EFFECT OF DRUGS ON RESISTANCE OF THE MYOCARDIUM TO ANOXIA

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The search for substances increasing the resistance of the myocardium to oxygen deficiency, and the study of the effect of known drugs on resistance of the myocardium to anoxia are important problems in cardiac pharmacology [4]. Methods recommended for the search for antianginal drugs [3] envisage the use of several different objects [5-8, 10] and different criteria of the anoxic state [5-7]. The method of evaluation of the mechanograms of an isolated preparation [10], which reflects virtually completely the state of its energy metabolism under anoxic conditions [9], is a relatively simple and informative method. A method of evaluation of the effect of drugs on the resistance of the myocardium to anoxia, developed by the present writer, is described below.

EXPERIMENTAL METHOD

The suggested method is based on detection of the action of test substances on the duration of work of isolated, spontaneously contracting atrial preparations (SCAP) of rats during two consecutive periods of anoxia. SCAP were isolated after decapitation of rats weighing 180-220 g and immersed in a constant-temperature (29°C) bath (volume 33 ml) containing modified Krebs–Henseleit nutrient solution (in mM): NaCl 119.4; KCl 4.8; Ca_2 2.6; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 2.5; glucose 5.6. The nutrient solution was perfused with oxygen at a constant rate (45.4 ± 1.5 ml/min) by means of a gas flowmeter from an anesthetic apparatus (the flowmeter was calibrated against the volume of gas supplied by means of a measuring cylinder). Contraction of the SCAP was recorded under isometric conditions, in the presence of diastolic loading of 1 g by means of the strain-gauge technique usually adopted [2], on a quick-acting self-writer of the N-327-1 type (the mechanogram was recorded when the speed of the tape-winding mechanism was 1-2 mm/sec). The SCAP was adapted in nutrient solution for 1 h (the nutrient solution was changed every 15 min). After stabilization of the mechanograms of the SCAP (amplitude and frequency of contractions) the action of the first period of anoxia was determined. To displace oxygen from the Krebs–Henseleit solution [10] it was perfused with nitrogen at a constant rate (36.3 ± 1.3 ml/min). The mechanogram was recorded up to 10% of the initial amplitude. Later the anoxic nutrient solution was replaced by one saturated with oxygen. After restoration of the working parameters of the SCAP, the test substance was added (0.2-0.4 ml) to the nutrient medium. Changes in the mechanogram of the preparation were recorded 10 min after addition of the substance, in a concentration changing the amplitude and frequency of contractions of the SCAP by not more than 15-20%, and the action of the second exposure to anoxia was studied.

The effect of anoxia on work of the SCAP was estimated from the 50% reduction of amplitude of atrial contractions during the 1st (t^1_{50}) and 2nd (t^2_{50}) exposures to anoxia. In the control group: t^1_{50} = 138.9 ± 5.7 sec, t^2_{50} = 136 ± 4.4 sec. The effect of drugs on resistance of SCAP was estimated from the difference in time to reduce the amplitude by 50% (Δt_{50}) between the 1st and 2nd exposures to anoxia, by the equation

$$\Delta t_{50} = t^2_{50} - t^1_{50} \text{ (in sec)}$$

In the control series of experiments Δt_{50} was found to be 2.6 ± 3.2 sec, evidence of good recovery of the working parameters of SCAP during reperfusion with oxygen.

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TABLE 1. Effect of Inhibitors of Glycolysis, the Pentose Cycle, and Aerobic Oxidation on Resistance of Isolated, Spontaneously Contracting Rat Atria to Anoxia (M ± m)

Inhibitor	Site of action of inhibitor	Concentration ($\times 10^{-5}$) g/ml	No. of experiments	Change in working time during anoxia (ΔI_{50}), sec	I, %	CHR, %	(P^{50}_{SCAP}) conv. units	(P^{50}_{SCAP}) % of control
Control	—	—	14	1,9±1,3	1,5±3,4	—4,5±6,1	0,74	—
Copper sulfate	Pentose cycle	5,0	3	2,0±1,1	11,1±5,9	0,4±6,4	0,85	+14,8
Monoiodoacetate	Glycolysis	9,0	5	—24,8±11,3	—47,6±4,1*	—43,1±25,1	0,18	—75,7
Malonate	Krebs' cycle	26,6	5	2,2±7,6	13,7±6,9	—8,5±2,2	0,78	+5,4
Potassium cyanide	Aerobic oxidation	3,0	8	3,9±7,6	—22,4±2,4*	—25,4±10,7	0,45	—39,2
2,4-Dinitrophenol	Uncoupling of oxidation from phosphorylation	0,02	3	—7,3±9,5	—21,2±9,1*	—3,9±3,0	0,54	—28,1

Legend *$p < 0.05$ Relative to control.

In individual cases, to allow for energy expenditure of SCAP, the parameter of total work done by the isolated atria during exposure to the second anoxia, when the amplitude was reduced by 50% (P^{50}_{SCAP}) was calculated by the formula:

$$P^{50}_{SCAP} = 0{,}75 \cdot \left(A + \frac{A \cdot I}{100}\right) \cdot \frac{t^2_{50}}{t^1_{50}} \cdot \left(HR + \frac{HR \times CHR}{100}\right),$$

where A is the original amplitude of contractions (in mm), HR the initial number of contractions of the preparation in 1 min, I the inotropic effect of the test substance (in %), CHR_{50} the chronotropic effect of the test substance (in %), 0.75 is a standard coefficient. P^{50}_{SCAP} in these investigations in the control series of experiments had a value of 0.75 conventional units.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that inhibition of the pentose phosphate pathway of carbohydrate oxidation by copper sulfate ($5 \cdot 10^{-5}$ g/ml) and of the Krebs' cycle at the succinate dehydrogenase level by malonate ($2.6 \cdot 10^{-4}$ g/ml) had no significant effect on the duration or volume of work done by SCAP during anoxia. Blocking of the glycolytic mechanism of energy formation by monoiodoacetate ($9 \cdot 10^{-5}$ g/ml), blocking of aerobic oxidation by potassium cyanide ($3 \cdot 10^{-5}$ g/ml), and uncoupling of oxidative phosphorylation by 2,4-dinitrophenol ($2 \cdot 10^{-7}$ g/ml) considerably shortened the duration of work of SCAP during anoxia, i.e., reduced the resistance of the myocardium to oxygen deficiency. This confirms the view that this model of myocardial anoxia adequately reflects dependence of the system of heart tissue to oxygen deficiency on the basic stages of energy production, and it can accordingly be used to search for or study the effectiveness of myocardial antianoxic agents.

Of the various drugs which increase the sensitivity of the myocardium to anoxia we tested adrenalin ($6 \cdot 10^{-8}$ g/ml), and of substances reducing ischemic myocardial damage, we chose antianginal agents such as propanolol ($2.5 \cdot 10^{-6}$ g/ml), viloxazine ($1.25 \cdot 10^{-5}$ g/ml), intencordin ($2.5 \cdot 10^{-5}$ g/ml), and corontin ($6.2 \cdot 10^{-5}$ g/ml), and also the antianoxic agent gutimin ($3 \cdot 10^{-4}$ g/ml). The results showed that adrenalin in vitro reduced the resistance of the myocardium to oxygen deficiency just as it did in the intact organism. This action of adrenalin on resistance of the myocardium to anoxia can be explained, in Gatsura's opinion [1], by the considerable accumulation of cAMP, the overintensification of glycolysis and lowering of the efficiency of ATP production, an increase in the "oxygen debt," a fall in the carbohydrate reserves of the tissues, the development of acidosis due to lactic acid accumulation and, finally, uncoupling of oxidative phosphorylation.

All the antianginal agents studied increased the duration and volume of work of the SCAP during anoxia. The most active of the substances tested were viloxazine, intencordin, and the antianoxic agent gutimin. It can be tentatively suggested that their antianginal action in ischemic heart disease is due to their metabolic effect.

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