EFFECT OF CREATINE PHOSPHATE ON ACTION POTENTIAL DURATION AND CONTRACTILITY OF THE FROG MYOCARDIUM IN HYPOXIA

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KEY WORDS: creatine phosphate; hypoxia; duration of action potentials; force of contraction; frog myocardium.

The fall in the creatine phosphate concentration in anoxia or ischemia runs parallel to the decrease in the force of contraction of the heart muscle [5, 6]. At the same time it has been shown [2, 3] that contraction and the calcium currents of the frog myocardium, when depressed by prolonged perfusion, exhaustion, or potassium cyanide, can be restored by the addition of creatine phosphate to the perfusion fluid. On this basis it has been suggested that creatine phosphate participates in the supplying of energy to the contractile system and to phosphorylation of the calcium channels of the sarcolemma.

It may accordingly be expected that contraction and calcium currents of the frog myocardium depressed by hypoxia [1] must also be restored after the addition of creatine phosphate to the perfusion fluid. The investigation described below was carried out to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on muscle strips of the frog ventricle 3-5 mm long, placed in a continuous-flow chamber with volume 3 ml. Mechanical activity of the strip was recorded by means of a 6M × 2B mechanotron and N-338-2 automatic writer. Square pulses 10 msec in duration with a frequency of 0.3 Hz were generated by an ESL-2 stimulator. The strength of the stimuli was 10 times above threshold, for hypoxia has been shown to increase the threshold amplitude of contraction considerably [7]. Transmembrane action potentials (APs) were recorded by glass microelectrodes on an FOR-2 camera from the screen of an S1-18 oscilloscope. The duration of the APs was measured at the half-amplitude level. The original physiological saline had the following composition (in mM): NaCl 110, KCl 2.5, $CaCl_2$ 1.08, Tris-HCl 10, glucose 5.5, pH 7.4-7.5. Calcium channels were blocked by means of nifedipine in a dose of 3.5×10^{-5} M [4]. (The necessary amount of nifedipine was dissolved in 0.5 ml ethyl alcohol and added to the physiological saline.) Calcium APs were obtained by adding 18 mM K^+ and 10^{-5} M adrenalin to the original solution. Hypoxic solutions were made up by displacing oxygen from the solutions with nitrogen. The residual oxygen concentration was measured with an oxygen-sensitive electrode (from Radiometer, Denmark), using the PU-1 polarograph. (It amounted to 3-10% of the original oxygen concentration in the solution.) Creatine phosphate was added in concentrations of 1, 11, 22, and 44 mM (from Reanal, Hungary). Since the disodium salt of creatine phosphate was used, the sodium ion concentration in the physiological saline was balanced to correspond to the added concentration of creatine phosphate. The calcium ion concentration in the physiological saline containing different concentrations of creatine phosphate was measured with a Ca-selective electrode (from Radiometer). The measurements showed that with an increase in the creatine phosphate concentration, the calcium ion concentration fell. For instance, when the creatine phosphate concentration was 1 mM, concentration of calcium ions was 1.0 mM, with a concentration of 11 mM, concentration was 0.79 ± 0.13 mM; with 22 mM it was 0.59 ± 0.10 mM, and with 44 mM it was 0.36 ± 0.10 mM. Accordingly the calcium ion concentration in the solutions with creatine phosphate was corrected. All experiments were carried out at room temperature (18-24°C). The results are shown in graphic form and values are expressed as percentages of the normal (P = 0.95).

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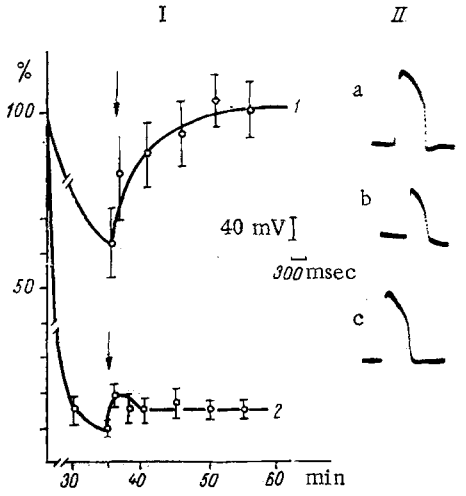

Fig. 1

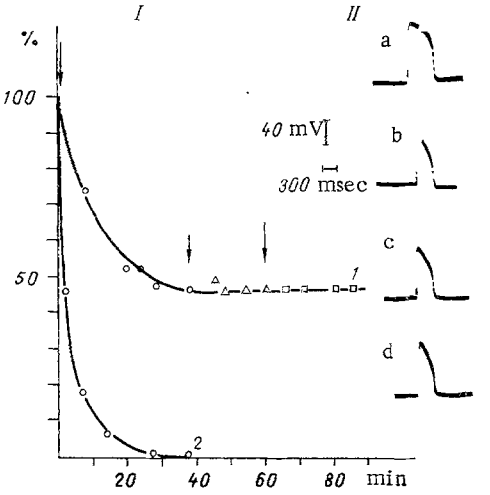

Fig. 2

Fig. 1. Effect of creatine phosphate on AP duration and force of contraction of muscle strip of frog ventricle in hypoxia. I) Change in AP duration (1) and force of contraction (2) during perfusion with hypoxic Ringer's solution containing 22 mM creatine phosphate (averaged results of five experiments); II) traces of AP: a) under normal conditions, b) after exposure to hypoxia for 35 min, c) after exposure to hypoxia for 15 min and addition of 22 mM creatine phosphate. Arrow indicates replacement of hypoxic Ringer's solution containing 22 mM creatine phosphate. Abscissa, duration of perfusion (in min); ordinate, change in AP duration and force of contraction (in % of initial level, taken as 100).

Fig. 2. Effect of creatine phosphate on AP duration and force of contraction of muscle strip of frog ventricle in hypoxia (after preliminary blocking of calcium channels with nifedipine). I) Change in AP duration (1) and force of contraction (2) during perfusion with Ringer's physiological saline containing 3.5×10^{-5} M nifedipine (circles), with hypoxic Ringer's solution without nifedipine (triangles), and with hypoxic solution containing 22 mM creatine phosphate (squares); II) traces of AP: a) under normal conditions, b) after perfusion for 30 min with nifedipine, c) after exposure to hypoxia for 20 min and addition of 22 mM creatine phosphate. Remainder of legend as to Fig. 1. [No reference to (d) in Russian original — Consultants Bureau.]

EXPERIMENTAL RESULTS

The writers showed previously that after perfusion of a muscle strip of frog ventricle for 40 min with hypoxic Ringer's solution the AP duration falls by 37 ± 10% and the force of contraction at this time is completely suppressed [1]. Addition of 1 mM creatine phosphate to the hypoxic solution after exposure to hypoxia for 35 min did not affect the duration of AP or the force of contraction in any of the three experiments; creatine phosphate in a dose of 11 mM (results of five experiments) restored the AP duration after 10-15 min to its original value but had virtually no effect on the force of contraction. The kinetics of the AP duration and force of contraction after addition of 22 mM creatine phosphate to the hypoxic solution (averaged results of four experiments) is shown in Fig. 1. As Fig. 1 shows, this concentration of creatine phosphate acting for 15 min fully restored the AP duration. The force of contraction increased during the first 2 min by 9.7 ± 3%, but after 5 min this increase slowed down and the force of contraction became stabilized at a level 6.6 ± 1.8% higher than in hypoxia. Examples of experimental traces of AP are given in Fig. 1, II.

An example of the kinetics of the AP duration and force of contraction following addition of 22 mM creatine phosphate and exposure to hypoxia for 40 min, preceded by blocking of the calcium channels (with VAU-1040, nifedipine) in a dose of 3.5×10^{-5} M [4] is given in Fig. 2. As Fig. 2 shows, 22 mM creatine phosphate under these conditions did not restore the AP duration or force of contraction. Examples of experimental traces of AP under these condi-

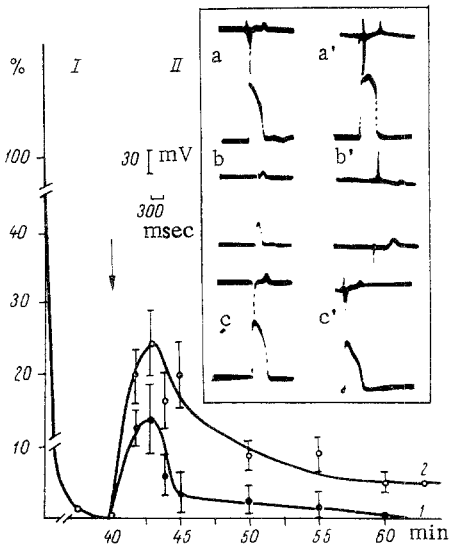

Fig. 3. Effect of creatine phosphate on force of contraction of muscle strip of frog ventricle in hypoxia (with sodium current inactivated and calcium current activated). I) Change in force of contraction during perfusion with hypoxic solution containing creatine phosphate in doses of 11 (1) and 22 mM (2); II) traces of calcium APs and their first derivative: a, a') under normal conditions, b, b') after exposure to hypoxia for 20 min, c, c') after treatment with creatine phosphate in doses of 11 and 22 mM respectively and exposure to hypoxia for 5 min. Top curves) first derivative of calcium potentials, bottom curves) calcium APs.

tions are given in Fig. 2, II. The results obtained in all three experiments were similar. These results are evidence that creatine phosphate, under hypoxic conditions, restores the AP duration through activation of calcium channels. If this is so, then creatine phosphate ought to restore calcium APs when suppressed by hypoxia. The kinetics of the force of contraction during hypoxia on addition of creatine phosphate in doses of 11 mM (averaged data of four experiments) and 22 mM (averaged data of six experiments) is illustrated in Fig. 3, and curves of calcium potentials and their first derivatives are shown (Fig. 3, II). The results demonstrate that creatine phosphate restores calcium APs completely. With the concentrations of creatine phosphate mentioned and under hypoxic conditions only a brief and small increase in the force of contraction was observed (Fig. 3, I). It was expected that an increase in the creatine phosphate concentration to 44 mM in the hypoxic solution would restore the force of contraction by a greater degree. However, the results of the three experiments showed that the action of this concentration of creatine phosphate on the force of contraction was weaker than when used in a dose of 11 mM, i.e., after a temporary increase by 11.2 ± 3% during the first minute of action of creatine phosphate, the force of contraction fell to zero in the course of the next 10-15 min.

The results of this study of the effect of creatine phosphate on the duration of AP and on calcium potentials under hypoxic conditions can be explained on the grounds that the ATP utilized for phosphorylation of calcium channels is a product of the creatine phosphokinase reactions [2, 3]. However, the expected stable restoration of the force of contraction of the frog myocardium by creatine phosphate was not obtained under hypoxic conditions.

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CHANGES IN FUNCTIONAL ACTIVITY OF THE CEREBRAL CORTEX AND CENTRAL GRAY MATTER IN RESPONSE TO ELECTROACUPUNCTURE

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Experimental and clinical investigations suggest that the effectiveness of reflex analgesia can be determined largely by the degree of activation of the central gray matter (CGM), one of the principal antinociceptive structures of the brain, by electroacupuncture (EAP) [3, 9, 11].

Both facilitatory and inhibitory influences of the cerebral cortex are known to play an important role in determining the functional state of nonspecific brain structures [1, 2, 4]. However, there is no information as yet in the literature on the possible mechanisms of corticofugal control of excitability of antinociceptive structures and, in particular, of CGM, during the use of EAP.

The aim of the present investigation was accordingly to study integration of nociceptive and non-nociceptive afferent impulsation in CGM and the cerebral cortex during EAP stimulation.

EXPERIMENTAL METHOD

Acute experiments were carried out on 17 cats anesthetized with hexobarbital (40 mg/kg, intraperitoneally), immobilized with suxamethonium, and maintained on artificial ventilation of the lungs. After fixation of the animal in a stereotaxic apparatus and trephining of the skull all regions of operative procedures were infiltrated with local anesthetic. Recording and stimulating electrodes were arranged on the surface of the cortex at stereotaxic coordinates (A 1.0-2.0; L 0.5-2.0; H 0.5 to +1) in CGM. The interpolar distance between the cortical stimulating electrodes, to which a pulsed current was applied (0.1 msec, strength up to 1 mA), was 1-1.5 mm. A silver electrode (diameter of tip 0.8 mm) was used for recording. The subcortical recording electrode was a steel rod up to 200 μ in diameter, insulated throughout its length except at the tip, 50-70 μ in diameter. Nociceptive responses were evoked by single stimulation of the dental pulp (lower canine tooth) through a bipolar electrode, inserted into a hole drilled in the dentine and secured there with acrylic glue. The duration of the stimulating pulses was 1-3 msec and their intensity up to 20 mA. Non-nociceptive responses were evoked by stimulation of the lower lip by means of a bipolar needle electrode with a current with pulse duration 0.1 msec and strength up to 5 mA. EAP was applied through three acupuncture needles, inserted into the base of the concha auriculae of the cat, to which a current was applied (1.2 msec, strength up to 16 mA, frequency 1-3 Hz, for 15-30 min).

Electrical responses in the form of evoked potentials (EPs) were assessed after averaging of 16 presentations on a specialized computer. To monitor the animal's functional state the arterial blood pressure, ECG, and brain surface temperature were recorded. At the end of the experiment a lethal dose of hexobarbital was injected into the animal and the position of the subcortical electrodes was then verified histologically.

EXPERIMENTAL RESULTS

In the experiments of series I, with single stimulation of the frontal, motor, and first (SI) and second (SII) somatosensory areas of the cortex, cortical area SII was found to have the strongest corticofugal connections with CGM. This was proved by the fact that responses in CGM to stimulation of different parts of SII were most stable in form and had maximal amplitude if all areas of the cortical surface were stimulated by currents with equal parameters

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