EFFECT OF BRADYKININ ON ACTIVITY OF MICROSOMAL Na, K-ATPase OF RAT KIDNEY AND BRAIN

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Experiments in vitro showed that bradykinin, in a concentration of $10^{-3}-10^{-12}$ M, affects neither microsomal Mg- or Na, KATPase from the rat kidney. Activation of microsomal Na, K-ATPase from the kidney and cerebral cortex was activated (by 30-40%) 20 min after injection of bradykinin (8 μg/g body weight) into the caudal vein; the Mg-ATPase activity was unchanged under these conditions.
KEY WORDS: Na, K-ATPase; rat kidney and brain microsomes; bradykinin.

Active polypeptides (AP) are known to play an important role in the pathogenesis of inflammation [1, 2]. The early stages of inflammation are also known to be accompanied by an increase in the AP content in the focus of inflammation [3] and by a disturbance of membrane permeability. Besides histamine and other substances like it, in some inflammations it is possible that the AP induce changes in membrane permeability and also in the active transport of cations through the membrane and of the transport of metabolites linked with it. Papers have been published on the effect of angiotensin and vasopressin in experiments in vitro [4, 5] and in vivo [6] on the activity of the enzyme Na, K-ATPase, responsible for the active transport of cations through cell membranes. However, no data can be found in the literature on the effect of another AP, bradykinin, on ATPase.

The object of this investigation was to study the effect of bradykinin in vitro and in vivo on the activity of microsomal Na, K-ATPase from rat kidney and brain.

EXPERIMENTAL METHOD

Rats weighing 160-170 g were used. After decapitation of the animals the tissue of the whole kidneys and cerebral cortex (gray matter) was washed with physiological saline to remove blood and then homogenized in 10 volumes of 0.32 M sucrose containing 5 mM Tris-HCl buffer, pH 7.4, at 0-2°C. Successive fractions of nuclei (800 g, 10 min), coarse mitochondria (10,000 g, 10 min), and microsomes (60,000 g, 40 min) were obtained on the MSE Superspeed-65 (England) ultracentrifuge. The microsomes and coarse mitochondria (for the brain) were washed with 0.32 M sucrose containing 5 mM EDTA under the same conditions of centrifugation. The fractions were suspended in deionized water and, if necessary (bradykinin, experiments in vitro) stored at −10°C and used after a single freezing and thawing. Protein was determined by Lowry's method. For optimal detection of Na, K-ATPase activity the method [7] of treatment (immediately before determination) of a suspension of membrane fractions with Na deoxycholate (DOC) (Schuchardt – Munich, West Germany) was used. The suspension (3 mg protein/ml) was treated for 30 min (20°C) with DOC in a final concentration of 0.1% in 20 mM imidazole-HCl, pH 7.2. The activity of the ATPases was determined from the increase in the content of inorganic phosphorus in the course of the reaction (20 min, 37°C). The reaction was started by the addition of ATP after preincubation for 5 min. Inorganic phosphorus in the 5%

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TABLE 1. Effect of Bradykinin (8 μg/g) on ATPase Activity of Kidney and Brain Membrane Preparations (freshly isolated)

Object, fraction	Statistical index	ATPase activity (μmoles Pi/mg protein/h)					
		normal				bradykinin	
		Mg-ATPase		Na, ATPase		Mg-ATPase	Na, K-ATPase
		−DOC	+DOC	−DOC	+DOC	+DOC	+DOC
Kidneys, microsomes	M	17,4	9,2	2,2	6,0	11,1	8,8
	$\pm m$	2,2	0,7	1,0	0,5	1,0	0,6
	n	8	5	8	5	6	7
	%	—	100	—	100	120	145*
Cerebral cortex, microsomes	M	12,0	10,5	4,6	8,8	11,7	11,6
	$\pm m$	1,3	0,7	0,9	0,5	0,9	0,7
	n	6	7	6	7	8	5
	%	—	100	—	100	111	132*
Cerebral cortex, coarse mitochondria	M	10,4	8,1	3,6	5,8	8,9	7,2
	$\pm m$	1,2	0,6	0,6	0,7	0,7	0,75
	n	5	5	5	5	6	6
	%	—	100	—	100	109	124

Legend: 1) in the "normal" columns ATPase activity is shown without (−) and with (+) DOC treatment; 2) n gives number of experiments; 3) results for which $P < 0.05$ are marked by an asterisk.

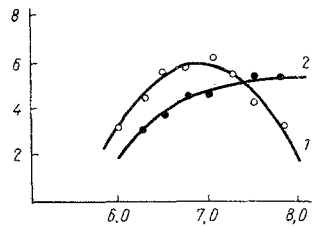

Fig. 1. Kidney microsomal ATPase activity as a function of pH (mean results from 4-6 experiments): 1) Na, K-ATPase, 2) Mg-ATPase. Abscissa, pH units; ordinate, activity of ATPases (in μmoles Pi/mg protein/h).

TCA-supernatant was determined photocolorimetrically [8] at 700 nm (Spekol, East Germany). The Na, K-ATPase activity in micromoles Pi/mg protein/h was calculated from the difference between the activities of total and Mg-ATPase; in addition, the Na, K-ATPase activity was verified by the addition of 0.1 mm ouabain (Calbiochem, USA) to the samples. The composition of the incubation sample (1 ml) for the brain fractions was (in millimoles): NaCl 100, KCl 20, $MgCl_2$ 5, ATP-Na_2 (Reanal, Hungary) 2, imidazole-HCl, pH 6.6, 50, and protein 100-400 μg; for kidney microsomes: NaCl 170, KCl 6, $MgCl_2$ 7.5, the remaining components in the same amounts. The pH of the incubation medium was 6.6. Bradykinin triacetate (Reanal, Hungary) was injected into the caudal vein of the rats in a dose of 100 μg in 0.1 ml 0.14 M NaCl (8 μg/g body weight). Exposure lasted 20 min.

EXPERIMENTAL RESULTS AND DISCUSSION

It must first be pointed out that the treatment of kidney and brain microsomes and the fraction of coarse brain mitochondria with DOC led to a marked decrease in Mg-ATPase activity and an increase in Na, K-ATPase activity, which was particularly marked in the case of the rat kidney microsomes (Table 1). When membrane preparations are treated with DOC it is important to allow for the concentrations of DOC and protein in the suspension and the pH of the medium. The pH optimum for the determination of ATPases in the present experiments for kidney microsomes was 6.6 (Fig. 1).

The investigations showed that in the experiments in vitro bradykinin, over a wide range of concentrations ($10^{-3}-10^{-12}$ M) did not affect the activity of rat kidney microsomal Mg- and Na, K-ATPases even after preliminary incubation of the polypeptide with the enzyme preparation for 10 min.

A statistically significant activation of the microsomal Na, K-ATPase of the brain (32%) and kidneys (45%) and a tendency for this activity to increase in the fraction of coarse brain mitochondria were observed 20 min after the injection of bradyinin into the caudal vein of the rats. The Mg-ATPase activity in the test fractions was unchanged after the action of bradykinin. These findings indicate a specific and unidirectional change in the activity of the transport Na, K-ATPase of kidney and brain membrane preparations.

Evidence that AP (angiotensin and vasopressin) influence the transport of Na^{22} and K^{40} through cell membranes ig given in the literature [9, 10]. However, the question of whether the action of AP on transport Na, K-ATPase is direct or indirect has not yet been finally settled. It is stated that vasopressin [4] and angiotensin [4, 5], in experiments in vitro in concentrations of $10^{-8}-10^{-2}$ M, moderately activate microsomal Na, K-ATPase from the hypothalamus, intestine, and adrenals but do not affect the microsomal Na, K-ATPase of the cerebral cortex. Vasopressin in high concentrations in vivo inhibits the microsomal Na, K-ATPase activity of the kidneys [6]. The action of AP on membrane permeability is evidently specific in different tissues and, in addition, the action of different APs may be selective.

The activation of microsomal Na, K-ATPase of the rat kidney and brain on contact with bradykinin observed in the present experiments can be attributed to various causes: disturbance of the conformation of the membranes and of the functions of the endogenous regulators of Na, K-ATPase activity and of active transport of cations, such as catecholamines and hormones [11] or Ca^{++} ions. The intracellular Ca^{++} is an effective inhibitor of the Na, K-ATPase of the kidneys [12], brain [13], and erythrocytes [14]. The effect of AP on Ca^{++} transport through membranes has been described in the literature in the case of angiotensin [15], which intensifies the outflow of Ca^{++} from Ca^{45}-loaded microsomes of the muscular coat of the rabbit aorta. Further experiments will reveal the finer details of the mechanism of the activation by bradykinin of the microsomal Na, K-ATPase of certain tissues; this is of great importance to research into the way in which the various plasma kinins participate in the disturbances of membrane permeability of endothelial and other cells during the development of acute inflammation.

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