EFFECT OF INTERMEDIATES OF THE KREBS CYCLE ON BLOOD SUPPLY AND ENERGY METABOLISM OF THE ISCHEMIZED MYOCARDIUM

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The effect of α-ketoglutarate and of sodium succinate on the collateral coronary circulation (CCC) of the ischemic myocardium, the systemic arterial pressure, and the concentrations of lactic and pyruvic acids and glucose in the blood flowing from the zone of ischemia was investigated in acute experiments on dogs. A positive effect of α-ketoglutarate in doses of 6 mg/kg (into the coronary artery) and 60 mg/kg (intravenously) on CCC and on contraction was discovered. Intravenous injection of α-ketoglutarate (12 mg/kg) and succinate (100 mg/kg) considerably reduced the glucose consumption of the ischemized myocardium, which was increased after ligation. Succinate, in all doses, prevented the increase in the lactate concentration in the blood flowing from the zone of ischemia more intensively than α-ketoglutarate. Consequently, α-ketoglutarate and succinate, in certain doses, can be used to correct CCC and the energy provision for the ischemized myocardium.

KEY WORDS: myocardial ischemia; bioenergetics of the heart; succinate; α-ketoglutarate.

Accumulation of reduced forms of NAD in the ischemized myocardium sharply reduces energy production in the Embden–Meyerhof and Krebs cycles. Mobilization of the potential of partial reactions of the Krebs cycle under these conditions may contribute to the maintenance of homeostasis of the ischemized myocardial cell [2, 4, 6]. From this point of view the FAD-dependent oxidation substrate succinate and α-ketoglutarate, oxidation of which gives the maximal energy yield (4 moles ATP to 1 mole of substrate), deserve attention.

In the investigation described below correlation between changes in the parameters of the blood supply and carbohydrate metabolism arising under the influence of these two intermediates during the period of acute ischemia of the myocardium was compared.

EXPERIMENTAL METHOD

Experiments were carried out on 79 adult mongrel dogs anesthetized with pentobarbital sodium (40 mg/kg intraperitoneally). To assess the collateral coronary circulation (CCC) the volume velocity of CC and the retrograde pressure in the left coronary artery were recorded alternately [3]. The systemic arterial pressure (SAP) was recorded in the carotid artery by a mercury manometer, and the first derivative of the left-ventricular pressure ($\Delta p/\Delta t$) was recorded by means of a differentiator. The ECG was recorded in the epicardial lead. These parameters were recorded synchronously on the ÉLKAR-6 electrocardiograph. Sodium succinate was injected into the coronary artery in doses of 2 and 10 mg/kg and α-ketoglutaric acid in doses of 1.2 and 6 mg/kg. The solutions were infused by means of an automatic system uniformly over a period of 15 min. Sodium succinate and α-ketoglutarate were injected intravenously by a jet method in doses of 20 and 100 mg/kg and 12 and 60 mg/kg respectively. Observations were continued for 60 min after ligation of the descending branch of the left coronary artery, and the parameters mentioned above were recorded 5, 10, 15, 30 and 60 min after ligation of the vessel. In the same experiments the concentrations of lactic and pyruvic acid and also of glucose were determined in blood flowing from the zone of ischemia and in samples taken from the aorta.

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TABLE 1. Effect of Sodium Succinate and α-Ketoglutarate on Blood Supply and Some Indices of Carbohydrate Metabolism of Ischemized Myocardium (60th minute of observation; in % of initial level), M ± m

Compound and mode of injection	Dose of compound, mg/kg	n	CCC	SAP	PR	Δp/Δt	Coefficient K	Lactate	Coefficient of glucose elimination
Control		24	−23,4±5,4 (1,5—0,2)	−16,0±3,3 (109±6)	+3,5±1,2 (162±9)	−14,1±6,2 (2275±252)	0,91	+261,5±40,4 (1,98±0,16)	24,4±1,5 (3,8±1,1)
Sodium succinate into coronary artery	2,0	10	−15,4±5,1	−32,0±6,9	−11,4±2,6*	+9,7±9,4	1,24	+87,7±28,5*	15,8±4,4
"	10,0	10	−3,1±11,3	−13,7±4,0	+7,3±3,0	+39,1±7,4*	1,12	+50,1±20,0*	17,2±3,8
intravenously	20,0	11	−4,2±8,3	−21,8±4,6	−4,8±2,8	−0,2±5,0	1,11		
"	100,0	10	−16,4±3,5	−32,7±2,6*	−2,4±4,0	+3,3±11,7	1,24	+65,0±26,8*	3,0±8,7*
α-ketoglutarate into coronary artery	1,2	11	−6,3±7,3	−24,0±4,1	−7,3±4,4	+0,4±5,6	1,23	+161,7±22,2	26,6±8,4
"	6,0	10	+27,4±16,5*	−18,4±5,4	−3,2±4,2	+15,4±4,0*	1,56	+142,4±18,6	21,2±7,4
intravenously	12,0	8	−1,8±5,8*	−19,4±4,0	−5,3±7,8	−0,6±5,4	1,22	+140,7±23,1	4,2±7,6*
"	60,0	9	+8,0±10,1*	−17,1±4,0	−8,5±4,2	+12,7±4,8*	1,30	+114,6±23,5	11,9±5,9

Legend. 1) Coefficient (%) of elimination of glucose given in absolute figures, coefficient K (—) as the ratio between the values of CCC and SAP, expressed as a percentage of the initial values. 2) Initial absolute values of parameters CCC (in ml/min), SAP (in mm Hg), PR, as the number of contractions per minute, lactate (in mmoles/liter), and Δp/Δt (in mm Hg/sec) given in parentheses. 3) * $P < 0.05$ compared with control.

EXPERIMENTAL RESULTS

Analysis of the experimental results shows that neither compound caused significant changes in pulse rate (PR) aside from the slight bradycardia (−11.4 ± 2.6 beats/min) observed after intracoronary injection of sodium succinate in a dose of 2 mg/kg (Table 1). The changes in SAP after injection of these compounds showed a slight tendency toward an increase of hypotension. The only exceptions were the experiments with intracoronary injection of sodium succinate in a dose of 10 mg/kg. In a series of experiments with intravenous injection of succinate in a dose of 100 mg/kg the hypotensive effect was statistically significant.

Sodium succinate, compared with the results of the control groups of experiments, significantly inhibited the decrease in CCC, and combined with the tendency towards strengthening of arterial hypotension, this was reflected in a significant rise in the value of the coefficient K (from 0.91 in the control to 1.24).

Succinate prevented the decrease in contractile power of the myocardium observed in the control, and after intracoronary injection in a dose of 10 mg/kg it increased Δp/Δt statistically significantly.

The functional shifts mentioned above developed against the background of a decrease in lactate elimination ($P < 0.05$) from the zone of ischemia and some decrease in the glucose consumption of the myocardium. The decrease in the elimination of glucose by the ischemized myocardium after intravenous injection of succinate in a dose of 100 mg/kg was statistically significant.

The positive effect of α-ketoglutarate on CCC in three series of experiments was accompanied by an increase in the coefficient K, which indicates a significant fall in tone of the interarterial anastomoses. After injection of α-ketoglutarate in large doses, a marked increase in the rate of rise of the left-ventricular pressure also was observed ($P < 0.05$).

The changes mentioned above developed against the background of a stable tendency toward a decrease in the lactate concentration in blood flowing from the zone of ischemia. After intravenous injection of α-ketoglutarate in a small dose, the elimination of glucose by the ischemized myocardium fell sharply ($P < 0.05$).

The beneficial effect of certain doses of these two intermediates of the Krebs cycle on CCC, on the myocardial contractility, on the decrease in elimination of lactate, and the decrease in glucose consumption by the ischemized myocardium are evidence that succinate and α-ketoglutarate participate in energy formation in the mitochondria. The results confirm the view that succinate plays an important role in the processes of energy production during myocardial hypoxia [4, 5] and of the possible compensatory role of partial reactions of the Krebs cycle in the provision of energy for the ischemized myocardium and its survival [1, 2, 6, 7].

The sharp decrease in the glucose consumption of the ischemized myocardium under the influence of intravenous injection of sodium succinate and α-ketoglutarate, established by these experiments, calls for further analysis, as also does the demonstration of the wide range of doses of α-ketoglutarate causing maximal stimulation of CCC and the study of the mechanism of this effect.

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GLUCOSE TOLERANCE AND INSULIN SENSITIVITY OF RATS POISONED WITH *Amanita phalloides*

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The effect of *Amanita phalloides* toxins on glucose tolerance and insulin sensitivity was studied. *Amanita* toxins were injected intraperitoneally in a dose of LD_{50} into male albino rats. Amanita toxins were found to disturb glucose tolerance, to increase the utilization of glucose by the tissues, and to make the animals more sensitive to insulin. These effects may be the result of depression of the insulin-activating capacity of the liver and potentiation of the function of the islet-cell apparatus of the pancreas.

KEY WORDS: *Amanita phalloides* toxins; glucose tolerance; insulin sensitivity.

The toxins of *Amanita phalloides*, namely amanitines and phalloidines, if administered to animals, cause degenerative changes in many organs and disturbances of all types of metabolism [2, 3]. Profound disturbances of the protein-synthesizing and carbohydrate functions of the liver, manifested by hypoglycemia, depletion of the glycogen reserves in the liver, and hyperinsulinemia, have been found [5-7].

The object of the present investigation to study glucose tolerance and insulin sensitivity in rats poisoned with *Amanita* toxins.

EXPERIMENTAL METHOD

Experiments were carried out on 110 noninbred male albino rats weighing 160-180 g. The toxins were injected intraperitoneally into the animals in a dose of LD_{50}. Physiological saline was injected into the control rats. In the glucose tolerance test, glucose was injected intraperitoneally in a dose of 1 mg/g body weight of the 40% solution [1] and the blood sugar was determined in the fasting state and 15, 30, 60, and 120 min after loading. Insulin sensitivity was judged from changes in the blood sugar level 10, 30, and 60 min after subcutaneous injection of insulin in a dose of 0.07 unit per animal. Blood sugar was determined by the orthotoluidine method. Analysis of the blood sugar curves involved determination of: the hyperglycemic coefficient

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