# SOME ECG AND METABOLIC PARAMETERS FOR RATS ADAPTED TO COMBINED HYPOXIA AND HYPERCAPNIA DURING EXPERIMENTAL CORONARY SPASM

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It is stated in the literature that different types of adaptation, notably adaptation to transient hypoxia, prevent the onset of pituitrin-induced coronary insufficiency [5]. Previously, on the basis of electrocardiographic data, the present writers found an increase in resistance of heart muscle to pituitrin myocardial ischemia after adaptation for 30 days to combined hypoxia and hypercapnia, and the effect was attributed to a marked increase in the blood supply to the myocardium [4].

However, ECG changes connected with myocardial ischemia are known to be based ultimately on metabolic changes [9]. In particular, glycolysis is intensified and intermediate metabolic products, including lactate, accumulate [10, 11].

The object of this investigation was to study the intensity of the final stage of glycolysis, namely interconversion of lactic and pyruvic acids (LA and PA respectively) and lactate dehydrogenase activity in rats with experimental pituitrin myocardial ischemia after adaptation to combined hypoxia and hypercapnia or to hypoxia alone.

## EXPERIMENTAL METHOD

Experiments were carried out on 60 male albino rats weighing 200 ± 20 g, divided into four groups. Group 1 consisted of control animals, receiving one or two (at an interval of 30 days) injections of pituitrin. The animals of group 2 were exposed to a combination of hypoxia with hypercapnia by keeping them daily (on average for 90 min) in a closed space (exsiccators with a volume of 7.6 liters, with airtight ports for taking gas samples), in which by the end of the exposure the oxygen concentration was 10% and the $CO_2$ concentration 8%. The animals of group 3 were exposed to hypoxia alone and adapted under the same conditions, but with absorption of the $CO_2$ by a chemical absorbent. Gas analysis in the closed space was carried out every 15-20 min on an apparatus of the Haldane type. The animals were adapted 5 days a week for 30 days. Intact animals constituted group 4.

Pituitrin was injected 24 h after the last adaptation exercise into the lateral vein of the tail in a dose of 0.02 Unit/100 g body weight. The degree of myocardial ischemia of the rats before and after adaptation was judged from the ECG, recorded under ether anesthesia on an Élkar six-channel electrocardiograph, with preliminary signal calibration at 1 mV = 15 mm and with a tape winding speed of 100 mm/sec, in three standard deviations and three amplified derivations from the limbs.

Levels of LA (in mg%) [8], PA (in mg%) by Babaskin's method [1], and lactate dehydrogenase (LDH) activity (in mmoles PA/ml serum or /100 mg tissue per hour at 37°C) [12] were determined in the blood and tissue from the right and left side of the rats' heart. The results were subjected to statistical analysis. The significance of differences was determined by Student's t test ($P < 0.05$).

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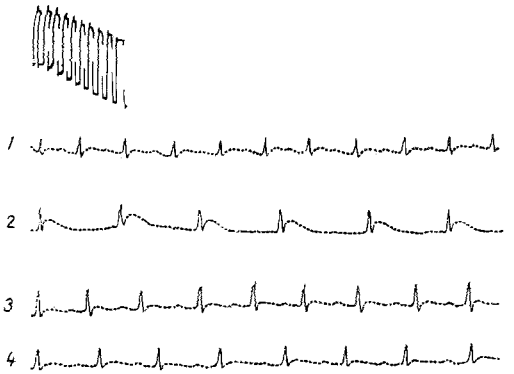

Fig. 1. ECG changes in response to injection of pituitrin before and after adaptation to hypoxic and hypercapnic conditions. 1) Initial ECG of rat; 2) injection of pituitrin before adaptation; 3) background ECG of same rat after adaptation; 4) injection of pituitrin after adaptation.

## EXPERIMENTAL RESULTS

Injection of pituitrin into unadapted animals caused changes in the ECG characteristic of myocardial ischemia [7]. For instance, on average the ST segment was displaced upward from the isoelectric line by 1.00 ± 0.04 mm and the T wave was more pointed in 68.7 ± 1.3% of cases. In 31.3 ± 0.9% of cases the ST segment was depressed below the isoelectric line on average by 1.20 ± 0.03 mm and the T wave was smoothed. The heart rate was reduced on average by 31.6 ± 2.4%.

The LA level in the unadapted animals increased in pituitrin-induced ischemia both in the myocardial tissue and in the blood serum; this increase in the blood was more marked (196.1 ± 17.2% in blood from the right, and 224.9 ± 11.6% in blood from the left heart). The PA level was lowered in all samples, but LDH activity was increased, by a greater degree, moreover, in the blood serum (147.5 ± 16.8% in the right and 132.1 ± 20.2% in the left heart). Intensification of glycolysis, as shown by elevation of the LA level and an increase in LDH activity, is a compensatory mechanism [6] helping to maintain cardiac function in coronary insufficiency.

When pituitrin was injected into the animals after adaptation to hypoxia and hypercapnia, in 100% of cases the ECG parameters studied remained virtually unchanged (Fig. 1). The bradycardia was less marked compared with that after the first injection of pituitrin. The heart rate before adaptation was reduced by 32.2%, and after adaptation by 10.4%.

The LA level and LDH activity were lower in the rats of this group than after the first injection of pituitrin (for example, in the animals of group 1 these parameters in the tissue of the right and left heart were 108.5 ± 12.3 and 128.2 ± 9.6%, and 106.1 ± 4.7 and 103.8 ± 5.3% respectively; in the animals of group 2 the corresponding values were 73.1 ± 8.0 and 94.8 ± 14.0%, and 87.0 ± 5.2 and 89.6 ± 5.4%). Changes in the PA concentration in the heart after injection of putuitrin in the animals of group 2 compared with the control were very small and not statistically significant (Fig. 2).

Unlike rats adapted to a combination of hypoxia with hypercapnia, ECG changes characteristic of myocardial ischemia were nevertheless observed in 20% of animals receiving pituitrin after adaptation to hypoxia alone. The heart rate in these animals, like that in the rate of group 2, was reduced by 10.4%. The lactate level in the animals of this group was lower than after the first injection of pituitrin, but higher than in animals adapted to hypoxia with hypercapnia: the LA level in the tissue of the right heart in the rats of group 1 was 108.5 ± 12.3%, in group 2 it was 73.09 ± 8.0%, and in group 3 107.4 ± 15.5%. The pyruvate level in the rats of group 3 was almost the same as in the animals of group 1 and lower than in those of group 2, adapted to a combination of hypoxia and hypercapnia. For instance, the PA level in blood from the right heart was 98.1 ± 2.9% in the rats of group 1, 102.9 ± 4.5% in those of group 2, and 97.1 ± 3.9% in the rats of group 3.

Injection of pituitrin into rats after adaptation to hypoxia showed that the LDH activity in the heart of these animals was lower than in the rats of all other groups: LDH activity in blood from the left heart was

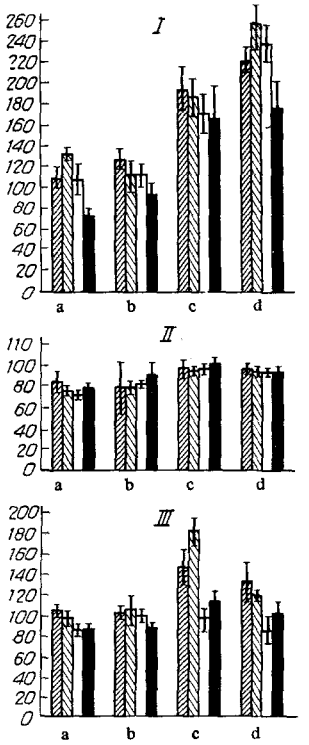


Fig. 2. LA (I) and PA (II) levels and LDH activity (III) in heart of rats with pituitrin-induced myocardial ischemia (in % of control). a, b) Tissue from right and left heart respectively, c, d) blood from right and left heart respectively. Columns from left to right correspond to animals of groups 1, 2, 3, and 4.

132.1 ± 20.2% in rats of group 1, 100.9 ± 14.3% in group 2, and 84.0 ± 14.9% in group 3.

Injection of pituitrin into rats adapted to hypoxia with hypercapnia thus caused accumulation of less lactate than it did in animals adapted to hypoxia alone, and the pyruvate level and LDH activity remained at a higher level in the former. The basic function of glycolysis, as a supplier of pyruvate for the tricarboxylic acid cycle, was thus more intensively expressed in animals adapted to modified atmospheric conditions in the shape of hypoxia combined with hypercapnia. The role of PA in the adaptive modifications of metabolism, on the other hand, is extremely important in connection with the intensification of $CO_2$ fixation by pyruvate during hypercapnia [2].

To rule out the possibility of a change in response of the vessels to a second injection of pituitrin, the control animals were given two injections of pituitrin separated by an interval of 30 days. ECG changes in these animals were identical with those after the first injection. LA and PA levels and LDH activity under these circumstances were comparable with those in rats after a single injection of pituitrin and they differed significantly from the value obtained in the rats of groups 2 and 3.

It can be concluded from these results that adaptation for 30 days to a combination of hypoxia with hypercapnia leads to a more marked increase than exposure to hypoxia alone in the resistance of animals with pituitrin-induced ischemia, and this effect is probably based on more rational modifications of myocardial metabolism.

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# PROTECTIVE ACTION OF SUPEROXIDE DISMUTASE IN EXPERIMENTAL MYOCARDITIS

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The method of hyperbaric oxygenation (HBO) is being applied on an ever-increasing scale for the treatment of cardiovascular diseases. However, the problem of protection of the patient against overdosage of HBO is still unsolved. The more intensive formation of the superoxide anion ($O_2^-$), which appears in such cases, may lead to oxidative destruction of the cell and, in particular, to stimulation of lipid peroxidation (LPO) [6, 9]. Administration of superoxide dismutase (SOD), which renders $O_2^-$ harmless, has a protective action against certain pathological processes with an inflammatory component [2, 3, 12].

The investigation described below showed that injection of exogenous SOD can reduce the severity of the functional and morphological manifestations of adrenalin myocarditis during periodic exposure to increased doses of HBO.

## EXPERIMENTAL METHOD

Adrenalin myocarditis was induced in chinchilla rabbits by slow (in the course of 2-3 min) intravenous injection of 1% caffeine solution (20 mg/kg) and 0.1% adrenalin solution (0.2 ml) with an interval of 2 min. Control animals received physiological saline. Sessions of HBO were given under a pressure of 2.5 atm for 1 h daily for 3 days. The contractile function of the left ventricle was assessed from the pressure inside the ventricle recorded by a Mingograph-82 electromagnometer under conditions of relative rest, and also at the 5th second after complete occlusion of the ascending aorta. The rate of contraction and relaxation of the myocardium of the left ventricle also was determined. The potential working capacity of the left ventricle was calculated by the formula in [10] and the intensity of functioning of structures by the method in [5].

SOD activity was determined by a method based on the ability of the enzyme to inhibit $O_2^-$-mediated autooxidation of adrenalin [1]. To study the effect of exogenous SOD on cardiac activity a purified preparation of SOD from bovine blood was injected intravenously into rabbits in a dose of 1 mg 3 times a day for 3 days. The first injection of SOD was given 30 min before injection of adrenalin. The enzyme, isolated from bovine blood by a modified method of McCord and Fridovich [11], had activity of 2000 Units/mg protein. Isoelectric focusing in polyacrylamide gel revealed two bands with isoelectric points of 4.6 and 4.8, possessing SOD activity.

To determine endogenous SOD activity the tissues were ground in a mortar with liquid nitrogen, homogenized on ice in a Potter homogenizer (glass–glass) for 5 min at 5000 rpm in K-phosphate buffer with 0.9%

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