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MODULATION OF RENAL ATPase ACTIVITIES BY CYCLIC AMP

E.M. GIESEN, J.L. IMBS, M. GRIMA, M. SCHMIDT and J. SCHWARTZ

Institut de Pharmacologie, Faculté de Médecine,
11, rue Humann, 67000 Strasbourg, France

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A possible link between cellular cyclic AMP content and Na^+K^+ATPase activity was investigated in homogenates of rat kidney. Enzyme kinetics of Mg^{2+} and Na^+K^+ATPase were run in the presence of cyclic AMP, dibutyryl cAMP and compounds expected to elevate cyclic AMP levels such as forskolin, a potent adenylate cyclase activator, IBMX,an inhibitor of phosphodiesterases, and the beta-agonist isoproterenol. Medullary Na^+K^+ATPase is strongly inhibited by cyclic AMP whereas cortical Na^+K^+ATPase was stimulated in the same conditions. The correlation between ATPase activity and cellular cyclic AMP content supports the concept of a possible regulation of the enzyme by cyclic AMP.

Even though the involvement of Na^+K^+ATPase in active Na^+ transport is well established now (1), the regulation of this sodium pump is still poorly understood. Cyclic AMP plays an unquestionnable role in the regulation of membrane permeability and passive Na^+ flux in the kidney but the implication of cyclic AMP in active Na^+ transport has been investigated relatively little, although in other systems the possibility of a link between the adenylate cyclase system and Na^+K^+ATPase had already attracted attention in 1969 (2).

Here, enzyme kinetics of Mg^{2+} and Na^+K^+ATPase were investigated in the presence of cyclic AMP, dibutyryl cAMP and of compounds expected to elevate cyclic AMP levels such as forskolin, a potent adenylate cyclase activator, IBMX, an inhibitor of phosphodiesterases and the beta-agonist isoproterenol. The responses of cortical and medullary ATPases to cyclic AMP were opposite.

METHODS

Male Wistar rats (CESAL, Montmedy, France), weighing 170 g to 200 g were killed by decapitation. The left kidney was rapidly removed and dissected on ice. The kidney was totally stripped and the papilla was discarded. Cortex and medulla were dissected and homogenized separately with a polytron blender

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(setting 4, 60 sec) in 10 ml icecold Tris-EDTA buffer (Tris 50 mM, EDTA 1 mM, pH 7.4). The homogenate was then diluted with the same buffer in order to obtain a suspension containing 1 mg protein per ml. Homogenate of the cortex was referred to as "cortex", homogenate of the medulla as "medulla". Medulla was not further purified and occasionally contaminated with some glomeruli. No attempt was made to characterize both fractions histologically.

For the determination of ATPase activity, 50 µl samples of homogenate (or 100 µl in the case of 1 min incubation time) were incubated at 37°C in 1 ml incubation medium containing 50 mM Tris, 5 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl, 1 mM EDTA, 2 mM ATP and 1 mM ouabain if necessary, pH 7.4. Na^+K^+ATPase activity was estimated by substracting Mg^{2+}ATPase activity, measured in the presence of ouabain, from total ATPase activity. All drugs were dissolved in Tris-EDTA buffer except for forskolin which was dissolved in ethanol (95% V/W). Ten µl of these solutions were added to the incubation medium to obtain a 10^{-6} M concentration of cyclic AMP or dibutyryl cAMP and a 10^{-4} M concentration of forskolin, isoproterenol or IBMX. We checked that 10 µl of ethanol did not modify ATPase activity. Untreated samples were run in parallel for control ATPase activities. At the end of the incubation, 1 ml TCA (10%) was added and the samples were centrifuged (10 min, 3000 rpm) in the cold. Inorganic phosphate (P_i) was determined by the method of King (3) on 750 µl samples of supernatent. In our hands, the variation of the method was 2%. Protein content was estimated according to Lowry et al. (4). Student's t test was used for statistical analyses.

RESULTS

We first investigated whether medullary Na^+K^+ATPase is inhibited by cAMP (Fig. 1). In control samples, the addition of ATP enhances P_i formation instantaneously, no lag period of enzyme activity was observed. The accumulation of P_i is very rapid during the first minutes of the experiment. When the medulla was treated with cyclic AMP, dibutyryl cAMP, forskolin or IBMX, P_i accumulation was reduced, not instantaneously but only after a 2 min lag period. Then, Na^+K^+ATPase was inhibited by about 50%. Isoproterenol inhibited Na^+K^+ATPase faster than the other compounds; already after one min P_i accumulation was significantly reduced. After 5 min the inhibition was overcome by the system and Na^+K^+ATPase activity in the presence of cyclic AMP became identical to the control value (Fig. 1, A). The differences between control activity and activities measured in the presence of a high cyclic AMP level, although still apparent, were statistically nonsignificant (Fig. 1,B to E). When the incubation was prolonged over 10 min (Table 1,A) or 30 min (Table 1,B) no difference between treated and control samples was observed. Only the combined action of IBMX and cyclic AMP, forskolin or isoproterenol causes a very slight but nonsignificant decrease of P_i. IBMX alone led to a minor, nonsignificant, increase in P_i production.

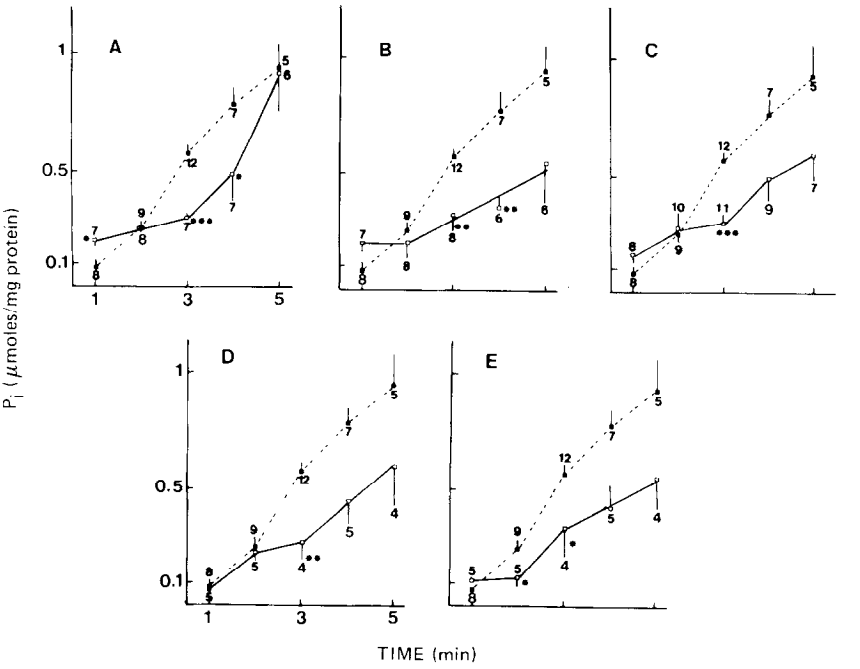

FIGURE 1 Inhibition of Na^+K^+ATPase in the medulla by cyclic AMP.

Na^+K^+ATPase was measured at different times (1 min to 5 min) and under different conditions : in the presence of A) cyclic AMP, B) dibutyryl cAMP, C) forskolin, D) IBMX, E) isoproterenol. The number of experiments is indicated on the graphic, bars are SEM. Asterisks stand for statistical significance.

*** $=P < 0.001$, ** $=P < 0.01$, * $=P < 0.05$.

TABLE 1,A and 1,B

ATPase activity in renal medulla

A	IBMX		B	IBMX	
Additif	-	+	Additif	-	+
	1.01 ± 0.09 (5)	1.3 ± 0.23 (5)		3.48 ± 0.35 (10)	3.83 ± 0.29 (8)
cyclicAMP	1.37 ± 0.4 (7)	0.86 ± 0.16 (5)	cyclicAMP	3.35 ± 0.27 (12)	3.6 ± 0.25 (9)
DIBUTYRYL cAMP	0.95 ± 0.19 (5)		DIBUTYRYL cAMP	3.74 ± 0.26 (11)	
FORSKOLIN	1.06 ± 0.10 (8)	0.86 ± 0.07 (5)	FORSKOLIN	4.08 ± 0.23 (12)	3.73 ± 0.27 (10)
ISOPROTE-RENOL	1.06 ± 0.11 (5)	0.76 ± 0.08 (5)	ISOPROTE-RENOL	4.00 ± 0.48 (6)	3.47 ± 0.28 (10)

P_i (µmoles per mg protein) that had accumulated by the activity of Na^+K^+ATPase under various conditions and after a 10 min (A) or 30 min (B) incubation period was measured. Values are averages ± SEM, the number of experiments is given in brackets.

TABLE 2

ATPase activity in renal cortex

	Mg^{2+} ATPase		$Na^+ K^+$ATPase	
	2 min	3 min	2 min	3 min
CONTROL	1.05 ± 0.04	1.53 ± 0.06	0.11 ± 0.03	0.28 ± 0.05
cyclic AMP	1.11 ± 0.08	1.52 ± 0.07	0.17 ± 0.01	0.36 ± 0.07
DIBUTYRYL cAMP	1.11 ± 0.05	1.50 ± 0.05	0.15 ± 0.01	0.45 ± 0.05*
FORSKOLIN	1.08 ± 0.04	1.47 ± 0.06	0.17 ± 0.05	0.51 ± 0.05*
IBMX	1.13 ± 0.06	1.65 ± 0.04	0.19 ± 0.03	0.38 ± 0.04
ISOPROTERENOL	1.12 ± 0.06	1.71 ± 0.04	0.21 ± 0.06	0.45 ± 0.07

Cortex homogenates were incubated for 2 min or 3 min. Mg^{2+} ATPase and Na^+K^+ATPase activities were determined as μmoles P_i formed per mg protein. Each result is the average of 3 experiments (2 min) or 5 experiments (3 min) ± SEM. Asterisks indicate significant differences ($p < 0.001$).

Medullary Mg^{2+} ATPase, unlike Na^+K^+ATPase, is completely insensitive to an increase of cyclic AMP level. No modification of Mg^{2+} ATPase was noticed under the same conditions used for Na^+K^+ATPase (data not shown).

Cortical Mg^{2+} and Na^+K^+ATPase were assayed after a 2 and 3 min incubation period, when the effect of cyclic AMP was maximal in the medulla. Contrary to medullary Na^+K^+ATPase, cortical Na^+K^+ATPase was stimulated by cyclic AMP, dibutyryl cAMP or one of the compounds that enhance cyclic AMP production. This stimulation was particularly pronounced and statistically highly significant in the presence of forskolin and dibutyryl cAMP (Table 2). Cortical Mg^{2+}ATPase activity remained unchanged.

DISCUSSION

Forskolin is well known as a potent activator of adenylate cyclase in many systems, including kidney membranes (5) and a kidney derived cell line (6). It also inhibits Na^+K^+ATPase in rat heart (7). Na^+K^+ATPase in renal medulla but not in the cortex was inhibited by forskolin (8) but the question whether the forskolin effect is direct or mediated by adenylate cyclase remained unanswered. Here we demonstrate that cyclic AMP, dibutyryl cAMP and

all the tested compounds which elevate cyclic AMP levels inhibit medullary Na^+K^+ATPase as does forskolin. We therefore propose the concept that cyclic AMP is responsible for the inhibition of medullary Na^+K^+ATPase. This result is in agreement with the inhibition of Na^+K^+ATPase by cyclic AMP in other cellular systems, such as liver plasma membranes (9,10), myocardial sarcolemn (11), adenocarcinoma (12), fibroblasts (13) and rat heart (7). It remains undetermined if cyclic AMP modulated active Na^+ transport directly, involving the phosphorylation of a specific protein by a cyclic AMP dependent phosphokinase, as it was suggested for phosphate transports (14). An alternative interpretation is based on the assessment that cyclic AMP primarily modulates cellular ion content (15,16,17) and that the changes in Na^+ and Cl^- concentration stimulate or inhibit the Na^+ pump (18,19). If the inhibition of medullary Na^+K^+ATPase by cyclic AMP reflects a physiologically relevant process, it should be interpreted as a means to ensure a rapid and reversible adaptation of the active Na^+ transport to changes in cellular ion content because the inhibition of Na^+K^+ATPase disappeared already after 5 min. The disappearance of ATPase inhibition by cyclic AMP cannot be explained by the degradation of cyclic AMP by phosphodiesterases as we initially suspected because the same effect occurred in the presence of dibutyryl cAMP and the phosphodiesterase inhibitor IBMX.

Surprisingly, cyclic AMP has the opposite effect on cortical as compared to medullary Na^+K^+ATPase. This divergence is difficult to interprete although differences in the responsiveness of cortex and medulla to certain stimuli have occasionnally been reported in the literature, vasopressin for example increases the concentration of cyclic AMP in both inner and outer medulla of rat kidney but not in the cortex (20). Medullary Na^+K^+ATPase is more sensitive to adrenalectomy and aldosterone treatment than the cortical enzyme (21). Using the technique of microdissection, Katz et al. (21) and Garg et al. (23) demonstrated important differences in ATPase activities along the nephron but did not investigate the effect of cyclic AMP on Na^+K^+ATPase. Besides these differences in the enzymatic equipment of renal epithelia, it should not be

neglected that an important part in renal functions is played by the hemodynamics of the kidney. In this context, it seems noteworthy to stress that the cortex is much richer in smooth muscle cells of vascular origin than the medulla and that isolated smooth muscle cells appear to respond to cyclic AMP by a stimulation of Na^+K^+ATPase activity (24). A preparation of vascular smooth muscle or of purified glomeruli and of purified medullary tubules would clearly help localize the observed differences.

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