the stimulating pulse. However, this caused only transient recovery of regular AP generation or equally transient and slight recovery of the shape of AP. Qualitative effects of stretching, however, remained unchanged. In some experiments, adrenalin ($5 \cdot 10^{-8}$ M) was added to increase excitability after the action of the phenothiazine and the effect of stretching on electrogenesis in the cardiomyocytes was observed to continue.

The results evidently indicate that the increase in sensitivity of AP to stretching under the influence of ethmozine or E-DAA is the result of blockade of the fast inward sodium current. This phenomenon can therefore be explained by an additional lowering of excitability of the myocardium during its stretching, including inhibition of the slow Ca channels. The fact that stretching itself can reduce excitability is confirmed by data obtained in [4]. One result of lowering of myocardial excitability after the combined action of phenothiazine and stretching may be prolonged postrepolarization refactoriness, which easily explains the absence of electrical response to the next stimulus (Fig. 3b). However the data given in Figs. 1 and 2 are difficult to explain from this standpoint. It therefore seems most likely that against the background of ethmozine and E-DAA, stretching also affects the Ca current.

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LITERATURE CITED

1. V. S. Markhasin and T. F. Shklyar, Byull. Éksp. Biol. Med., No. 3, 21 (1982).
2. A. I. Undrovinas, L. V. Rozenshtraukh, and A. V. Yushmanova, Byull. Eksp. Biol. Med., No. 10, 72 (1982).
3. I. A. Juravicius, L. V. Rozenshtraukh, A. I. Undrovinas, et al., Kardiologiya, No. 9, 118 (1978).
4. L. J. Penefsy and B. F. Hoffman, Am. J. Physiol., 204, 433 (1963).