B2 receptor-mediated dual effect of bradykinin on proximal tubule Na+-ATPase: Sequential activation of the phosphoinositide-specific phospholipase Cβ/protein kinase C and Ca2+-independent phospholipase A2 pathways


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Received 13 November 2007; received in revised form 10 January 2008; accepted 24 January 2008

Available online 7 February 2008

Abstract

In a previous paper we showed that bradykinin (BK), interacting with its B2 receptor, inhibits proximal tubule Na+-ATPase activity but does not change (Na+ +K+)ATPase activity. The aim of this paper was to investigate the molecular mechanisms involved in B2-mediated modulation of proximal tubule Na+-ATPase by BK. To abolish B1 receptor-mediated effects, all experiments were carried out in the presence of (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu), des-Arg9-[Leu8]-BK (DALBK), a specific antagonist of B1 receptor. A dual effect on the Na+-ATPase activity through the B2 receptor was found: short incubation times (1–10 min) stimulate the enzyme activity; long incubation times (10–60 min) inhibit it. The stimulatory effect of BK is mediated by activation of phosphoinositide-specific phospholipase Cβ (PI-PLCβ)/protein kinase C (PKC); its inhibitory action is mediated by Ca2+-independent phospholipase A2 (iPLA2). Prior activation of the PI-PLCβ/PKC pathway is required to activate the iPLA2-mediated inhibitory phase. These results reveal a new mechanism by which BK can modulate renal sodium excretion: coupling between B2 receptor and activation of membrane-associated iPLA2.

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Keywords: Second sodium pump; Extracellular volume regulation; Sodium transport; Kinase; Receptors

1. Introduction

Bradykinin (BK) is a component of the kallikrein–kinin system and plays an important role in electrolyte balance as well as blood pressure regulation [1]. These effects are associated with the modulation of renal sodium excretion and are due, at least in part, to modulation of transepithelial sodium reabsorption [2]. Because most filtered sodium is reabsorbed in proximal tubules, small changes in this process will have major consequences for the overall sodium metabolism in the body. Furthermore, the pathologies correlated to renal sodium excretion, such as primary hypertension, have been associated with changes in proximal tubule sodium reabsorption [3].

The limiting step for sodium reabsorption in the proximal tubule is its active basolateral transport through the primary active transporters [4]. Two primary active transporters involved in the genesis of the Na+ electrochemical gradient have been described: ouabain-sensitive (Na+ +K+)ATPase and ouabain-insensitive, furosemide-sensitive Na+-ATPase [5–7]. In a previous paper our group showed that BK inhibits proximal tubule Na+-ATPase activity through B2 receptors but does not change the (Na+ +K+)ATPase activity [8]. This inhibitory effect

Abbreviations: AA, arachidonic acid; ACA, N-(p-amlycinnamoyl) anthranilic acid; BEL, bromoeno lactone; BK, bradykinin; BLM, basolateral membranes; Cph C, calphostin C; cPLA2, cytosolic PLA2; DALBK, (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu), des-Arg9-[Leu8]-BK; DPPC, 1,2-dipalmitoyl phosphatidylcholine; EGTA, ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; GDPγS, guanosine 5′-O(2-thiodiphosphate); HEPES, N-2-hydroxyethylpiperazine N′-2-ethanesulfonic acid; iPLA2, Ca2+-independent PLA2; PACOCF3, palmitoyl trifluoromethyl ketone; PI-PLCβ, phosphoinositide-specific phospholipase C β; PKC, protein kinase C; PLA2, phospholipase A2; PMA, phorbol 12-myristate 13-acetate; sPLA2, secretory PLA2; TLC, thin layer chromatography; Tris, tris(hydroxymethyl)aminomethane

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0005-2736/$ - see front matter © 2008 Elsevier B.V. All rights reserved. doi:10.1016/j.bbamem.2008.01.017
of BK on proximal tubule Na\(^+\)-ATPase activity is compatible with B\(_2\)-mediated natriuresis and diuresis [9].

The effects of BK are mediated by two types of kinin receptors: B\(_1\) and B\(_2\) [10,11]. Normally, B\(_2\) receptors are constitutively expressed in tissues; B\(_1\) is expressed only in specific situations. Both receptors have seven transmembrane domains and belong to the family of G protein-coupled receptors. Previous studies indicate that the B\(_2\)-mediated bradykinin effects on renal sodium excretion are, at least in part, mediated by activation of phospholipase A\(_2\) (PLA\(_2\)) [12]. However, the type of PLA\(_2\) and the molecular mechanism underlying the coupling among bradykinin receptor, PLA\(_2\) activity and active sodium transporters are still to be determined. There are three classes of PLA\(_2\), based on the Ca\(^{2+}\)-requirement for enzymatic activity: secretory PLA\(_2\) (sPLA\(_2\)), which requires millimolar concentrations of Ca\(^{2+}\); cytosolic PLA\(_2\) (cPLA\(_2\)), which requires micromolar concentrations of Ca\(^{2+}\); and Ca\(^{2+}\)-independent PLA\(_2\) (iPLA\(_2\)) [13]. So far nothing is known about the regulation of iPLA\(_2\) by peptide hormones, in particular BK.

The aim of this study was to test the involvement of membrane-associated iPLA\(_2\) on the B\(_2\)-mediated BK inhibition of proximal tubule Na\(^+\)-ATPase activity. To avoid the PLA\(_2\) present in the cytosol fraction we used basolateral membranes (BLM) isolated from porcine proximal tubule cells. This preparation contains components of different signaling pathways including G proteins, phospholipases and protein kinases [14–16]. Interestingly, it was observed that BK at the B\(_2\) receptor has a dual effect on the Na\(^+\)-ATPase activity that is mediated by sequential activation of the membrane-associated phosphoinositide-specific phospholipase C \(\beta\) (PI-PLC\(\beta\))/protein kinase C (PKC) and iPLA\(_2\) pathways. These results reveal an additional mechanism by which BK can modulate renal sodium excretion: coupling between B\(_2\) receptor and membrane-associated iPLA\(_2\) activation.

2. Materials and methods

2.1. Materials

ATP, ouabain, furosemide, EGTA, HEPES, Tris, bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro–Phe-Arg), des-Arg\(_9\)–[Leu\(_8\)]-BK (DALBK), diclofenac sodium salt, indomethacin, and 1,2-dipalmitoyl-phosphatidylcholine (DPPC) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Percoll was from Pharmacia Biotech, Uppsala, Sweden. Palmitoyl trifluoromethyl ketone (PACOCF\(_3\)), N-(\(\alpha\)-amylcinnamoyl) anthranilic acid (ACA) and quinacrine dihydrochloride (Mepacrin) were purchased from Calbiochem-Novabiochem Co., San Diego, CA, USA. Rabbit polyclonal antibody anti-iPLA\(_2\) was purchased from Upstate Biotechnology, Lake Placid, NY, USA and peroxidase-conjugated sheep anti-rabbit antibody was purchased from Calbiochem, San Diego, CA, USA. All other reagents were of the highest purity available. \(^{32}\)PiP\(_i\) was obtained from the Institute of Energetic and Nuclear Research, São Paulo, SP, Brazil. \(\gamma\)-3-phosphatidylcholine, 1-palmitoyl-2-[\(\gamma\)-3\(\(^{14}\)C]palmitoyl) was obtained from Amersham Pharmacia Biotech UK Limited. Silica gel 60 thin layer chromatography (TLC) plates and all solvents used were obtained from Merck KGaA, Darmstadt. All solutions were prepared with deionized glass-distilled water. \([\gamma\)-3\(\(^{2}\)P]ATP was prepared as described by Maia et al. [17].

2.2. Isolated basolateral membranes

The basolateral membranes (BLM) of proximal tubules were isolated and purified using the Percoll gradient method as described previously [18]. The membrane preparation was resuspended in 250 mM sucrose at a final concentration of 5–10 mg protein/ml and stored at –20 °C. The (Na\(^+\) + K\(^+\))ATPase activity, a marker for basolateral membranes, was 10–12 times higher than the activity found in the cortex. Residual contamination with other subcellular membrane fractions was minimal. Protein concentration was determined by the Folin phenol method [19] using bovine serum albumin as a standard.

2.3. Measurement of ATPase activity

The composition of the assay medium for the measurement of the Na\(^+\)-ATPase activity (0.1 ml) was: 4 mM MgCl\(_2\), 4 mM ATP (specific activity about 10\(^{5}\) Bq/nmol ATP), 20 mM HEPES–Tris (pH 7.0); 90 mM NaCl; 10 \(^{–}\) M DALBK; and 1 mM ouabain. The ATPase activity was measured according to the method described by Grubmeyer and Penefsky [20]. The reaction was started by the addition of the membranes to the assay medium to a final protein concentration of 0.3–0.5 mg/ml, and stopped after 10 min by the addition of 0.1 N HCl-activated charcoal. The \([^{32}\text{P}]\text{Pi}\) released was measured in an aliquot of the supernatant obtained after centrifugation of the charcoal suspension for 5 min at 2000 rpm in a clinical centrifuge. Spontaneous hydrolysis of \([^{32}\text{P}]\text{ATP}\) was measured simultaneously in tubes to which protein was added after the acid. The Na\(^+\)-ATPase activity was calculated from the difference between the \([^{32}\text{P}]\text{Pi}\) released in the absence and in the presence of 2 mM furosemide, both in the presence of 1 mM ouabain [21].

2.4. Measurement of PL\(_2\) activity

The PL\(_2\) activity was measured according to the method described by Yang et al. [22]. The assay utilized 100 \(\mu\)M DPPC (containing 5.0 \(\times\) 10\(^{5}\) cpm of \(\gamma\)-3-phosphatidylcholine, 1-palmitoyl-2-[\(\gamma\)-3\(\(^{14}\)C]palmitoyl) in 0.8 mM Triton X-100, mixed micelles in 200 mM HEPES (pH 7.0), 5 mM EDTA, 2 mM DTT, 1 mM ATP and 10 \(^{–}\) M DALBK. The reaction was started by the addition of isolated basolateral membranes to a final protein concentration of 0.3 mg/ml. The free fatty acid released was extracted from isolated BLM following the method of Horwitz and Perlman, modified by Malaquias and Oliveira [23]. The lipid samples were applied to TLC silica gel plates along with phospholipid standards. Prior to lipid loading, the plates were activated at 110 °C for 10 min. TLC was developed in two solvent mixtures: CH\(_3\)OH/CH\(_3\)OH/H\(_2\)O (65:35:5 v/v) and CH\(_3\)H\(_2)/CH\(_3\)CH\(_2\)OCH\(_2\)CH\(_3)/CH\(_3\)OOH (90:60:4 v/v). The region of the TLC plate containing free fatty acid was identified with iodine vapor and scraped into scintillation vials. The radioactivity was quantified in a liquid scintillation counter (Packard Tri-Carb model A2100TR, Illinois, USA).

2.5. Measurement of PLC activity

BML phospholipids were labeled by incubating them at 37 °C for 4 h with 4 mM ATP (40,000 cpm/mmol) \([^{32}\text{P}]\text{ATP}\), 4 mM MgCl\(_2\), 20 mM HEPES–Tris (pH 7.0) and 90 mM NaCl. The reaction was started by the addition of 10 \(^{–}\) M BK or vehicle (10 mM HCl). Total phospholipids were extracted from isolated BLM following the method of Horwitz and Perlman, modified by Malaquias and Oliveira [23]. Lipid samples were dissolved in 20 \(\mu\)l of a mixture of CH\(_3\)OH/CH\(_3\)OH/H\(_2\)O (75:25:2 v/v) and spotted along with phospholipid standards on silica gel G plates that were pre-activated at 110 °C for 10 min. TLC was developed in solvent mixture CH\(_3\)OH/CH\(_3\)OH/CH\(_3\)CH\(_2\)OCH\(_2\)CH\(_3\) (90:60:4 v/v). The region of the TLC plate containing free fatty acid was identified with iodine vapor and scraped into scintillation vials. The radioactivity was quantified in a liquid scintillation counter (Packard Tri-Carb model A2100TR, Illinois, USA).

2.6. Measurement of PK\(_C\) activity

The PK\(_C\) activity of isolated BLM was measured by calphostin C (C\(_p\)C)–sensitive incorporation of \(^{32}\)Pi from \([^{32}\text{P}]\text{ATP}\) (7 \(\mu\)Ci/\(\mu\)mol), using histone as substrate. The composition of the reaction medium was: 4 mM MgCl\(_2\), 20 mM HEPES–Tris (pH 7.0), 1.5 mg/ml histone and 0.7 mg/ml protein. The reaction was started with 40% TCA and the sample immediately placed on ice. An aliquot (0.1 ml) was filtered through a Millipore filter (0.45 \(\mu\)m) and washed with ice-cold 20% TCA solution and 0.1 M phosphate buffer (pH 7.0). The radioactivity was quantified by liquid scintillation counting (Packard Tri-Carb 2100 TR). The specific PK\(_C\) activity was calculated from the difference between
the activity in the absence and in the presence of 10⁻⁶ M Cph C. The phorbol ester, phorbol myristate acetate (PMA), was used as the activator of PKC.

2.7. Immunoblotting

The presence in basolateral membranes of iPLA₂ protein was determined by immunoblotting. Proteins were resolved on 7.5% SDS-PAGE gels and transferred to nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer’s instructions. The membranes were incubated overnight with the anti-iPLA₂ antibody 1:500 with agitation at 4 °C, washed three times and then incubated with anti-rabbit peroxidase-conjugated antibody (Amersham Pharmacia) diluted 1:5000 in PBST plus 3% non-fat dry milk for 1 h at room temperature, with agitation. After antibody labeling, detection was performed with ECL (Amersham Biosciences, Piscataway, NJ, USA).

2.8. Data analysis

The means were compared by one-way variance analysis (ANOVA) taking into account the treatment of the experimental groups. The magnitudes of the differences were evaluated using the multiple comparative Bonferroni test. The data are presented as the mean ± standard error. The α corresponds to the results obtained from different basolateral membrane preparations.

3. Results

In a previous paper we showed that BK has a biphasic effect on proximal tubule Na⁺-ATPase activity, with an inhibitory effect mediated by B₂ receptor and a stimulatory effect mediated by B₁ receptor [8]. Here we examined the possibility that the B₂-mediated inhibition of the Na⁺-ATPase by BK could be mediated by activation of PLA₂. All experiments were carried out in the presence of 10⁻⁸ M DALBK, a specific antagonist of B₁ receptor that completely abolishes the B₁-mediated stimulation of enzyme activity by BK [24]. The pre-incubation of the enzyme with 10⁻⁹ M BK for 30 min decreased Na⁺-ATPase activity from 27.5±3.1 to 13.9±2.5 nmol Pi × mg⁻¹ × min⁻¹ (Fig. 1A). This effect was completely abolished by the PLA₂ non-specific inhibitors, quinacrine and ACA, at 10⁻⁶ M.

Since all experiments were done in the absence of added Ca²⁺ and in the presence of 1 mM EGTA, this result indicates the involvement of Ca²⁺-independent PLA₂ (iPLA₂) in the BK effect. To test this hypothesis, the experiments were performed in the presence of PACOCF₃ and bromoenol lactone (BEL), specific inhibitors of iPLA₂ [25] (Fig. 1B). PACOCF₃ and BEL at 10⁻⁶ M completely reversed the inhibitory effect of BK 10⁻⁹ M on Na⁺-ATPase activity. Quinacrine, ACA, BEL and PACOCF₃ at 10⁻⁶ M did not affect Na⁺-ATPase activity when added separately, without BK (data not shown).

To determine the presence of iPLA₂ in BLM we measured its activity directly (Fig. 2). The isolated BLM were incubated with 10⁻⁹ M BK for different times (1, 3, 5, 10, 20 and 30 min). After the reaction, the phospholipids were separated and PLA₂ activity was quantified. Fig. 2A shows the effect of 10⁻⁹ M BK on PLA₂ activity. BK increased PLA₂ activity by 120% with the maximal effect observed at 5 min. This was completely reversed after 20 min of incubation. To further confirm the presence of iPLA₂ in BLM, immunoblotting using rabbit anti-iPLA₂ antibody was carried out (Fig. 2B). A band at 85 kDa which corresponds to the correct molecular mass for iPLA₂ [26] was detected in cortex homogenate and BLM. Rat cerebellum was used as a positive control for iPLA₂ [27]. The correlation between the effect of BK on Na⁺-ATPase activity and activation of iPLA₂ was tested in the next experimental group (Fig. 2C). The BK-stimulated PLA₂ activity was completely reversed by PACOCF₃ and BEL at 10⁻⁶ M, specific inhibitors of iPLA₂ (Fig. 2C). Furthermore, it was observed that both PACOCF₃ and BEL completely abolished the basal PLA₂ activity (data not shown).

B₂ receptor belongs to the G protein-coupled receptor family, and consequently, is associated with trimeric G proteins [11]. To confirm that the effect of 10⁻⁹ M BK on the BLM-associated iPLA₂ is mediated by B₂ receptor, we performed experiments in the presence of 10⁻⁶ M HOE140, a specific antagonist for B₂, and 10⁻⁶ M GDPβS, a non-hydrolyzable GDP analog that inhibits trimeric G proteins (Fig. 2C). Both compounds completely abolished the stimulatory effect of BK on the BLM-associated iPLA₂ activity. Similarly, these compounds abolished the inhibitory effect of 10⁻⁹ M BK on Na⁺-ATPase activity (data not shown).

It is well known that PLA₂ catalyzes the hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate arachidonic acid (AA) [13]. The increase in concentration of AA from
10^{-12} M to 10^{-8} M decreased the Na^+-ATPase activity by 60% (Fig. 2D). This effect is similar and non-additive to the BK effect. Fig. 3 shows the modulation of the effect of 10^{-9} M BK or 10^{-8} M AA on Na^+-ATPase activity by the cyclooxygenase (COX) inhibitors, 10^{-9} M diclofenac and 10^{-6} M indomethacin. It was observed that the inhibitory effect of BK or AA was completely reversed by both COX inhibitors. The addition of diclofenac or indomethacin alone did not change the Na^+-ATPase activity (data not shown).

These data indicate that a membrane-associated, Ca^{2+}-independent PLA2 isozyme is involved in the inhibitory effect of BK on Na^+-ATPase activity. Fig. 4A shows the effect of BK on Na^+-ATPase activity in the presence of 10^{-8} M DALBK, a specific antagonist of B_1 receptor. BK at 10^{-9} M had a dual effect on Na^+-ATPase activity. The incubation of the enzyme with 10^{-9} M BK for a short time (1–10 min) increased Na^+-ATPase activity, with a maximal effect observed at 1 min. After 15 min of incubation, 10^{-9} M BK inhibited the enzyme activity with a maximal effect observed at 30 min. This inhibitory effect was maintained for at least 60 min (data not shown). To be sure that the stimulatory effect is mediated by B_2 receptor, we tested the effect of 10^{-6} M HOE140 and 10^{-6} M GDPβS. The enzyme activity was measured as described in Materials and methods (n=6). Dose-dependent effect of AA on Na^+-ATPase activity. The concentration of AA was increased from 10^{-12} to 10^{-8} M and this effect is similar and non-additive to the BK effect (open circle). Controls are represented by open triangle. The enzyme activity was measured as described in Materials and methods (n=13). Data are shown as means±SE. *Statistically significant when compared to respective controls (p<0.05).
consistent with sequential activation of both enzymes. The effect of BK on PI-PLCβ and PKC was completely abolished by addition of $5 \times 10^{-8}$ M U73122 (data not shown).

The next experiments were carried out to study the requirement for prior activation of the PI-PLCβ/PKC pathway for PLA2-mediated inhibition of the Na+-ATPase by $10^{-9}$ M BK after 30 min of incubation (Fig. 6A). The inhibitory effect of $10^{-9}$ M BK was reversed by $10^{-8}$ M Cph C and $5 \times 10^{-8}$ M U73122 when these were added before BK. On the other hand, the addition of $10^{-8}$ M Cph C or $5 \times 10^{-8}$ M U73122 after 15 min of incubation with BK, when activation of the PI-PLCβ/PKC pathway was complete, did not change the inhibitory effect of BK on the enzyme activity.

Fig. 6B shows the modulation of BK-stimulated iPLA2 activity by an agonist and two antagonists of the PI-PLCβ/PKC pathway. BK ($10^{-9}$ M) increased iPLA2 activity in a similar manner to PMA, an activator of PKC, increased iPLA2 activity in a similar manner to BK. These data show that activation of the PI-PLCβ/PKC pathway is a critical step prior to Ca$^{2+}$-independent, membrane-associated iPLA2-mediated inhibition of Na+-ATPase by BK.

4. Discussion

In this paper we show that BK, through its B2 receptor, has a dual effect on proximal tubule Na+-ATPase in a time-dependent manner.
Na\(^{+}\)-ATPase belongs to the P-type ATPase family and several studies have shown biochemical differences between this enzyme and the classical (Na\(^{+}\)-K\(^{+}\)) ATPase [6–8,30,31]. This enzyme is completely inhibited by furosemide but is insensitive to ouabain, a classic (Na\(^{+}\)-K\(^{+}\)) ATPase inhibitor. The possible effect of furosemide in other ATPases can also be ruled out because it was observed that furosemide does not change the (Na\(^{+}\)-K\(^{+}\)) ATPase, Mg\(^{2+}\)-ATPase, Ca\(^{2+}\)-ATPase and Ecto-ATPase activities [7,32]. So, it is plausible to postulate that furosemide, in the preparation used in the present manuscript, works as a “specific inhibitor” of Na\(^{+}\)-ATPase. We also observed that bradykinin has the same effect on Na\(^{+}\)-ATPase measured by Na\(^{+}\) stimulation in the presence of ouabain 1 mM. The link between Na\(^{+}\)-stimulated ATPase activity and Na\(^{+}\) transport was also shown by Malnic et al. [33]. They observed that furosemide inhibits Na\(^{+}\) reabsorption in the proximal tubule.

It has been demonstrated that ouabain-insensitive Na\(^{+}\)-ATPase is a primary active transporter target for compounds that are involved in the regulation of renal Na\(^{+}\) excretion, such as the peptides of the kallikrein–kinin system [10,15,34–36]. Na\(^{+}\)-ATPase is about 10 times less active than (Na\(^{+}\)-K\(^{+}\)) ATPase [31], which suggests that this enzyme may be involved in fine tuning, whereas (Na\(^{+}\)-K\(^{+}\)) ATPase is responsible for most of the Na\(^{+}\) reabsorption in the proximal tubule. The data reported in the present paper, together with observations reported by our group in previous papers, indicate that Na\(^{+}\)-ATPase is involved, at least in part, in the effect of BK on proximal tubule sodium reabsorption [8,10,36]. In addition, because B\(_2\) receptor mediates a dual effect of BK on Na\(^{+}\)-ATPase, we postulate that this effect may permit fine tuning of sodium reabsorption in proximal tubules.

The mechanisms involved in the modulation of sodium reabsorption in proximal tubule cells by BK are still to be determined [2,9]. The results obtained in this paper show that: (a) the stimulatory phase involves activation of the PI-PLC\(_\beta\)-PKC pathway, and the inhibitory phase involves a Ca\(^{2+}\)-independent, BLM-associated PLA\(_2\); (b) prior activation of the PI-PLC\(_\beta\)-PKC pathway is required for activation of the iPLA\(_2\)-mediated inhibitory phase; and (c) the inhibitory effect of BK depends on the metabolism of AA by COX. These results represent a new mechanism of regulation of renal sodium excretion by BK.

In the present manuscript, we used isolated BLM which avoids the possible translocation of PLA\(_2\) from cytosol to plasma membrane after its activation. Furthermore, all experiments were carried out in the absence of Ca\(^{2+}\). In this way, the involvement of Ca\(^{2+}\)-dependent group IV cPLA\(_2\) (\(\alpha\) and \(\beta\)) can be ruled out because its activation involves Ca\(^{2+}\)-dependent translocation to plasma membranes. On the other hand, cPLA\(_2\)-\(\gamma\), which belongs to group IV, lacks a C2 domain (Ca\(^{2+}\)-independent) and could be constitutively associated with cell membrane through a palmitoylation site. The molecular weight of cPLA\(_2\)-\(\gamma\) is 61 kDa; we showed the presence of only one band of 85 kDa in BLM using polyclonal antibody anti-iPLA\(_2\). Furthermore, the observation that PACOCF\(_3\) and BEL abolished both basal and BK-stimulated PLA\(_2\) activity associated with BLM could indicate the presence of group VI iPLA\(_2\) in the proximal tubule BLM. This hypothesis is strengthened by observation that a BEL-sensitive group VI iPLA\(_2\).
with molecular weight around 85 kDa, has been shown in renal cortex [26,37–41]. However, the involvement of group IV ePLA2;γ cannot be completely ruled out. Further experiments are necessary to clarify this issue.

The activation of PLA2 could lead to either an increase or a decrease in Na\(^+\) reabsorption in proximal tubule cells, depending on the agonist and the cellular distribution of PLA2 [12,42]. It was observed that BK modulates Ca\(^{2+}\)-dependent PLA2 activity in rabbit isolated proximal tubule; BK-stimulated cPLA2 activity but decreased Ca\(^{2+}\)-dependent, membrane-associated cPLA2 activity [43]. On the other hand, the role of iPLA2 is still not completely known. It was proposed that group VI iPLA2 regulates phospholipid turnover, cell growth and apoptosis. In a previous paper, it was shown that the iPLA2 located in endoplasmic reticulum plays an important role against oxidant-induced lipid peroxidation and oncosis in proximal tubules [38]. However, its modulation by hormones and its involvement in sodium homeostasis are yet to be determined. Here we show that BK induces the activation of BLM-associated iPLA2 activity. These data agree with the observation that BK, via the B2 receptor, inhibits Na\(^+\) reabsorption in the proximal tubule, showing a close correlation between sodium excretion and iPLA2 activation. This result opens up new possibilities for understanding the role of this enzyme in renal sodium excretion as well as blood pressure regulation.

The molecular mechanisms involved in the activation of iPLA2 are poorly understood. In the present study, we observed that the inhibition of PKC (with Cph C) and PI-PLC\(\gamma\) (with U73122) abolished the BK-induced activation of PLA2. Furthermore, the phorbol ester, PMA, an activator of PKC, increased the PLA2 activity in a similar manner to BK. Because we used isolated BLM, the presence of a membrane-associated PKC pool is required. In a previous paper, we showed that BLM has a resident pool of PKC activated by phorbol ester and insensitive to Ca\(^{2+}\) [44]. In a similar way, using membrane fractions of ventricular myocytes, Steer et al. [45] showed that PMA increases iPLA2 through activation of a resident pool of PKC. These results show that PKC is the link between the activation of PI-PLC\(\gamma\) and Ca\(^{2+}\)-independent, membrane-associated PLA2 by BK. However, there is a question still to be answered: does PKC mediate the activation of iPLA2 through direct phosphorylation or is this effect mediated by another protein? Although no phosphorylation consensus sequences have been described in iPLA2, different studies suggest the involvement of phosphorylation in the regulation of this enzyme [37,40,46–48].

In summary, we have shown that BK, through its B2 receptor, has a dual effect on proximal tubule Na\(^+\)-ATPase activity, mediated by the integrated activation of two different pathways: PI-PLC\(\gamma\)/PKC and iPLA2. These results open new possibilities in our understanding of the molecular mechanisms underlying BK regulation of renal sodium excretion and the role of iPLA2 in this process.

Acknowledgments

We thank Dr Martha Sorensen (Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro) for her critical review of the manuscript. This work was supported by grants from Programa de Apoio ao Desenvolvimento Científico e Tecnológico (PADCT), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Programa de Apoio a Núcleos de Excelência (PRONEX/CNPq), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Technical support was provided by Shanserley Leite do Espírito Santo and Marta Peres Teixeira (CNPq fellowships).

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