Some Mechanisms of Antiarrhythmic Effect of Phosphoenolpyruvate

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Depolarization automaticity was modeled on the papillary muscle from the guinea pig heart. Phosphoenolpyruvate (0.1 mM) 2-fold decreased the high-frequency calcium-dependent automaticity, but only weakly affected frequency of action potentials, whose upstroke was formed by fast sodium current or had a mixed nature. Phosphoenolpyruvate shifted the diastolic potential toward negative values, which depended on the amplitude of depolarization step. These effects developed 10-15 min after application of the preparation.

Key Words: *phosphoenolpyruvate; depolarization automaticity; action potential*

Glycolysis intermediates produced pronounced antiischemic and antiarrhythmic effect in animals with acute coronary occlusion and reperfusion [1,8,9,11]. Taking into consideration the involvement of cell metabolism in the genesis of cardiac arrhythmias [5,12], the antiarrhythmic effect of these substances can be explained by prolongation of the glycolytic energy production in cardiomyocytes [3,6,7]. However, participation of common electrophysiological mechanisms [13] in the antiarrhythmic effect of glycolysis intermediates was not yet assessed.

Our aim was to study the effect of phosphoenolpyruvate (PEP) on electrical activity of isolated myocardial ventricles.

MATERIALS AND METHODS

The study was carried out on guinea pigs weighing 250-350 g. The papillary muscles were isolated from the right ventricle under nembutal narcosis. The muscle were mounted in a chamber (2 ml) and perfused with oxygenated Tyrode solution (35°C; pH 7.4) containing (in mM): 130 NaCl, 2.7 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 10 glucose, and 5 HEPES-NaOH.

Membrane potential was measured with 10-20-MΩ glass microelectrodes. The signal and its first derivative were recorded with an automatic differentiator (Micromed). The duration of action potentials (AP) corresponding to 50 and 90% repolarization was measured. The preparations were stimulated with the trains of rectangular pulses applied via bipolar silver electrodes. The duration and frequency of pulses in the train were 1 msec and 1 Hz, respectively.

The depolarization automaticity was modeled according to [2]. The rectangular direct current impulses were applied at a rate of 1 Hz via silver-chloride electrodes and an agar salt bridge from an ESU-2 electric stimulator (4.5-6.5 sec pulse duration). Membrane potential was recorded by the same method.

In this study we used PEP tricyclohexylammonium salt (Sigma).

RESULTS

Initially we evaluated the effect of PEP on some parameters of AP in persistently stimulated guinea pig papillary muscle. PEP (0.1 mM) produced a moderate, but significant decrease in AP duration by 11 and 7% at the 50 and 90% repolarization level, respectively. These changes developed 3-5 min after application of PEP in Tyrode solution and reached a steady-state level after 10-15 min. The PEP-induced shortening of AP became reversible only after 30-60-min washout. The test substance did not change resting potential, AP amplitude, and maximum depolarization rate.

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Then we studied the effect of PEP on depolarization automaticity of guinea pig papillary muscles. In control series, direct current steps (one rheobase unit) elicited single AP. More intense stimulation evoked repeated AP, whose shape and frequency depended on membrane depolarization. The current step stimulus of 5-6 rheobase units evoked typical high-frequency calcium responses with small upstroke rate and diastolic potential of about -60 mV [4].

In accordance with previous data, PEP significantly shortened the duration of the first (basic) potential. At threshold stimulus, it was 174±61 msec 15 min after application of PEP, while the initial duration was 211±78 msec. PEP did not change AP amplitude and the rate of rapid depolarization of the papillary muscles.

The effect of PEP on depolarization automaticity was analyzed in dependence on initial frequency of repeated responses evoked by a single step of direct current (Table 1).

In the first high-frequency group of papillary muscles, PEP significantly (by 50±9.9%) decreased the frequency of calcium AP evoked by direct current stimulation (Fig. 1). PEP did not affect the frequency of depolarization automaticity in other groups, where the upstroke was formed by fast sodium current or had a mixed genesis. A tendency toward enhanced automaticity in the low-frequency group seems to be of little importance, because in the whole organism the first-order pacemakers usually suppress their activity.

Another pronounced effect of PEP was a large shift of diastolic potential toward negative values, which depended on the amplitude value of depolarization step (Fig. 2). Hyperpolarization was minimal for

TABLE 1. Effect of PEP (47 mg/liter) on Depolarization Automaticity of Guinea Pig Papillary Muscle

No.	Number of experiments	Number of responses		Changes, %
		control	test	
1	10	7.9±0.3	3.8±0.8	50±9.9*
2	13	4.9±0.2	4.0±0.6	81±10
3	9	2.2±0.3	3.2±1.2	181±74

Note. *$p<0.05$ compared to the control value (Student's t test).

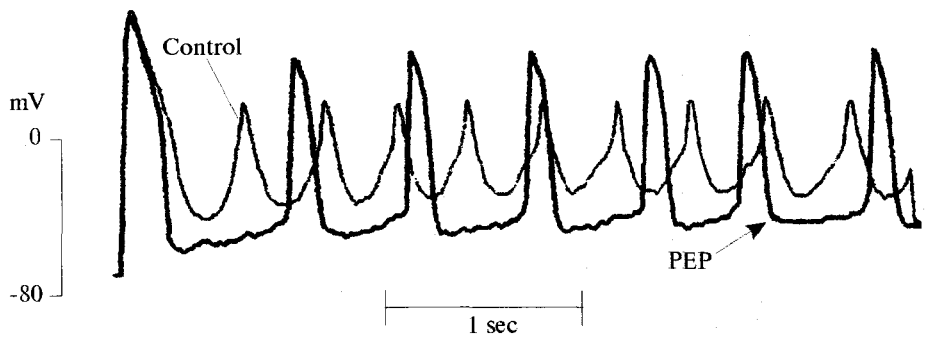

Fig. 1. Effect of phosphoenolpyruvate (PEP, 47 mg/liter) on the frequency of depolarization automaticity and maximum diastolic potential in guinea pig papillary muscles 10 min after the start of the experiment (6 rheobases current).

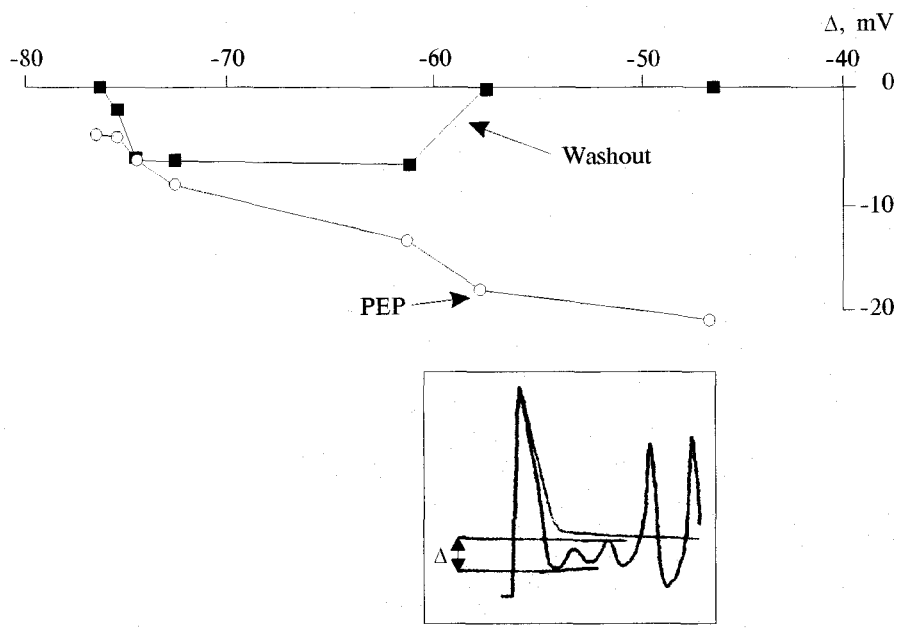

Fig. 2. Effect of phosphoenolpyruvate (PEP, 47 mg/liter) on the changes (Δ) in the maximum diastolic potential in guinea pig papillary muscles stimulated with direct current.

subthreshold and rheobase values of stimulating current and was 14±9 mV for the current, which evoked the calcium responses. It seems that PEP-induced shift in diastolic potential prevented optimal activation of calcium current, which is the basic determinant of the high-frequency responses. The described phenomena developed 10-15 min after application of PEP.

Inhibition of repetitive responses is characteristic of some conventional antiarrhythmic preparations [4]. However, in this case the inhibition results from direct blockade of ionic currents responsible for their generation. Therefore, the examined electrophysiological feature of antiarrhythmic effect of PEP seems to be original.

The mechanism of antiarrhythmic effect of PEP cannot be easily attributed to modulation of electrophysiological characteristics of intact myocardium. In contrast to conventional antiarrhythmic drugs, it does affect the upstroke and duration of AP. However, experiments on the model of depolarization automaticity can partially explain the effect of PEP capacity on cardiac rhythm disturbances. This model adequately reproduces the mechanisms of arrhythmogenesis at the boundary of intact and ischemic myocardium [10]. Therefore, the pronounced hyperpolarizing effect of PEP and consequently, the decrease in the frequency of the repetitive responses are probably important components of the antiarrhythmic effect of this substance.

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