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EFFECT OF THE ANTIHYPERTENSIVE ACTION OF CAPTOPRIL ON LEVEL OF HYPERTROPHY AND SOME METABOLIC PARAMETERS OF HEART MUSCLE

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KEY WORDS: captopril; heart muscle; fenal hypertension; metabolism

Captopril, an inhibitor of angiotensin converting enzyme, is known to limit renal hypertension to a significant degree or to abolish it [3, 4, 7]. However, the question of to what extent this antihypertensive effect influences the hypertrophy of the myocardium and its energy metabolism has been the subject only of sporadic research [6], and many aspects of it remain unexplained.

The aim of this investigation was to discover how the antihypertensive effect of captopril in experimental renal hypertension affects the mass of the heart muscle, activity of creatine kinase and glycolytic enzymes, the tricarboxylic acid cycle, and the pentose phosphate shunt.

EXPERIMENTAL METHOD

Experiments were carried out on 28 male Wistar rats, divided into three series: I) control animals, II) animals with experimental hypertension, and III) animals with experimental hypertension treated with captopril. There were 10 rats in each series. Renal hypertension was induced by the method in [8], i.e., by constricting the abdominal aorta between the two renal arteries. The operation was performed retroperitoneally in the modification in [1]. The rats received a standard diet and water ad libitum. The animals were used in the experiments 30 days after the operation for electromanometric recording of the pressure in the carotid artery. Pentobarbital anesthesia was used for the experiments. Animals of series III were given captopril in a dose of 30 mg/kg twice a day per os. The drug was obtained from "Farmakhim" (Bulgaria). The myocardium of the left ventricle was homogenized in a "Pottez" homogenizer with teflon pestle. The homogenate was diluted with bidistilled water in the ratio of 1:10 and centrifuged in the cold for 30 min at 10,000 rpm. Activity of the enzymes was determined in samples taken from the supernatant with the aid of test kits from "Böhringer." Activity of the following enzymes was determined and expressed in international units (IU) per gram protein: a) glycolytic – lactate dehydrogenase (LDH, EC 1.1.1.27, cat. no. 124893), α-hydroxybutyzate dehydrogenase (α-HBDH) or lactate dehydrogenase (LDH-1-isozyme, EC 1.1.1.27, cat. no. 124818), and pyzuvate kinase (PK, EC 2.7.1.40, cat. no. 126047); b) of the pentose cycle – glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49, cat. no. 124672); c) of their tricarboxylic acid cycle – isocitrate dehydrogenase (ICDH, EC 1.1.1.42, cat. no. 125989), and malate dehydrogenase (MDH, EC 1.1.1.37, cat. no. 124940); d) creatine phosphokinase (CPK, EC 2.7.3.2, cat. no. 126322), and creatine phosphokinase MB isozyme (CPK-MB, EC 2.7.3.2, cat. no. 189219). The weight of the whole heart of animals of all three series was expressed per 100 g body weight. The results were subjected to statistical analysis.

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TABLE 1. Effect of Captopril on Activity of Enzymes of Energy Metabolism in Myocardium of Rats with Spontaneous Hereditary Hypertension ($M \pm S$)

Series of experiment	Enzyme activity, IU/g protein/liter $\times 10^3$					
	CPK	CPK-MB	PK	G6PD	ICDH	MFH
I	1060±59	154±36	107±9	347±58	72±8	1560±98
II	1123±130	207±45	118±22	584±107	67±10	1430±90
p		<0,05	<0,05	<0,001		<0,05
III	1081±57	120±16	89±5	352±50	62±6	1670±99
p		<0,01	<0,001	<0,001		<0,001
Significance		<0,05	<0,05	<0,001		<0,05

EXPERIMENTAL RESULTS

After 30 days the animals undergoing the operation were in a state of marked hypertension, manifested as elevation of the systolic blood pressure to 209 ± 8.4 mm Hg and the diastolic to 149 ± 9.2 mm Hg. At the same time hypertrophy of the heart was observed, its weight being increased by 29%. In animals receiving captopril after the operation to constrict the aorta, neither hypertension nor hypertrophy of the heart was present.

The results of determination of enzyme activity are given in Table 1. They show that against the background of a very small increase in CPK activity in the hypertrophied myocardium of an animal with hypertension, a marked increase in the activity of its isozyme CPK-MB (by 34.6%) was found.

Changes in activity of the glycolytic enzymes were not significant, but G6PD activity, the limiting stage of the pentose phosphate shunt, rose about twofold. A study of enzymes of the Krebs' cycle revealed only one small shift, namely a decrease of 9% in MDH activity.

Captopril, which in these experiments prevented hypertension and hypertrophy of the myocardium, at the same time prevented changes in CPK-MB activity and actually reduced this activity below the control level (Table 1). The drug also completely prevented G6PD activation. Another, rather unexpected but significant effect of captopril was to depress PK activity, so that it was lower than in the control animals. In the Krebs' cycle captopril lowered ICDH activity but, conversely, increased MDH activity.

Thus captopril mainly prevented changes in activity of enzymes connected with hyperfunction and hypertrophy of the myocardium during hypertension, and also increased MDH activity and reduced PK activity to a certain extent.

During hyperfunction and hypertrophy of the heart, which arose in these experiments as a result of renal hypertension, we observed two significant shifts noted previously in the emergency stage of compensatory hyperfunction of the heart during aortic stenosis [5], but not previously discovered during long-lasting hypertension. The first of these changes was an increase in activity of the MB isozyme of CPK, which has high affinity for ADP [9] and may thus play a role in the intensification of transfer of the phosphate group from creatine phosphate to ADP, essential for the supply of energy for hyperfunction of the heart. The second change, namely an increase in activity of G6PD, the key enzyme of the pentose phosphate shunt, demonstrates that during hypertension, just as during hyperfunction of the heart caused by other factors, activity of the pentose phosphate shunt plays an important role in the increase of ribose formation necessary for RNA synthesis, and thus in the development of hypertrophy of the heart also. It is clear, moreover, that captopril, which abolished hypertension, thereby abolished hyperfunction of the heart and made the changes in enzyme activity examined above unnecessary.

The effect of captopril itself, expressed as depression of activity of the glycolytic enzyme PK, accompanied by increased activity of an important enzyme of the tricarboxylic acid cycle (MDH), is particularly interesting. When this fact is evaluated, it must be recalled that captopril activates prostacycline synthesis in the myocardium [4] and, as a result, may increase the coronary blood flow, and thus create a shift toward aerobiosis. It can be tentatively suggested that it is this state of affairs which led at the same time to depression of activity of the glycolytic enzyme and increased activity of one of the key enzymes of the tricarboxylic acid cycle.

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DISTURBANCE OF INTERACTION BETWEEN THROMBIN AND THE VESSEL WALL AND ITS INACTIVATION BY ANTITHROMBIN III IN AN EXPERIMENTAL NEPHROTIC SYNDROME

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KEY WORDS: thrombin; antithrombin III; nephrotic syndrome

In the modern view inactivation of thrombin in vivo is effected in several ways: through interaction of the enzyme with plasma inhibitors — chiefly with antithrombin III [3], highly specific reversible binding with receptor structures of the endothelium [6], and through activation of the anticlotting system, leading to the secretion of heparin, which catalyzes the inactivation of thrombin by antithrombin III [5], from mast cells into the blood stream. It has been suggested that the endothelium plays an important role in the secretion and inactivation of thrombin. Heparin-like binding sites of the enzyme located on the surface of the endothelium [11] and the membrane protein thrombomodulin evidently behave as cofactors in the action of inhibition of thrombin by antithrombin III [7]. In addition, thrombomodulin and an unidentified protein of endothelial cells with mol. wt. of 30 kD are unique receptor proteins, responsible for endocytosis of the enzyme by the endothelium [15, 13]. The change in the binding capacity of the vessel wall in relation to thrombin may probably lead to disturbances of thrombin clearance from the blood stream and its inactivation by plasma antithrombin III. The unusually high frequency of thromboembolic complications accompanying the nephrotic syndrome [12] suggests a disturbance of the mechanisms of biological inactivation and elimination of thrombin from the blood stream in this pathology. The investigation described below was carried out to test this hypothesis.

EXPERIMENTAL METHOD

Bovine α-thrombin was obtained by purification of a commercial preparation by ion-exchange chromatography on SP-Sephadex A-50 ("Pharmacia," Sweden) [4]. The α-thrombin was homogeneous on polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate (PAG — SDS) and its molecular weight was 37 ± 1 kD. The clotting activity of the enzyme was 2000 NIH units/mg protein. The protein was labeled with ^{125}I ("Izotop," USSR) with the aid of "Iodo-Gen" (Pierce and Warriner, England), jointly with A. V. Rudin, by the method in [9]. To remove free ^{125}I and denatured protein, the ^{125}I-α-thrombin was subjected to chromatography on a column with Sephadex G-25. The ^{125}I-α-thrombin used in the subsequent experiments had specific radioactivity of 600 μCi/μM and its fibrinogen-clotting and amidase activity, determined spectrophotometrically by hydrolysis of D-phenylalanyl-pipecolyl-arginine paranitroanilide ("Serva," West Germany), was 1440 NIH units/mg and its molecular weight 37 ± 1 kD. Precipitation led to precipitation of 98% of the radioactivity together with protein.

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