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EFFECT OF CARBOCYCLIN ON CARDIAC ATP-ASE ACTIVITY IN NORMOTENSIVE AND STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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It has been suggested that one cause of essential hypertension is a disturbance, for some reason or other, of ionic homeostasis of the cells [5, 6]. Investigations have shown that prostaglandins (PG) play a direct role in the regulation of the electrolyte composition of cells [2, 13]. Prostacyclin (PGI_2) is known to be the principal PG synthesized by the rat heart. In rats with hereditary predisposition to hypertension PGI_2 is formed in smaller quantities than in normotensive animals [4]. An intensive search is currently in progress world-wide for analogs of PG which can be used in medical practice. One stable analog of PGI_2 is carbocyclin (6a-carboprostacyclin) (CC).

The aim of this investigation was to study the effect of CC on ATPase activity in the heart of normotensive Wistar-Kyoto (WKY) and stroke-prone spontaneously hypertensive Okamoto (SHR-SP) rats.

EXPERIMENTAL METHOD

Adult male rats aged 10-12 months had blood pressures of: 125.1 ± 4.2 mm Hg (WKY) and 198.6 ± 5.6 mm Hg (SHR-SP; $p < 0.001$).

BP was measured in the caudal artery by a plethysmographic method using an instrument from "Natsume" (Japan). The animals were decapitated, the heart homogenized in ice-cold isolation medium, and subsequent procedures were carried out in the cold at 2-4°C. Membrane fractions were isolated by fractional ultracentrifugation, using Triton X-100 as detergent. Succinate dehydrogenase activity was measured as the marker enzyme. Its activity in the plasma membrane (PM) fraction was zero, and in

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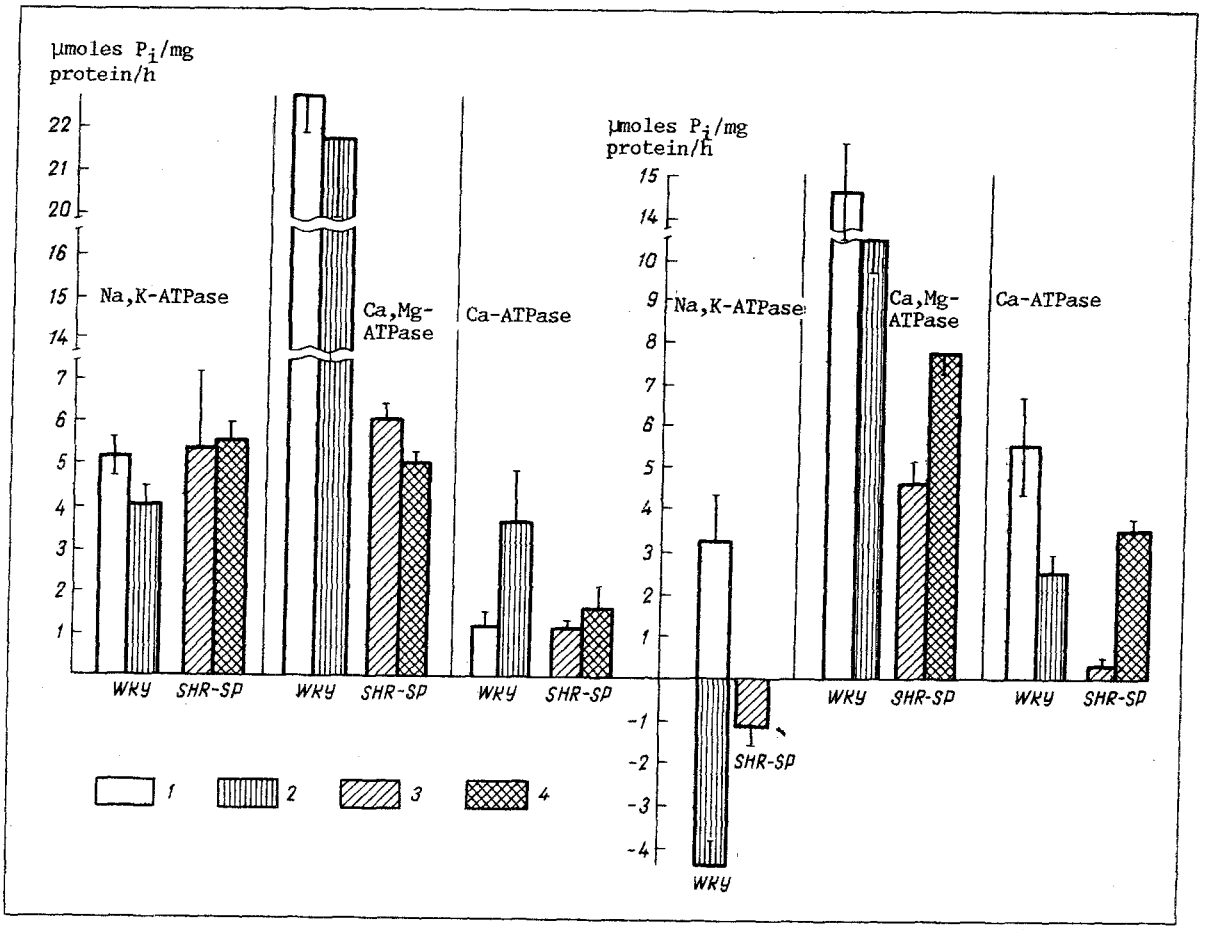

Fig. 1. Effect of CC on ATPase activity in different fractions of the heart of normotensive (WKY) and stroke-prone spontaneously hypertensive (SHR-SP) rats. 1) WKY; 2) WKY + CC; 3) SHR-SP; 4) SHR-SP + CC.

the fraction of heavy sarcoplasmic reticulum it was 15-20% of its activity in the mitochondrial fraction. ATPase activity was judged by the increase in inorganic phosphate in the incubation medium. Media of the following composition were used to determine enzyme activity (in mM): imidazole 30, NaCl 100, KCl 20, $MgCl_2$ 3, Na_2ATP (pH 7.4) 3, for total ATPase; imidazole 30, NaCl 140, $MgCl_2$ 3, Na_2ATP 3, ouabain 3 (pH 7.4) for Ca,Mg-ATPase. Na,K-ATPase activity was determined as the difference between total and Ca,Mg-ATPase activity. Ca-ATPase activity was determined as the difference between activity of the enzyme in medium containing (in mM): imidazole 30, KCl 30, $CaCl_2$ 0.5, $MgCl_2$ 4, ouabain 3.0 (pH 7.0) in the presence and in the absence of 1 mM EGTA. Carbocyclin (from Upjohn Co.) was dissolved in 70° ethanol to a concentration of 10^{-5} g/ml, and immediately before use it was again diluted with deionized water to a concentration of 10^{-6} g/ml, and added to the incubation medium in an amount so that its final concentration was 10^{-7} g/ml. After preincubation for 6 min at 37°C 3 mM Na_2ATP was added and incubation continued for a further 10 min. All the solutions were made up in deionized water. Phosphorus was determined by the Lowry–Lopez method and protein by Lowry's method. The results were subjected to statistical analysis by Student's *t* test.

EXPERIMENTAL RESULTS

Activity of Na,K-ATPase in WKY and SHR-SP rats in the PM fraction did not differ. This is in agreement with data in [15], according to which the membrane potential in SHR-SP rats is the same as in WKY.

CC had opposite actions on Na,K-ATPase activity in PM of WKY and SHR-SP rats (Fig. 1). Activity of the enzyme under these circumstances was 25% higher ($p < 0.05$) in SHR-SP than in WKY rats.

"Negative" value of Na,K-ATPase activity appeared under the influence of CC in the fraction of heavy sarcoplasmic reticulum (HSR) under the influence of CC. As was pointed out above, Na,K-ATPase activity was judged as the difference between activity of the enzyme in the presence and in the absence of ouabain. Addition of CC in the presence of ouabain led not to a decrease but, on the contrary, to an increase in the activity of this enzyme by 48.3%. It can be tentatively suggested that CC either modifies the affinity of Na,K-ATPase for ouabain or itself binds with ouabain. The latter, in small doses [1], may under certain conditions not inhibit, but activate the enzyme. Unlike WKY rats, activity of Na,K-ATPase in the HSR fraction of SHR-SP rats was initially "negative." This is in agreement with data on compensatory activation of Na,K-ATPase and an increase in ouabain-insensitive Na^+ transport in AHR-SP rats even in the early stages of development of hypertension [15]. CC abolished the activating action of ouabain, and Na,K-ATPase activity in SHR-SP rats under the influence of CC was zero ($p < 0.05$).

Ca,Mg-ATPase activity in PM and HSR of normotensive rats was almost 3.5 times higher than in SHR-SP rats ($p < 0.001$). Meanwhile in SHR-SP rats Ca,Mg-ATPase activity was also considerably reduced (by almost 11 times, $p < 0.001$). According to some workers the development of arterial hypertension is linked with excessive Na^+ accumulation in the cell [5, 9]. Sodium ions are known to reduce by 8-10 times the affinity of Ca-ATPase for Ca^{2+}, thereby inhibiting activity of the enzyme [12]. Data on an increase in the area of SR in SHR rats to almost twice that of normotensive rats [3] and a decrease in the content of high-energy compounds in the myocardium during hypertrophy of the heart in SHR, against the background of depression of Ca^{2+} transport [11], also have been obtained. It will be evident that "hyperaccumulation" of calcium phosphates takes place in SHR-SP rats, leading ultimately to reduction of Ca-ATPase activity. It is suggested that in arterial hypertension the ability of calmodulin either to bind calcium [7] or to activate Ca,Mg-ATPase [14] is depressed, and this may lead to a decrease in Ca,Mg-ATPase activity, to an increase in the Ca^{2+} concentration in the cell, and to an even greater increase in vascular tone, and to a rise of BP.

At the beginning of development of arterial hypertension, i.e., in the prehypertensive and early hypertensive stages, Na,K-ATPase activity is perhaps somewhat inhibited [6]. Later, however, with the development and stabilization of hypertension, in order to maintain the viability of the cells and the body as a whole, corresponding changes must evidently take place in the mechanisms regulating the excess of sodium ions in the cell and increased vascular tone. In other words, at this stage definite changes must take place in the body aimed at survival, and including an increase in Na,K-ATPase activity [7], increased release of PGI_2 from the aortic wall [10] — processes which have been found to be interconnected [13].

Injection of CC did not affect Ca,Mg-ATPase activity in the plasma membrane of normotensive animals, but depressed activity of the enzyme by 18% in SHR-SP rats ($p < 0.05$). Ca,Mg-ATPase activity increased following injection of CC only in WKY rats (more than twofold compared with the control, $p < 0.05$).

In the HSR fraction CC lowered Ca,Mg- and Ca-ATPase activity of WKY by 27.6% ($p < 0.01$) and by 55.4% ($p < 0.01$), respectively. Activity of Ca,Mg-ATPase in WSR in SHR-SP rats increased by 67.4% following injection of CC ($p < 0.001$), whereas Ca-ATPase activity increased by 17.5 times ($p < 0.001$). There is evidence [10] that PGI_2, injected intravenously, increases the vascular resistance of the ischemic myocardium and reduces that of the normal heart [8].

We thus demonstrated the opposite effect of the synthetic PGI_2 analog (CC) on activity of Na,K-, Ca,Mg-, and Ca-ATPases studied in the HSR fraction from WKY and SHR-SP rats. The effect of CC on Ca,Mg- and Ca-ATPase activity in WKY rats and SHR-SP rats in the PM fraction was similar in direction but greater in degree in the WKY rats, possibly due to the greater powers of adaptation of these animals.

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BLOOD LEVELS OF MET-ENKEPHALIN AND BETA-ENDORPHIN IN THE EARLY PERIOD AFTER ACUTE BLOOD LOSS IN RATS

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Data on increased secretion of beta-endorphin (BE) and enkephalins in various types of shock have been published in recent years [12-14]. A broad spectrum of physiological effects, namely a marked antistressor action [8], ability to inhibit secretion [16] and peripheral effects [11] of catecholamines, and the character of the effect on the hemodynamics and metabolism [2, 6, 13], – suggests the possibility that enkephalins and BE may be involved in the development of shock. Nevertheless, information in the literature on this subject [13, 14] is quite contradictory, due perhaps to the different conditions of the investigations and differences in the pathogenesis of the various types of shock. The aim of this investigation was to study blood levels of Met-enkephalin (ME) and BE in rats in the early period after acute blood loss and to analyze dependence of the character of the course of the pathological process on the blood opioid levels.

EXPERIMENTAL METHOD

Experiments were carried out on 174 Wistar rats weighing 330-350 g and 110 CBWH albino mice weighing 22-25 g. A model of acute blood loss was created by bleeding from the right common carotid artery in a volume corresponding to 3% of body weight. The carotid artery was catheterized under superficial ether anesthesia. BP in the carotid artery was recorded by the direct method on a "Thomson" polygraph (France). Blood samples were taken immediately before hemorrhage (background values) and 1 and 30 min after bleeding. These time intervals were chosen allowing for the known phasic nature of changes in hemodynamic parameters in the early period after acute blood loss [3]. Intact rats served as the control group.

Concentrations of BE and ME in samples of blood plasma were determined by radioimmunoassay using standard kits from "Immuno Nuclear Corporation" (USA). Preliminary treatment of the blood plasma for investigation of ME including extraction with methanol, whereas BE were isolated by chromatography using reagents supplied with the kits. Radioactivity was counted on a "Tracor" gamma-spectrometer (USA). The blood lactate concentration was determined by an enzymic method using kits from "Boehringer" (West Germany). Optical density was recorded on a "Specord M-40" spectrophotometer (East Germany). Hypoxic hypoxia was induced in the animals by placing them in an airtight chamber with a capacity of 75 ml for mice and 1400 ml for rats, carbon dioxide being absorbed by soda lime, and the duration of survival of the mice was recorded by determining cessation of respiratory movements [1]. The delta-receptor agonist DADLE, the mu-receptor agonist DAGO (obtained at the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR) and

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