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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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TABLE 1. Effect of PH in vivo on ATPase Activity of Membrane Preparation from Rat Brain and Kidneys (in μ moles P_i/mg protein/h; M ± m)

Experimental conditions	Kidneys			Brain		
	Ca-AtPase	Mg-ATPase	Na,K-ATPase	Ca-ATPase	Mg-ATPase	Na,K-ATPase
Intact rats (n = 11)	9,4±0,3	13,3±0,3	6,0±0,6	6,0±0,2	11,4±0,2	5,8±0,4
PH injected for 5 days (n = 5), % of control	9,7±0,3 104	12,2±0,4 92	10,6±0,4 177*	7,4±0,1 123*	12,4±0,3 109	8,2±0,4 141*
PH injected for 10 days (n = 5), % of control	11,1±0,2 118*	14,0±0,4 105	8,1±0,4 135*	8,3±0,6 138*	11,6±0,4 102	9,5±0,6 164*
PH injected for 20 days (n = 5), % of control	10,8±1,0 115	14,8±0,5 111*	6.8±1,0 113	9,4±0,5 157*	12,2±0,5 107	9,3±1,0 160*
PH injected for 30 days (n = 7), % of control	10,7±0,4 114*	14,9±0,4 112*	8,6±0,5 143*	8,6±0,7 143*	12,2±0,6 107	8,3±0,5 143*
PH injected for 40 days (n = 5), % of control	10,7±0,3 114*	14,0±0,5 105	7,7±0,3 128*	8,8±0,4 147*	12,9±0,5 113*	6,9±1,1 119

Legend to Tables 1 and 2: Membrane preparations isolated from tissues of one rat. Three parallel experiments set up in each case. 2. n) Number of animals. 3. Statistically significant results, compared with control, at the $P < 0.05$ level indicated by asterisk.

TABLE 2. Effect of PH in vitro on ATPase Activity of Membrane Preparation from Rat Brain and Kidneys (in μ moles P_i/mg protein/h; M ± m)

Experimental conditions	Ca-ATPase	Mg-ATPase	Na,K-ATPase
Without PH (n = 6)	6,4±0,2	11,3±0,4	7,2±0,6
Addition of 0.0002 i.u. PH (n = 6), % of initial activity	7,5±0,3 117*	12,2±0,3 108	9,3±0,9 129*
Addition of 0.02 i.u. PH (n = 6), % of initial activity	7,3±0,4 114*	10,9±0,5 97	9,6±0,8 133*
Addition of 2 i.u. PH (n = 6), % of initial activity	8,9±0,3 139*	10,5±0,5 93	6,0±0,8 83*

*Same as in Table 1.

experiments were an unpurified fraction of mitochondria (10,000g, 10 min) after preliminary removal of the nuclear fraction (1000g, 10 min) from homogenates of the cerebral cortex and renal cortex of rats. The fractions were washed twice with isolation medium (0.25 M sucrose, containing 10 mM Tris-HCl, pH 7.4). Activity of the ATPases was determined [5] from the degree of increase in the inorganic phosphorus (P_i) concentration during incubation (10 min, 37°C). The composition of the incubation medium (in mM) was: ATP-Na_2 3, NaCl 100, KCl 20, $MgCl_2$ 5, $CaCl_2$ 5, Tris-HCl, pH 7.4, 50; protein 300-600 μg per sample (2 ml). Activity of Na,K-ATPase was calculated from the difference between the total and Mg-ATPase. Hydrolysis of ATP in the presence of Mg^{2+} and Ca^{2+} was taken to be due to the activity of Mg- or Ca-ATPase, respectively. P_i [11] and protein [13] were determined colorimetrically. The experimental results were subjected to statistical analysis using the Student–Fisher criterion [6].

EXPERIMENTAL RESULTS AND DISCUSSION

After administration of PH in vivo for different times, considerable activation of Na,K-ATPase and Ca-ATPase was found in the membranes of both brain and kidneys; in the latter case the activation was more marked for brain tissue (Table 1). It is interesting to note that, during prolonged administration of PH, the effect of Na,K-ATPase activation gradually diminished, possibly on account of the accumulation of a high intracellular Ca^{2+} ion concentration. Activity of Mg-ATPase in the brain and kidney tissue was virtually unchanged

after the action of PH in vivo. The possibility cannot be ruled out that PH interacts directly with membranous structures possessing Na,K-ATPase activity, for in experiments on membrane preparations of rat cerebral cortex in which various doses of PH were added in vitro, a similar activating effect was found (Table 2). In that case also, Ca-ATPase was activated, whereas Mg-ATPase activity was unchanged. Just as in the experiments in vivo, in experiments in vitro in which large doses of PH were tested, the effect of activation of brain Na,K-ATPase was reduced.

Less regular changes in ATPase activity in the membrane preparations from brain and kidney were found in response to the action of TCT. A significant increase in activity of Na,K-ATPase in brain membrane preparations and an increase in Ca-ATPase activity in kidney preparations were observed only in the early stages of administration of this hormone (2nd and 5th days). In the doses used, TCT evidently had no direct effect on ATPase, and the effects that were observed could have been due to compensatory hyperproduction of PH. In preliminary experiments in vitro TCT had no significant effect on ATPase activity in membrane preparations from the rat cerebral cortex.

There are interesting data in the literature on activation of Na,K-ATPase in the membranes of the liver, kidneys, and skeletal muscles of rats by thyroid hormones [10], in the microsomes of the rat hypothalamus by angiotensin II [12], in the microsomes of the rat kidney and cerebral cortex by bradykinin [8], in the microsomes of the rabbit heart by insulin [7], and in membranes of the rat kidney by vasopressin [1]. The action of these hormones and active polypeptides, as of the hormones PH and TCT studied in the present investigation, on the Na,K-ATPase of cell membranes may be exerted indirectly through functional systems of cyclic nucleotides. By interacting with receptors, PH is known to cause the accumulation of cyclic AMP in the tissues [9], and cyclic AMP-dependent protein kinase can phosphorylate the proteins of cell membranes, thereby controlling their permeability.

PH thus stimulates the function of transport ATPase systems of the kidney and brain cell membranes. The action of PH thus revealed may be considered to be connected with one of the clinical manifestations of hyperparathyroidism, namely the massive deposition of Ca^{2+} in the tissues.

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