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ACTION OF VASOPRESSIN ON ATPase ACTIVITY OF MICROSOMAL FRACTIONS OF RABBIT HEART AND LIVER

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Intravenous injection of vasopressin in a dose of 5 pressor units/kg body weight led after 1 h to changes in the ATPase activity of rabbit heart and liver microsomes. These changes differed in direction: Mg- or Ca-activated ATPase activity of the cardiac microsomes was very slightly increased, whereas ATPase activity of the hepatic microsomes was reduced.

KEY WORDS: ATPase activity; microsomes of heart and liver; vasopressin.

In recent years, vasopressin in combination with macromolecular dextran has been used in order to produce acute experimental disturbances of the coronary circulation. This use was based on the ability of vasopressin to constrict the small coronary vessels, to change the permeability of biological membranes, and thereby to increase the aggregating effect of macromolecular dextran [4-6]. Under these conditions changes were observed in cardiac function, accompanied by marked shifts in the protein and energy metabolism of the myocardium [2].

Since muscle contraction is influenced by the state of the sarcoplasmic reticulum (SPR) and, in particular, of its ATPase systems, changes in Ca- and Mg-ATPase activity of SPR fragments from heart muscle were studied during the action of vasopressin. To emphasize the role of SPR in the contractile activity of the cell,

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TABLE 1. Effect of Vasopressin on ATPase Activity of Rabbit Cardiac and Hepatic Microsomes (M ± m)

Experimental conditions	Total ATPase activity, μmoles P_i/mg protein/h			
	cardiac microsomes		hepatic microsomes	
	Mg-ATPase	Ca-ATPase	Mg-ATPase	Ca-ATPase
Normal (16 experiments)	23,52±1,11	22,63±1,45	9,09±0,64	6,54±0,62
Administration of vasopressin (11 experiments)	26,99±0,97	26,32±0,97	8,10±1,32	5,28±0,50

the results obtained for heart muscle were compared with those obtained with fragments of the endoplasmic reticulum of liver cells, which have no contractile ability.

EXPERIMENTAL METHOD

Male rabbits weighing 2-3 kg were used. The animals were deprived of food for 20-24 h before the experiment in order to deplete the liver glycogen. Vasopressin was injected intravenously in a dose of 5 pressor units/kg body weight. The animals were decapitated 1 h later, the heart and liver quickly removed, and the tissue rinsed free from blood with cold 0.14 M NaCl and homogenized with 9 volumes of isolation medium (0.25 M sucrose, 0.05 M Tris−HCl buffer, pH 7.4, 3 mM EDTA). The 10% homogenate was centrifuged at 600g (10 min) to remove unfragmented cells and nuclei. Mitochondria were sedimented for 10 min at 9000g. To obtain microsomes, the postmitochondrial fraction was centrifuged for 1 h at 105,000g on an MSE Superspeed-60 centrifuge or at 45,000g for 2 h on the TsVR-1 centrifuge. The residue of liver microsomes was suspended in 0.25 M sucrose, pH 7.4, and kept at −10°C. To remove contractile proteins the microsomes were treated for a further 15-18 h with 0.6 M KCl in 0.005 M histidine buffer, pH 7.2, at 2°C [1, 3]. The extracted contractile protein was separated by centrifugation at 105,000g for 1 h. The resulting residue of cardiac microsomes was washed 3 times with 0.01 M Tris−HCl buffer, pH 7.4, at 10,000g (10 min). The residue of cardiac microsomes was then suspended in 0.25 M sucrose, pH 7.4, and kept at −10°C. The writers have shown that the ATPase activity of microsomes remains virtually constant during keeping under these conditions for 30-40 days. ATPase activity was judged from the increase in concentration of inorganic phosphorus (P_i) in the course of the reaction (30 min, 37°C). The composition of the incubation medium (in 1 ml) was: 50-100 μg microsomal protein, 4 mM ATP-Na_2, 5 mM $MgCl_2$ (or 5 mM $CaCl_2$), 50 mM Tris-HCl, pH 7.4. Activity of the enzyme was expressed in micromoles P_i removed from ATP per hour per milligram protein. P_i was determined by the method of Lowry and Lopes [7] and the protein content by Lowry's method [8]. The results were subjected to statistical analysis, using the Student−Fisher criterion.

EXPERIMENTAL RESULTS

The results showed that the yield of microsomal protein, both under normal conditions and after injection of vasopressin, was 5.4 and 15 mg/g wet weight of tissue for the heart and liver, respectively. Treatment of cardiac microsomes with KCl solution of high ionic strength led to extraction of the contractile proteins, accounting for about 50% of the total weight of the microsomes.

As Table 1 shows, activities of Ca-ATPase (22.6 units of activity) and Mg-ATPase (23.5 units of activity) of the cardiac microsomes were almost identical. Similar results have been obtained by a study of the ATPase properties of the sarcolemma of rabbit skeletal muscles [1]. Intravenous injection of vasopressin caused an increase in ATPase activity of the cardiac microsomes: Mg-ATPase activity by 15%, Ca-ATPase by 16%. This increase was possible connected with changes in the membrane permeability of the heart cells which, in turn, led to changes in the contractile process in the relaxation phase. This hypothesis is supported by the results of a cardiographic investigation, which indicated slowing of the cardiac rhythm and of the respiration rate 1 h after injection of vasopressin. Comparison of the results with the changes in hepatic microsomal ATPase activity shows that normally there is a difference in the action of Mg^{2+} and Ca^{2+} ions on the ATPase activity of hepatic microsomes. Activity of Mg-ATPase (9.1 units of activity) was 28% higher than that of Ca-ATPase (6.5 units of activity). Injection of vasopressin caused a reduction in ATPase activity of the hepatic microsomes: Mg-ATPase activity by 11% and Ca-ATPase activity by 20% (Table 1).

Intravenous injection of vasopressin thus causes changes in the microsomal ATPase activity of the rabbit heart and liver, reflecting injury to the microsomal structures; these changes, moreover, differed in direction depending on the physiological activity of the cell.

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EFFECT OF PARATHORMONE AND THYROCALCITONIN ON Na,K-ATPase IN CELL MEMBRANES OF RAT BRAIN AND KIDNEY

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The effect of parathormone (PH) and thyrocalcitonin (TCT) on ATPase enzyme systems of membrane preparations from the cerebral cortex and renal cortex of rabbits was studied in vitro and in vivo. Both in vitro and in vivo PH was found to increase the activity of transport Na,K-ATPase and Ca-activated ATPase in the cell membranes of the kidneys and brain. TCT produced similar, but much less marked changes in these ATPases only in experiments in vivo. Both hormones were virtually without effect on Mg-ATPase activity of the brain and kidney membranes. It is suggested that PH acts directly on membranous structures possessing Na,K-ATPase activity, whereas TCT acts indirectly on them.

KEY WORDS: parathormone; thyrocalcitonin; rat cerebral and renal cortex; ATPase activity.

Under the influence of parathormone (PH) and thyrocalcitonin (TCT) the intracellular Ca^{2+} concentration undergoes changes in different directions in different organs and tissues [2, 3]. The mechanisms of action of these hormones are not yet clear. It was therefore decided to investigate the effect of these hormones on the activity of cell membrane Na,K-ATPase, an enzyme responsible for the active transport of monovalent cations. It is important to note that intracellular Ca^{2+} ions are regulators of Na,K-ATPase activity [4].

In this investigation the effect of PH and TCT was studied in vitro and in vivo on various ATPases, including Na,K-ATPase, from membrane fractions of rat brain and kidney tissue.

EXPERIMENTAL METHOD

Experiments were carried out on 64 rats weighing 150-200g. PH (Hormon-Chemie, West Germany) was injected subcutaneously into the hind limbs of the rats daily for 5-40 days, made up in 0.14 M NaCl in a volume of 0.2 ml, equivalent to a dose of 10 i.u. (2 Collip units)/100 g body weight. In the experiments in vitro PH was diluted with 20 mM Tris-HCl, pH 7.4, and added in doses of 0.0002, 0.02, and 2 i.u. TCT (from the All-Union Research Institute of Technology of Blood Substitutes and Hormonal Preparations) was injected intraperitoneally for 2-30 days in a daily dose of 5 i.u./100 g body weight. In the experiments in vitro, TCT was diluted with 0.14 M NaCl and added in doses of 0.0002 and 0.02 i.u. per sample. The membrane preparations used in the

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