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Bradykinin modulates the ouabain-insensitive Na^+-ATPase activity from basolateral membrane of the proximal tubule

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Abstract

This paper studies the modulation by bradykinin of the ouabain-insensitive Na^+-ATPase activity in both renal cortex homogenate and basolateral membrane from proximal tubule. The increase in bradykinin concentration from 10^{-14} to 10^{-10} M stimulated the ouabain-insensitive Na^+-ATPase activity in cortex homogenates about 2.2-fold, but inhibited the enzyme activity of basolateral membrane preparations by 60%. In both preparations, the maximal effect was obtained with 10^{-10} M bradykinin. Further increase in the concentration of bradykinin completely abolished these effects. The antagonist of the B_2 receptor, Hyp^3, completely abolished the effect of 10^{-10} M bradykinin on the Na^+-ATPase activity in the basolateral membrane preparation in a dose-dependent manner, but had no effect on the bradykinin stimulated enzyme activity of the cortex homogenate. Furthermore, in the presence of 10^{-7} M Hyp^3, 10^{-10} M bradykinin stimulated the Na^+-ATPase activity by 45% in the basolateral membrane preparations. The increase in des-Arg^9-bradykinin concentration from 10^{-12} to 10^{-7} M, an agonist of the B_1 receptor, stimulated the Na^+-ATPase activity of the cortex homogenates and of the basolateral membrane preparations by 105 and 148%, respectively. In the presence of 25 μM mergetpa, an inhibitor of kininase I, the increase in bradykinin concentration from 10^{-12} to 10^{-10} M promoted similar inhibition of the Na^+-ATPase activity of both cortex homogenates and basolateral membrane preparations. These results suggest that bradykinin stimulated the Na^+-ATPase activity of proximal tubule through the interaction with B_1 receptors and inhibited the enzyme through the interaction with B_2 receptors. Furthermore, the cortex homogenate expresses a kininase I activity that cleaves bradykinin to des-Arg^9-bradykinin.

Keywords: Na^+-ATPase; Bradykinin; Furosemide; Proximal tubule

1. Introduction

The kallikrein–kinin system is involved in several regulatory processes of the organism, such as hypertension, coagulation and inflammation [1–3]. In spite of the large amount of experimental data found in the literature, the physiological role of the renal kallikrein–kinin system remain unclear. It was proposed that kinins regulate natriuresis and diuresis through the modulation of the renal blood flow and osmotic gradient of the renal medulla [3–5]. On the other hand, the infusion of bradykinin into the renal artery induces natriuresis without altering glomerular flow

Abbreviations: EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine *N*′-2-ethanesulfonic acid; Tris, tris(trishydroxymethyl)aminomethane; ATP, adenosine triphosphate (sodium salt); PMSF, phenylmethylsulfonyl fluoride

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rate (GFR), suggesting a direct effect on tubular Na^+ transport [6,7].

It has been established that the effect of kinins is mediated by receptors distributed on the plasma membrane of many cell types [1,4,5]. Two types of kinin receptors have been described: B_1 and B_2 [1,4,5]. B_2 receptors have higher affinity for kallidin and bradykinin, whereas B_1 receptors are more sensitive to des-Arg kinins [2,5]. Experimental evidence indicates that the effects of bradykinin on ionic transport along the different renal tubular segments are mediated by B_2 receptors, while the effects on renal hemodynamics are mediated by B_1 receptors [2,8].

Two sodium pumps are expressed in the basolateral membrane of the renal proximal tubule cells: the ouabain-sensitive Na^+,K^+-ATPase and the ouabain-insensitive, furosemide-sensitive Na^+-ATPase [9–17]. The ouabain-insensitive Na^+-ATPase transports Na^+ against an electrochemical gradient, is not stimulated by K^+ [11,14] and its physiological role is not clear. Recently, we proposed that the inhibitory effect of adenosine on the Na^+ reabsorption in the proximal tubule could be mediated, at least in part, by the inhibition of the ouabain-insensitive Na^+-ATPase [12].

Since bradykinin modulates the Na^+ transport in different segments of the nephron, we investigated its effects on the Na^+-ATPase activity in basolateral membranes of the proximal tubule cells and cortex homogenate from kidney pig. The data obtained indicate that bradykinin modulates the ouabain-insensitive Na^+-ATPase through both B_1 and B_2 receptors.

2. Materials and methods

2.1. Materials

ATP, ouabain, furosemide, azide, mannitol, bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), des-Arg^9-bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe), D-Arg,[Hyp^3,Thi^{5,8}-D-Phe^7]-bradykinin (Hyp^3), captopril and D,L-2-mercaptomethyl-3-guanidinoethylthiopropanioic acid (mergetpa) were purchased from Sigma Chem; Percoll was from Pharmacia. All reagents were of the highest purity available. [^{32}P]Pi was obtained from the Institute of Energetic and Nuclear Research, Brazil. All solutions were prepared with deionized glass-distilled water. [γ-^{32}P]ATP was prepared as described by Maia et al. [18].

2.2. Preparation of cortex homogenates and isolated basolateral membrane

The cortex homogenates and basolateral membranes were prepared from adult pig kidney. The kidneys were obtained in a commercial slaughter house immediately after the death of the animals. The kidneys were removed immediately after animal death and maintained in cold isosmotic solution containing (mM): sucrose 250, HEPES-Tris (pH 7.6) 10, EDTA 2 and PMSF 1. Thin slices of the cortex (cortex–corticis) were removed with a scalpel. After dissection, slices were homogenized in the same cold isosmotic solution with a Teflon and glass homogenizer. The homogenate was centrifuged for 10 min at 3000 rpm in a Sorvall centrifuge using SS-34 rotor at 4°C. The supernatant was collected and stored at −4°C. The fraction containing the basolateral membrane was isolated by the Percoll gradient method [19] modified from that described by Scalera et al. [20] and Sacktor et al. [21]. The membrane preparation was resuspended in 250 mM sucrose at a final concentration of 20–30 mg of protein ml^{-1} and was stored at −4°C.

2.3. Measurement of ATPase activity

Except as noted in Section 3, the composition of the standard assay medium (0.2 ml) contained: 10 mM $MgCl_2$; 5 mM [γ-^{32}P]ATP; 20 mM HEPES-Tris (pH 7.0); 5 mM azide; and 90 mM NaCl for the measurement of the Na^+-ATPase activity. The final osmolality was adjusted with mannitol to 300 mOsm kg^{-1}.

The ATPase activity was measured according to the method described by Grubmeyer and Penefsky [22]. The reaction was started by the addition of cortex homogenate or isolated basolateral membrane to a final protein concentration of 0.3–0.5 mg ml^{-1}. The reaction was stopped after 30 min by the addition of charcoal activated by HCl (0.1 N). The [^{32}P]Pi released was measured in an aliquot of the supernatant obtained after centrifugation of the

charcoal suspension for 20 min at 3000 rpm in a clinical centrifuge. Spontaneous hydrolysis of [γ-^{32}P]ATP was measured simultaneously in tubes where protein was added after the acid. The Na^+-ATPase activity was calculated by the difference between the [^{32}P]Pi released in the absence and in the presence of 2 mM furosemide, both in the presence of 1 mM ouabain [9,10]. The control values were 1.9 ± 0.1 and 8.2 ± 0.6 nmol ^{32}Pi mg^{-1} min^{-1} for cortex homogenate and isolated basolateral membrane, respectively. The Na^+,K^+-ATPase activity measured in isolated basolateral membrane (69.2 ± 7.2 nmol ^{32}Pi mg^{-1} min^{-1}) is 8.9 times higher when compared to the activity found in cortex homogenate (7.8 ± 0.3 nmol ^{32}Pi mg^{-1} min^{-1}). Protein concentrations were determined by the Folin phenol method [23] using bovine serum albumin as a standard. Each experiment was performed in an independent preparation of basolateral membrane or cortex homogenate. The data were analyzed by two-way analysis of variance (ANOVA), considering the treatments as factors. The significance of the differences was verified by the Bonferroni *t*-test. Statistical analysis was made using absolute values and the results were expressed in percentage of the control.

3. Results

3.1. Effect of bradykinin on the Na^+-ATPase activity

To determine whether bradykinin regulates the furosemide-sensitive sodium pump present in the basolateral membrane from proximal tubule, we measured the ouabain-insensitive, furosemide-sensitive Na^+-ATPase activity of the cortex homogenate and basolateral membranes isolated from proximal tubule cells in the absence or in the presence of different bradykinin concentrations (from 10^{-6} to 10^{-14} M). The results illustrated in Fig. 1 show that the Na^+-ATPase modulation by bradykinin has a biphasic behavior. The increase in bradykinin concentration from 10^{-14} to 10^{-10} M stimulated the Na^+-ATPase activity in the homogenate preparation about 2.2-fold, but inhibited the enzyme activity in basolateral membrane preparation by 60%. In both preparations, the maximal effect was obtained with 10^{-10} M bradykinin. Further increase in the concentration

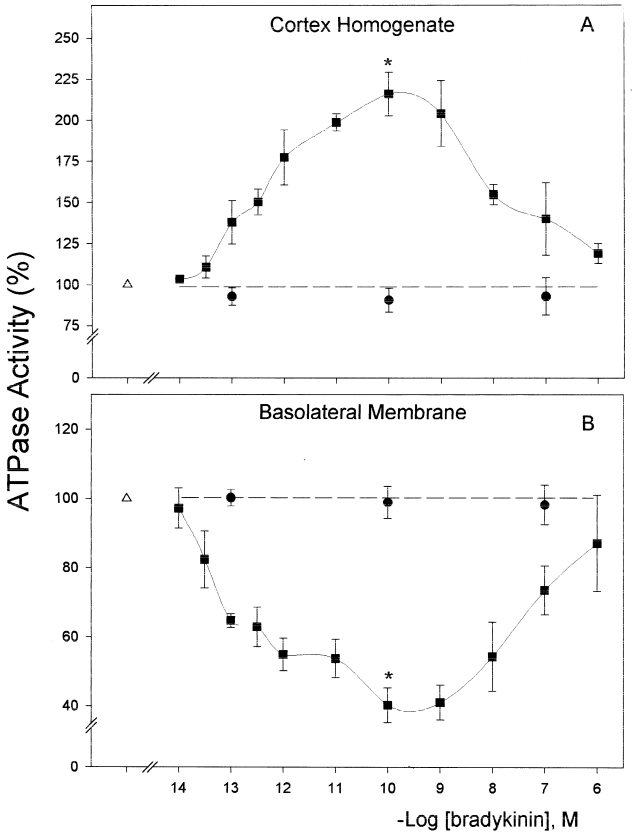
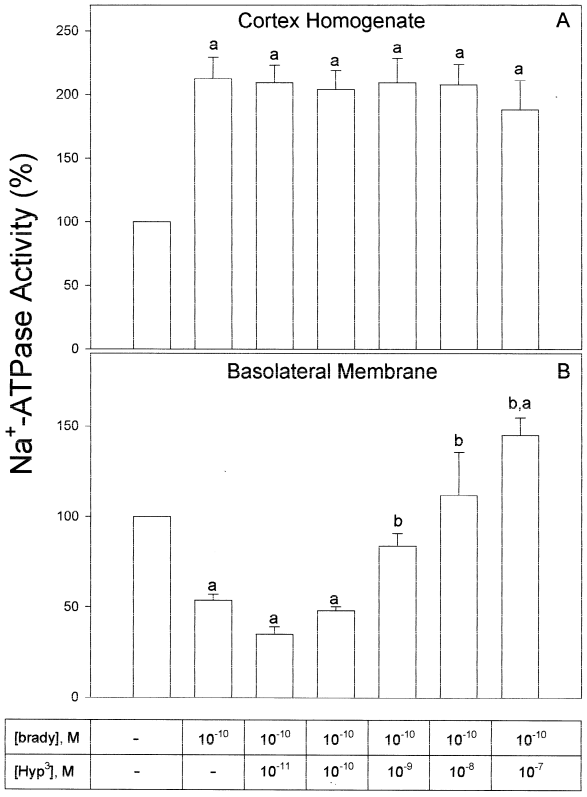


Fig. 1. Dependence of Na^+-ATPase (■) and Na^+,K^+-ATPase (●) activities in the cortex homogenate (A) and in isolated basolateral membrane (B) on bradykinin concentration. △, ATPase activity in the absence of bradykinin. The ATPase activity was measured as described in Section 2 ($n = 12$). The bradykinin concentration was increased from 10^{-14} to 10^{-6} M. The results are expressed as percentage of the control. *Statistically significant when compared to the control ($P < 0.05$).

of bradykinin completely abolished these effects in both preparations, and full reversal was obtained at a concentration of 10^{-6} M. In contrast, bradykinin used in the same concentrations range did not affect the ouabain-sensitive Na^+,K^+-ATPase activity, both in cortex homogenate and isolated basolateral membranes. Similar results were obtained when the proteins were preincubated with bradykinin during 30 min and the reaction was run in the absence of bradykinin (data not shown). Since the effects of bradykinin have been described to be mediated by receptors the generation of the signals and effects on the transport proteins might be expected to take some time. So, experiments were performed where the Na^+-ATPase activity was measured in the absence

or in the presence of bradykinin at different incubation periods (1, 3, 5, 10, 20 and 30 min). The effect of bradykinin was calculated as percentage in relation to the control (in the absence of bradykinin). Bradykinin only modulates the Na^+-ATPase activity after 5 min. After this point, the effect of bradykinin increases along the time of reaction reaching maximal effect in 20 min. These results are compatible with the hypothesis that the effects of bradykinin on the Na^+-ATPase activity of both cortex homogenate and basolateral membrane preparations are mediated by receptor.



[brady], M	-	10^{-10}	10^{-10}	10^{-10}	10^{-10}	10^{-10}	10^{-10}
[Hyp^3], M	-	-	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}

Fig. 2. Modulation of the effect of bradykinin on the ouabain-insensitive Na^+-ATPase activity in the cortex homogenate (A) and in isolated basolateral membrane (B) by Hyp^3. The ATPase activity was measured as described in Section 2 ($n=6$). The Hyp^3 concentration was increased from 10^{-11} to 10^{-7} M. Bradykinin 10^{-10} M (brady) was added where indicated. [a]Statistically significant when compared to the control ($P<0.05$). [b]Statistically significant when compared to the level of the Na^+-ATPase activity in the presence of 10^{-10} M bradykinin ($P<0.05$). The results are expressed as percentage of the control.

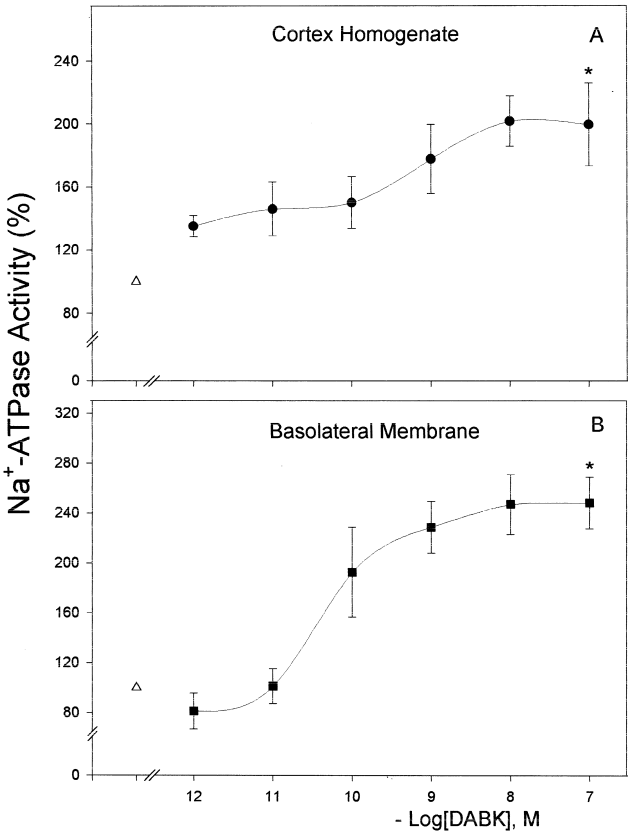


Fig. 3. Dependence of Na^+-ATPase activity in the cortex homogenate (A) and in isolated basolateral membrane (B) on des-Arg^9-bradykinin (DABK) concentration. The ATPase activity was measured as described in Section 2 ($n=5$). △, ATPase activity in the absence of bradykinin and des-Arg^9-bradykinin. The des-Arg^9-bradykinin concentration was increased from 10^{-12} to 10^{-7} M. The results are expressed as percentage of the control. *Statistically significant when compared to the control ($P<0.05$).

3.2. Bradykinin receptor agonist and antagonist

There are two known receptors for bradykinin: B_1 and B_2 [1,4,5]. Since bradykinin has different affinities for B_1 and B_2 receptors, the opposite effect of bradykinin on the Na^+-ATPase observed in homogenate and basolateral preparations could be explained by the binding of bradykinin to different receptors. To test this hypothesis, we studied the modulation of the effect of bradykinin by agonists and antagonists of the B_1 and B_2 receptors.

Fig. 2 shows the modulation of the effect of bradykinin on the Na^+-ATPase activity in both cortex homogenate and basolateral membrane preparations by Hyp^3, an antagonist of B_2 receptor. In the ho-

mogenate preparation, the stimulatory effect of 10^{-10} M bradykinin on the Na^+-ATPase activity was not modified by Hyp^3. On the other hand, the inhibitory effect of 10^{-10} M bradykinin on the Na^+-ATPase activity observed in basolateral membrane was completely reversed by Hyp^3 in a dose-dependent manner with maximal effect observed at the concentration of 10^{-8} M Hyp^3. In the presence of 10^{-7} M Hyp^3, 10^{-10} M bradykinin stimulated the Na^+-ATPase activity by 45%.

Fig. 3 shows that the addition of increasing concentrations (from 10^{-12} to 10^{-7} M) des-Arg^9-bradykinin, an agonist of B_1 receptor, stimulated the Na^+-ATPase activity of the cortex homogenate and basolateral membrane preparation by 105 and 148%, respectively. The maximal effect, in both preparations, was obtained at a concentration of 10^{-9} M des-Arg^9-bradykinin.

These data suggest that the interaction of bradykinin with B_2 receptor inhibits the Na^+-ATPase activity while its interaction with B_1 stimulates it.

3.3. Role of kininases

Bradykinin can be cleaved by kininases I and II. Kininase I (also called carboxypeptidase N) is an exopeptidase that removes the C-terminal Arg from bradykinin, while kininase II is a carboxydipeptidase that cleaves the dipeptide Phe^8-Arg^9 and thus inactivates bradykinin [1,5]. The differences in the effect of bradykinin on the Na^+-ATPase activity in basolateral membranes and homogenate preparations could be due to the cleavage of bradykinin by different kininases. In order to investigate this hypothesis, 25 μM mergetpa, an inhibitor of kininase I, and 25 μM captopril, an inhibitor of kininase II, were used in the presence of bradykinin [24,25]. The concentrations of the inhibitors were at least five times higher than those necessary to inhibit the kinases in other systems [24,25]. The data depicted in Fig. 4 show the modulation of the Na^+-ATPase by different concentrations of bradykinin (from 10^{-12} to 10^{-10} M) in the presence of mergetpa. It can be seen that an increase in bradykinin concentration up to 10^{-10} M, in the presence of 25 μM mergetpa, promoted a similar inhibition of the Na^+-ATPase activity of homogenate and basolateral membrane preparations. Further increase in the bradykinin concentration completely abolished this inhibition in both preparations, with maximal effect obtained with 10^{-6} M bradykinin. On the other hand, in the presence of 25 μM captopril the addition of 10^{-10} M bradykinin did not change the Na^+-ATPase activity of the basolateral membrane and homogenate preparations (Fig. 5).

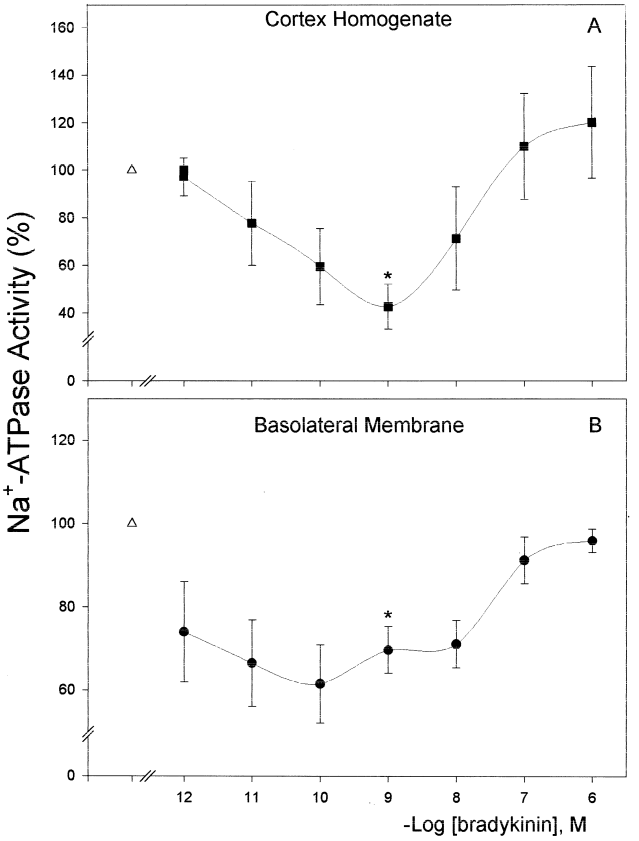


Fig. 4. Dependence of ouabain-insensitive Na^+-ATPase activity in the cortex homogenate (A) and in isolated basolateral membrane (B) on bradykinin concentration in the presence of 25 μM mergetpa. △, ATPase activity in the absence of bradykinin and mergetpa. The ATPase activity was measured as described in Section 2 ($n=5$). The bradykinin concentration was increased from 10^{-12} to 10^{-6} M. The results are expressed as percentage of the control. *Statistically significant when compared to the control ($P<0.05$).

These data suggest that the product of the cleavage of bradykinin by kininase II in cortex homogenate preparation stimulates the Na^+-ATPase activity by interaction with B_1 receptors. However, the bradykinin not cleaved to des-Arg^9-bradykinin or the des-Arg^9-bradykinin when in higher concentration could promote the inhibition of the enzyme activity medi-

ated by B_2 receptor. To verify if bradykinin, in lower concentrations (10^{-13} to 10^{-7}), could inhibit the Na^+-ATPase activity of the basolateral membrane in the presence of des-Arg^9-bradykinin (10^{-8} M) the experiments summarized in Fig. 6 were performed in both preparations. In all conditions, mergetpa 25 μM was added to the reaction medium. As shown in Fig. 6, the addition of des-Arg^9-bradykinin 10^{-8} M stimulates the Na^+-ATPase activity by 107 and 102% both in cortex homogenate and in isolated basolateral membrane, respectively. This effect, in both preparations, is reversed by bradykinin in a dose-dependent manner. The maximal effect observed in the presence of mergetpa was obtained with bradykinin 10^{-9} M. This concentration is 1000

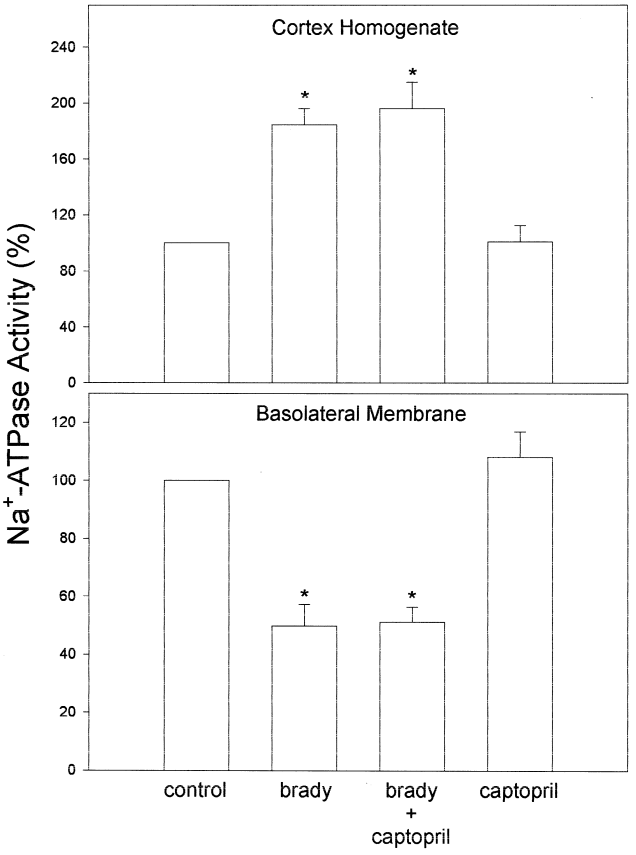


Fig. 5. Interaction between 10^{-10} bradykinin and 25 μM captopril on ouabain-insensitive Na^+-ATPase activity in the cortex homogenate (A) and in isolated basolateral membrane (B). The ATPase activity was measured as described in Section 2 ($n=5$). Bradykinin 10^{-10} (brady) was added where indicated. The results are expressed as percentage of the control. *Statistically significant when compared to the control ($P<0.05$).

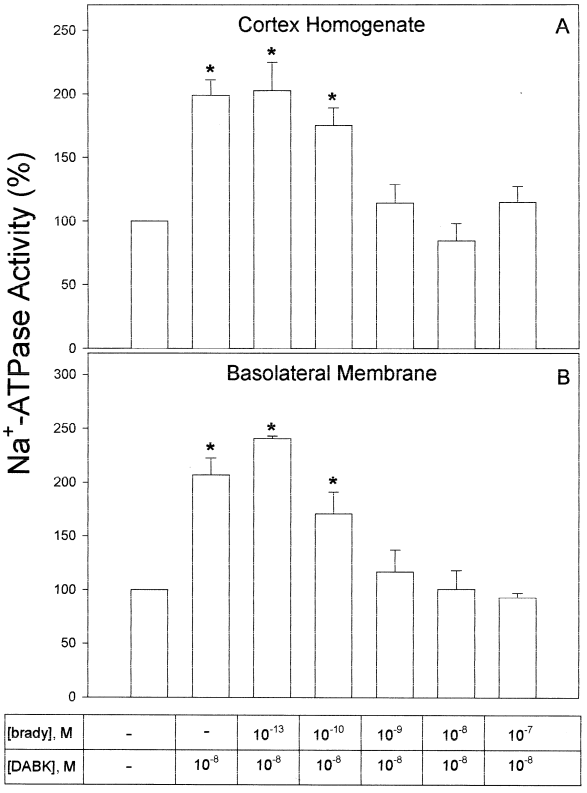


Fig. 6. Interaction between bradykinin and des-Arg^9-bradykinin on ouabain-insensitive Na^+-ATPase activity in the cortex homogenate (A) and in isolated basolateral membrane (B). In all conditions 25 μM mergetpa was added. The ATPase activity was measured as described in Section 2 ($n=6$). Bradykinin concentration from 10^{-13} to 10^{-7} M (brady) and des-Arg^9-bradykinin 10^{-8} M were added where indicated. The results are expressed as percentage of the control. *Statistically significant when compared to the control ($P<0.05$).

times lower than the concentration that promotes maximal inhibition of the Na^+-ATPase in cortex homogenate in the absence of mergetpa (Fig. 1). These data indicate that bradykinin in lower concentrations inhibits the Na^+-ATPase activity in the presence of des-Arg^9-bradykinin, explaining its inhibitory effect in cortex homogenate. However, we cannot rule out a possible inhibition of the Na^+-ATPase activity by the interaction of des-Arg^9-bradykinin with B_2 receptors [1,5].

4. Discussion

In the present work, we studied the regulation of the ouabain-insensitive Na^+-ATPase in cortex ho-

mogenates and isolated basolateral membranes preparations of proximal tubule by bradykinin.

The Na^+-ATPase was initially described in aged microsomal fractions from guinea-pig kidney cortex [26]. Later, the presence of this enzyme was demonstrated in basolateral membranes of the proximal tubule from different species [27]. Characteristically, this enzyme is not inhibited by ouabain and is inhibited by furosemide and ethacrynic acid [9].

Since bradykinin has multiple actions in the kidney [4–7,28], we studied the effect of bradykinin on the ouabain-insensitive Na^+-ATPase activity in cortex homogenates and basolateral membrane preparations of proximal tubule. In both preparations, we observed a biphasic effect of bradykinin on the Na^+-ATPase activity. It has been suggested that bradykinin has a much higher affinity for B_2 than for B_1 receptors [1,5,29]. Bachvarov et al. [30] observed that the K_d for bradykinin binding in cloned rabbit B_2 receptor expressed in COS-1 cells is 2.1×10^{-9} M. As shown in Fig. 1, the bradykinin concentration that inhibits the Na^+-ATPase activity in basolateral membrane preparation is close to the bradykinin concentration that binds to B_2 receptors, suggesting that the inhibitory effect of bradykinin is mediated by its interaction with B_2 receptor. This hypothesis is favored by the observation that the inhibitory effect of bradykinin on the enzyme activity is completely abolished by Hyp^3, an antagonist of B_2 receptor (Fig. 2). In addition, it was found that the B_2 receptor is located in both cortical and medullar segments of the nephron [8,31,32].

The fact that the inhibitory effect of bradykinin on the Na^+-ATPase in the basolateral membrane is reversed at higher concentrations of bradykinin (from 10^{-9} to 10^{-6} M), indicates a possible action mediated by interaction with B_1 receptor. This hypothesis is supported by the following observations: (1) in the presence of 10^{-7} M of Hyp^3, an antagonist of B_2 receptor, bradykinin 10^{-10} M stimulates the Na^+-ATPase activity in the isolated basolateral membrane (Fig. 2); and (2) des-Arg^9-bradykinin, an agonist of B_1 receptor, stimulates the Na^+-ATPase activity in the cortex homogenates and isolated basolateral membrane preparations (Fig. 3).

In cortex homogenates, lower concentrations of bradykinin ($<10^{-9}$ M) are also able to stimulate Na^+-ATPase activity. It has been suggested that brush border membrane of the proximal tubule from different species presents both kininases I and II [29]. Furthermore, it was observed that kininase I cleaves bradykinin in des-Arg^9-bradykinin, an agonist of B_1 receptor [1,5,29]. We observed that in the presence of mergetpa, an inhibitor of kininase I, lower bradykinin concentrations inhibit the Na^+-ATPase activity in the cortex homogenate as observed in isolated basolateral membrane (Fig. 4). In addition, the stimulatory effect of bradykinin on the Na^+-ATPase activity in the cortex homogenate was not modified in the presence of captopril, an inhibitor of kininase II (Fig. 5). So, we propose that in cortex homogenates bradykinin is cleaved to des-Arg^9-bradykinin and binds with higher affinity to B_1 receptor. Since des-Arg^9-bradykinin, an agonist of B_1 receptor, increases the Na^+-ATPase activity in the isolated basolateral membrane as well as in cortex homogenates, this effect cannot be linked to the B_1 receptor described in the cortical vascular tissue [33].

The concentration of bradykinin that stimulates the Na^+-ATPase activity in isolated basolateral membrane, in the presence of Hyp^3 (an antagonist of B_2 receptor), is far below the K_d for B_1 receptors (Fig. 2). The observed stimulation of 45% of the enzymatic activity is not maximal since des-Arg^9-bradykinin (agonist of B_1 receptor) stimulates the same activity by 148% (Fig. 3). On the other hand, des-Arg^9-bradykinin is a more potent agonist for B_1 receptor than bradykinin, which might explain the effect of bradykinin in cortex homogenate. These data indicate that bradykinin stimulates the enzyme activity even when used in a concentration far below the K_d for B_1 receptors. Furthermore, as shown in Fig. 1, the concentration of bradykinin that promotes half-maximal stimulation of the Na^+-ATPase activity in basolateral membrane (Fig. 1B) is in the same range of the K_d for B_1 receptor. In IMR-90 cells, a human cell line that spontaneously coexpresses both receptor types, the K_d for bradykinin for B_1 receptors is 7×10^{-6} M, while the K_d for des-Arg^9-bradykinin is 5×10^{-7} M [34].

Taken together, these data indicate that bradykinin stimulates the ouabain-insensitive Na^+-ATPase activity in proximal tubule through its interaction with B_1 receptors and inhibits the enzyme through its interaction with B_2 receptors.

It has been described that bradykinin stimulates phospholipase C and phospholipase A_2 which may provide a source of arachidonic acid for the production of eicosanoids. Recently, it was observed in our laboratory that activation of protein kinase C increases the Na^+-ATPase activity (data unpublished) and that inhibition of protein kinase A decreases it [12]. So, one of the possible pathways involved in the activation of the Na^+-ATPase by bradykinin could be via phospholipase C, which may activate a protein kinase C. In rabbit vascular smooth muscle cells, it was observed that interaction of bradykinin with B_1 receptor activates protein kinase C [34]. On the other hand, bradykinin could inhibit the Na^+-ATPase activity by decreasing the cAMP concentration and, consequently, the activity of protein kinase A. In accordance with this hypothesis, it is known that stimulation of the contraction of rat uterus by bradykinin is mediated by a decrease in the concentration of cAMP [35]. Since the effect of bradykinin on the Na^+-ATPase activity is the same in both preparations used in the experiments, it is plausible to postulate that the effect of bradykinin involves membrane-bound elements, such as phospholipases and adenylyl cyclase. In cortex homogenate preparation, only small amounts of the cytosolic components would be expect to be present due to the dilution of the medium along the different experimental steps. Based on this argument, the involvement of the cytosolic elements in the effect of bradykinin on the enzyme activity could be ruled out.

Stein et al. [36], proposed that bradykinin (5 μg min^{-1}) could decrease Na^+ reabsorption in the proximal tubule from deeper nephrons from dog kidney. The observation that bradykinin decreases the Na^+-ATPase activity, but does not change the Na^+,K^+-ATPase activity in basolateral membranes of the proximal tubule suggests that the modulation of Na^+ reabsorption by bradykinin could, in part, be due to the inhibition of the Na^+-ATPase via B_2 receptors. Recently, we observed that adenosine, a natriuretic compound, inhibits the ouabain-insensitive Na^+-ATPase activity, but does not change the Na^+,K^+-ATPase activity of the proximal tubule from pig kidney [12]. These observations could suggest that the primary active transport target involved in the regulation of the sodium reabsorption in the proximal tubule by natriuretic compounds is the ouabain-insensitive Na^+-ATPase and not the ouabain-sensitive Na^+,K^+-ATPase.

Acknowledgements

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