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J. BEŁTOWSKI, D. GÓRNY, A. MARCINIAK

BIPHASIC EFFECT OF PROTEIN KINASE C ON RAT RENAL CORTICAL Na^+, K^+-ATPase

Department of Pathophysiology, University School of Medicine, Lublin, Poland

We examined the dependence of rat renal Na^+, K^+-ATPase activity on protein kinase C (PKC) stimulation. Infusion of either phorbol 12, 13-dibutyrate (PDBu) or phorbol 12-myristate 13-acetate (PMA) into rat abdominal aorta resulted in dose-dependent changes of renal cortical Na^+, K^+-ATPase activity. Low doses of these esters (3×10^{-11} mol/kg/min) increased activity of Na^+, K^+-ATPase whereas high doses (3×10^{-9} mol/kg/min) decreased it. The changes in Na^+, K^+-ATPase activity induced by PDBu and PMA were prevented by staurosporine, a PKC inhibitor. 4α phorbol didecanoate (4α PDD), phorbol ester which does not activate PKC had no effect on cortical Na^+, K^+-ATPase. PDBu and PMA did not change Na^+, K^+-ATPase activity in the renal medulla. The stimulatory effect of PDBu (3×10^{-11} mol/kg/min) was neither mimicked by amphotericin B, a sodium ionophore nor blocked by amiloride, an inhibitor of Na^+/H^+-exchanger. The inhibitory effect of 3×10^{-9} mol/kg/min PDBu was not mimicked by amiloride indicating that the observed effects of PKC stimulation are not secondary to alterations in intracellular sodium concentration. The inhibitory effect of PDBu was prevented by infusion of ethoxyresorufin, an inhibitor of cytochrome P450-dependent arachidonate metabolism. These results suggest that the inhibitory effect of PKC on renal cortical Na^+, K^+-ATPase is mediated by cytochrome P450-dependent arachidonate metabolites.

Key words: *Na^+, K^+-ATPase, protein kinase C, kidney.*

INTRODUCTION

Sodium and potassium activated adenosine triphosphatase (Na^+, K^+-ATPase) provides the driving force for vectorial transepithelial sodium transport in renal tubules and its activity correlates with intensity of sodium transport in each nephron segment. Na^+, K^+-ATPase activity is mainly determined by the number of pump units, their internal activity and intracellular sodium concentration (1). Renal tubular Na^+, K^+-ATPase is a target for many hormones such as norepinephrine, angiotensin, dopamine, parathyroid hormone and atrial natriuretic peptide (2). However, intracellular mechanisms

responsible for this regulation are only partially defined. Among these mechanisms phospholipase C — protein kinase C signal transduction pathway seems to be one of the most important (2). The data concerning Na^+, K^+-ATPase regulation by protein kinase C (PKC) are controversial. Both stimulation (3—5) and inhibition (6—10) of Na^+, K^+-ATPase by PKC were described. Bertorello (11) described biphasic effect of PKC activators on Na^+, K^+-ATPase — dependent oxygen consumption in proximal tubular cells with an initial stimulation followed by an inhibition. Some authors (9, 12) observed no changes in Na^+, K^+-ATPase activity resulting from its phosphorylation by PKC. Most of these studies were performed on isolated renal Na^+, K^+-ATPase or on isolated native and cultured renal cells. The purpose of our study was to evaluate the effect of PKC activation *in vivo* on renal Na^+, K^+-ATPase activity.

MATERIALS AND METHODS

All studies were performed on the male Wistar rats weighing 250—300 g. The animals had free access to food and water before the experiments. They were anaesthetized with sodium pentobarbital (45 mg/kg i.p.) and were surgically prepared for the experiment. For the infusion of investigated drugs a thin cather was placed through the femoral artery into the abdominal aorta close to the renal arteries.

Drug administration

After the surgery the infusion with physiological saline was started at the rate of 66 µl/min for 30 minutes (stabilization period). All investigated substances were infused as saline solution at the rate of 66 µl/min (4 ml/hour). The total time of infusion was 60 minutes. The animals from the control group received 0.9% NaCl during the whole experiment (60 minutes). Each investigated drug was administered for 30 minutes, between 1 and 30 or between 31 and 60 minute of infusion. PKC activators (phorbol esters) were always administered between 31 and 60 minute of infusion. After the end of infusion the kidneys were removed and used for further experiments.

Tissue preparation

Na^+, K^+-ATPase activity was evaluated in the microsomal fraction of the rat kidney tissue, separately in the cortex and medulla. The membrane (microsomal) fraction was obtained by the method of Jörgensen (13). After the end of infusion the kidneys were removed immediately and stored in ice-cold solution containing 0.25 M sucrose plus 0.03 M histidine (pH 7.2). The medulla and cortex were separated by dissection and then homogenized in sucrose-histidine solution (10 ml per 1 g of tissue). The homogenate was centrifuged at 8000 × g for 20 minutes at 4°C. The sediment was resuspended in surcose-histidine solution (10 ml) and centrifuged again. The combined supernatants from two centrifugations were centrifuged at 48 000 × g for 30 minutes at 4°C. The pellet (microsomal fraction) was resuspended in 4 ml of sucrose-histidine solution and stored at −25°C. Enzyme assay was made within 24 hours.

Enzyme assay

ATP-ase activity was assayed by measuring the amount of inorganic phosphate (Pi) liberated from ATP during the incubation of the microsomal fraction at 37°C. The assay medium (1 ml) contained: 100 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 40 mM Tris-HCl (pH 7.1) and 50 μg of microsomal protein. Preincubation was carried out for 10 minutes and then 3 mM ATP was added. After 15 minutes of incubation time the enzymatic reaction was terminated by adding 0.35 ml of ice-cold 1 N $HClO_4$. Then inorganic phosphate (Pi) was assayed by the spectrophotometric method of Hurst (14). According to this method 0.2 ml of the incubation medium was diluted with water to 3 ml and 0.6 ml of sodium molybdate reagent (0.31 M Na_2MoO_4 in 3.0 N H_2SO_4) was added to form yellow phosphomolybdate complex which was subsequently reduced to molybdenum blue by adding 0.6 ml of stannous chloride-hydrazine sulphate reagent containing $SnCl_2$ (1.3 μM), hydrazine sulphate (0.023 M) and H_2SO_4 (1.0 N). Absorbance was read after 20 minutes at wavelength of 650 nm against blank samples using 10-by 75-mm cuvette. Blank samples were prepared as described above but the enzyme was inactivated by boiling for 5 minutes before adding to the incubation medium.

Two samples from each kidney were investigated, one in the presence of 1 mM ouabain in the incubation medium and one in the absence of this drug. Na^+, K^+-ATPase activity (ouabain-sensitive fraction) was calculated as the difference between total ATPase (assayed in the absence of ouabain) and ouabain-resistant fraction, assayed in the presence of ouabain. ATPase activity was expressed in μmol Pi liberated from ATP by 1 mg of microsomal protein during one hour (μmol Pi/mg protein/hour).

The relationship between incubation time and Na^+, K^+-ATPase activity was linear between 5 and 40 minute of incubation. The relationship between protein content in the assay medium and Na^+, K^+-ATPase activity was linear when protein content was between 20 and 80 μg.

Protein was assayed by the method of Lowry *et al.* (15) using bovine serum albumin as standard.

Reagents

The following drugs were obtained from Sigma Chemical Co. (St. Louis, USA): phorbol 12, 13-dibutyrate (PDBu), phorbol 12-myristate 13-acetate (PMA), 4α phorbol didecanoate (4α PDD), staurosporine, ethoxyresorufin, ibuprofen, amiloride, amphotericin B, adenosine triphosphate (ATP) disodium salt (crystalline, vanadium free), ouabain, L-histidine. Other reagents were obtained from POCH Gliwice (Poland) and were of research grade. Before the infusion, phorbol esters, staurosporine, amiloride and amphotericin B were dissolved in dimethylsulphoxide (DMSO) and then diluted in 0.9% NaCl. Ethoxyresorufin and ibuprofen were dissolved in ethanol. Final concentrations of DMSO and ethanol in infused solution were $< 0.1\%$ and were shown not to influence renal Na^+, K^+-ATPase activity in preliminary experiments using these solvents alone.

Statistics

Na^+, K^+-ATPase activity was assayed in three samples from each homogenate and means were used in further calculations. The data obtained in the investigation are illustrated as the mean $\pm$ SD from 8 experiments (animals) in each group. Statistical comparisons were performed by the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for comparison of different means.

RESULTS

After a 30-minute infusion, PDBu had dose-dependent effect on Na^+, K^+-ATPase in the renal cortex (*Fig. 1*). A low dose of PDBu (3×10^{-11} mol/kg/min) increased Na^+, K^+-ATPase activity by 34%. A higher dose (3×10^{-10} mol/kg/min) caused only 13% stimulation. After the infusion of the highest tested dose of PDBu (3×10^{-9} mol/kg/min) Na^+, K^+-ATPase activity was decreased by 23%. In contrast to cortical Na^+, K^+-ATPase, the activity of this enzyme in the renal medulla was not changed after the infusion of PDBu (*Fig. 1*). This phorbol ester did not change either cortical or medullary ouabain resistant ATPase (data not shown). To evaluate whether PDBu directly affects Na^+, K^+-ATPase activity, we added this compound to the incubation medium in vitro during enzyme assay. PDBu in the highest concentration in which it was present in infused solution (1.3 μM) had no effect on isolated either cortical or medullary Na^+, K^+-ATPase (data not presented).

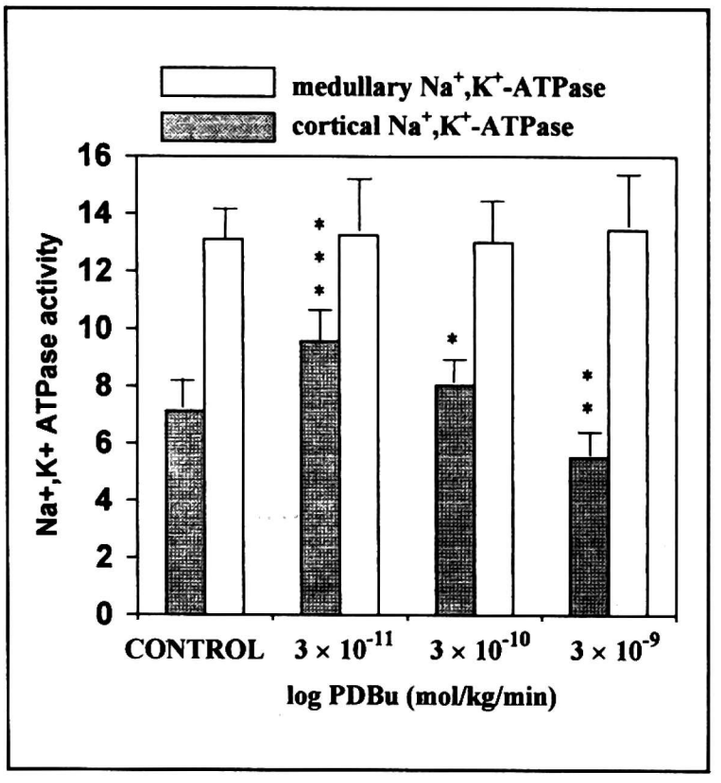

Fig. 1. The effect of phorbol 12, 13-dibutyrate (PDBu) on Na^+, K^+-ATPase activity in the rat renal cortex and medulla. Increasing doses of PDBu were infused for 30 minutes and then Na^+, K^+-ATPase was assayed in an isolated microsomal fraction of the renal cortex and medulla. Na^+, K^+-ATPase activity is expressed in μmol of inorganic phosphate liberated by the enzyme contained in 1 mg of microsomal protein during 1 hour (μmol Pi/mg protein-hour). n = 8 rats in each group. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (compared with control by ANOVA and Duncan's test).

Similar results were obtained when we tested another PKC-stimulating phorbol ester, phorbol 12-myristate 13-acetate (PMA). A low dose of PMA (3×10^{-11} mol/kg/min) elevated Na^+, K^+-ATPase activity by 28%. The stimulation was still observed after the infusion of a higher dose of PMA (3×10^{-10} mol/kg/min) but was less pronounced (+14%). Finally, 3×10^{-9} mol/kg/min PMA inhibited Na^+, K^+-ATPase by 25% (*Fig. 2*). PMA had no effect on renal medullary Na^+, K^+-ATPase and on either cortical or medullary oubain-resistant ATPase.

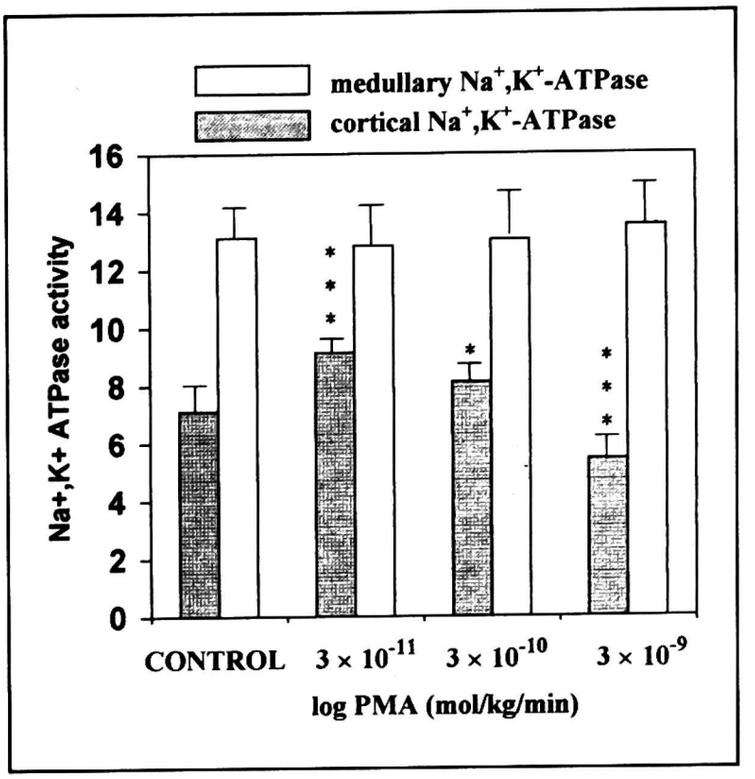

Fig. 2. The effect of phorbol 12-myristate 13-acetate (PMA) on Na^+, K^+-ATPase activity in the rat renal cortex and medulla. Increasing doses of PMA were infused for 30 minutes and then Na^+, K^+-ATPase was assayed in an isolated microsomal fraction of the renal cortex and medulla. Na^+, K^+-ATPase activity is expressed in μmol of inorganic phosphate liberated by the enzyme contained in 1 mg of microsomal protein during 1 hour (μmol Pi/mg protein/hour). n = 8 rats in each group. *P < 0.05, ***P < 0.001 (compared with control by ANOVA and Duncan's test).

Unlike PDBu and PMA, phorbol ester which does not stimulate protein kinase C (4α PDD) infused in the same doses ($3 \times 10^{-11} - 3 \times 10^{-9}$ mol/kg/min) did not change the acivity of cortical Na^+, K^+-ATPase (*Fig. 3*).

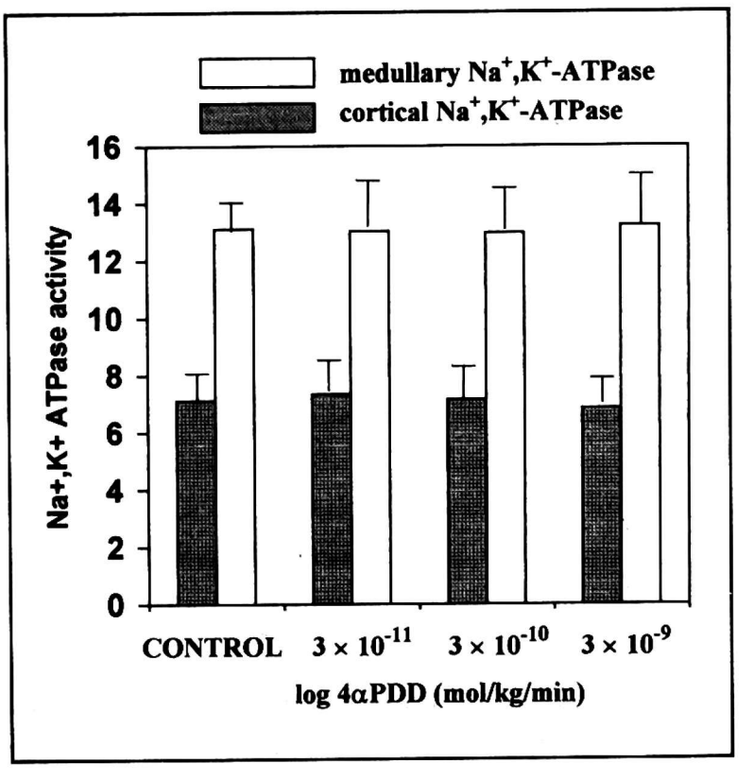

Fig. 3. The effect of 4α phorbol didecanoate (4αPDD) on Na^+, K^+-ATPase activity in the rat renal cortex and medulla. Increasing doses of 4 αPDD were infused for 30 minutes and then Na^+, K^+-ATPase was assayed in an isolated microsomal fraction of the renal cortex and medulla. Na^+, K^+-ATPase activity is expressed in μmol of inorganic phosphate liberated by the enzyme contained in 1 mg of microsomal protein during 1 hour (μmol Pi/mg protein/hour). n = 8 rats in each group.

Because the effects of PDBu and PMA were similar, only PDBu was used in further experiments.

Both stimulation and inhibition of renal cortical Na^+, K^+-ATPase were abolished by pretreatment with the specific inhibitor of protein kinase C,

staurosporine (*Fig. 4*). Staurosporine (10^{-10} mol/kg/min) infused for 30 minutes before the administration of PDBu (3×10^{-11} mol/kg/min) partially attenuated stimulation of Na^+, K^+-ATPase whereas a higher dose of staurosporine (10^{-9} mol/kg/min) completely abolished it. The increase in Na^+, K^+-ATPase activity induced by 3×10^{-10} mol/kg/min PDBu was completely blocked by the previous infusion of 10^{-10} mol/kg/min staurosporine. This inhibitor of PKC also dose-dependently abolished Na^+, K^+-ATPase inhibition by the highest tested dose of PDBu (*Fig. 4*). Staurosporine in both tested doses had no influence on Na^+, K^+-ATPase activity in animals not treated with phorbol esters (*Fig. 4*) suggesting that this enzyme is not significantly regulated by PKC under basal conditions.

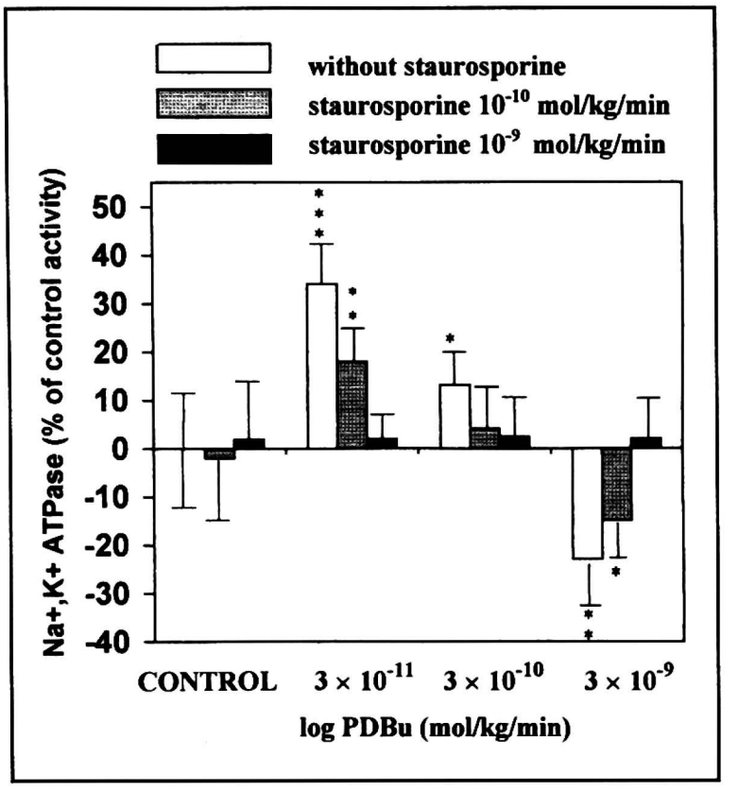

Fig. 4. The effect of protein kinase C inhibitor, staurosporine on the action of PDBu on renal cortical Na^+, K^+-ATPase. Staurosporine (10^{-10} or 10^{-9} mol/kg/min) was infused for 30 minutes and then PDBu in three different doses was administered for the next 30 minutes. After the end of infusion, Na^+, K^+-ATPase was assayed in the microsomal fraction isolated from the renal cortex. Na^+, K^+-ATPase activity is expressed in % of control value. n = 8 rats in each group. *P < 0.05, **P < 0.01, ***P < 0.001 (compared with the control group by ANOVA and Duncan's test).

Stimulation of cortical Na^+, K^+-ATPase by low doses of phorbol esters may be a direct effect of PKC on the pump itself or may be secondary to increased apical sodium entry. Activation of PKC stimulates Na^+/H^+-exchanger, the main pathway of apical sodium entry into PCT cells (16). It is well documented that prolonged increase in intracellular sodium concentration increases the number of active Na^+, K^+-ATPase units in the plasma membrane (17). Therefore we performed studies using sodium ionophore — amphotericin B and an inhibitor of sodium entry — amiloride. Neither amphotericin B (2—20 nmol/kg/min) nor amiloride (0.02—2 μmol/kg/min) infused for 30 minutes had any significant effect on cortical Na^+, K^+-ATPase activity (*Table 1*). These results indicate that changes of intracellular Na^+ do not influence Na^+, K^+-ATPase activity under these experimental conditions and suggest that observed effects of phorbol esters are not secondary to

stimulation or inhibition of sodium entry. This was further supported by the fact that amiloride (2 μmol/kg/min) administered for 30 minutes before the infusion of PDBu (3×10^{-11} mol/kg/min) did not present stimulation of Na^+, K^+-ATPase (*Table 1*).

Table 1. The effect of sodium ionophore, amphotericin B and an inhibitor of Na^+/H^+-exchanger, amiloride on Na^+, K^+-ATPase activity in the renal cortex. 1) Amphotericin B (2—20 nmol/kg/min) or amiloride (0.02—2 μmol/kg/min) was infused for 30 minutes and then Na^+, K^+-ATPase activity was assayed in the microsomal fraction isolated from the renal cortex. 2) In a separate experiment we evaluated whether amiloride is able to block the stimulatory effect of a low dose of PDBu on cortical Na^+, K^+-ATPase. PDBu (3×10^{-11} mol/kg/min) was infused for 30 minutes in rats previously treated or not treated with amiloride (2 μmol/kg/min). There was no significant difference in cortical Na^+, K^+-ATPase activity between these two groups. n = 8 rats in each group. ***P < 0.001 (compared with control group by ANOVA and Duncan's test).

	Na^+, K^+-ATPase activity in the renal cortex (μmol Pi/mg protein/hour)
Control	7.12 ± 0.70
Amphotericin B (2nmol/kg/min)	7.26 ± 0.81
Amphotericin B (20 nmol/kg/min)	7.34 ± 0.49
Amiloride (0.02 μmol/kg/min)	6.91 ± 0.71
Amiloride (0.2 μmol/kg/min)	6.83 ± 0.82
Amiloride (2 μmol/kg/min)	6.68 ± 0.72
PDBu (3×10^{-11} mol/kg/min)	9.56 ± 0.83 ***
Amiloride (2 μmol/kg/min) + PDBu (3×10^{-11} mol/kg/min)	9.72 ± 1.10 ***

Arachidonate metabolites produced by cytochrome P450-dependent monooxygenase play an important role in the regulation of renal Na^+, K^+-ATPase. They were also shown to mediate the effect of protein kinases in some experiments (2, 18). To evaluate whether the inhibition of Na^+, K^+-ATPase by high doses of PKC-stimulating phorbol esters was mediated by these eicosanoids, we tested the influence of inhibitors of arachidonate metabolism on the effect of PDBu. We used two inhibitors: ethoxyresorufin (ETX) — a specific inhibitor of cytochrome P450-dependent arachidonate metabolism and ibuprofen — the inhibitor of cytooxygenase. ETX (10^{-9} — 10^{-8} mol/kg/min) administered before the infusion of the lowest tested dose of PDBu (3×10^{-11} mol/kg/min) did not influence Na^+, K^+-ATPase stimulation

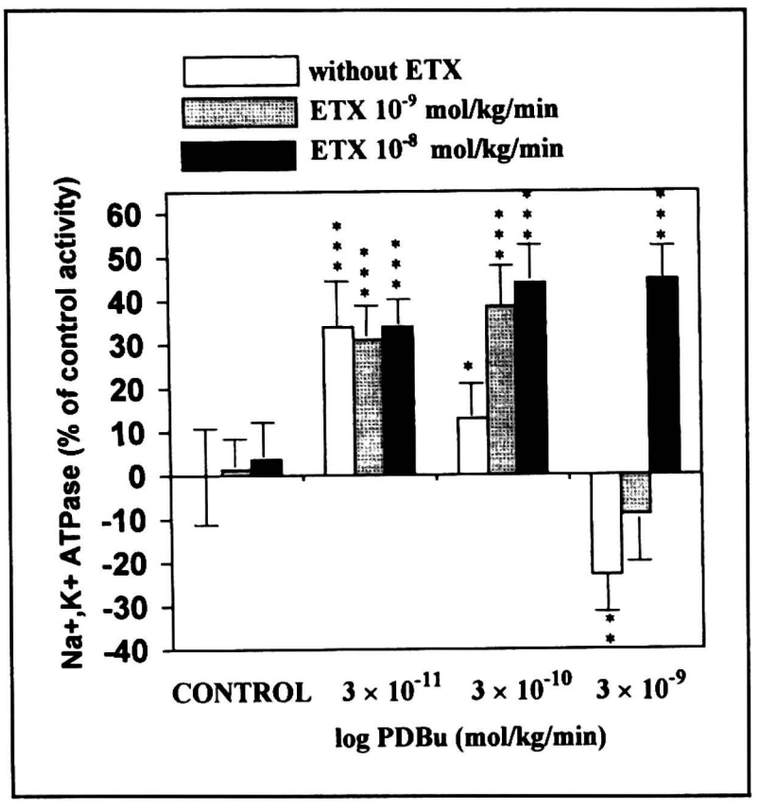

Fig. 5. The effect of ethoxyresorufin, the specific inhibitor of cytochrome P450-dependent arachidonate metabolism on Na^+, K^+-ATPase modulation by different doses of PDBu. Ethoxyresorufin (ETX) (10^{-9} or 10^{-8} mol/kg/min) was infused for 30 minutes and then different doses of PDBu were administered for another 30 minutes. $n = 8$ rats in each group. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (compared with control by ANOVA and Duncan's test).

by this PKC activator (*Fig. 5*). When ETX was infused before the administration of 3×10^{-10} mol/kg/min PDBu, Na^+, K^+-ATPase activity was significantly higher than in animals not treated within this inhibitor (+23%, $p < 0.01$ and +27%, $p < 0.001$ for 10^{-9} and 10^{-8} mol/kg/min ETX respectively). Moreover, Na^+, K^+-ATPase activity in animals receiving 10^{-8} mol/kg/min ETX and 3×10^{-10} mol/kg/min PDBu was significantly higher ($p < 0.05$) than after a lower dose of PDBu (3×10^{-11} mol/kg/min) without ETX (144% and 134% of control respectively). Finally, ethoxyresorufin dose-dependently blocked the inhibition of Na^+, K^+-ATPase by 3×10^{-9} mol/kg/min PDBu. Na^+, K^+-ATPase activity after pretreatment with 10^{-9} mol/kg/min ETX was not significantly different from control and was higher than after PDBu alone (+18%, $p < 0.01$). When a higher dose of ETX (10^{-8} mol/kg/min) was administered for 30 minutes before the infusion of PDBu (3×10^{-9} mol/kg/min), this PKC activator increased Na^+, K^+-ATPase activity by 45%. The difference in Na^+, K^+-ATPase activity in rats pretreated with 10^{-8} mol/kg/min ETX which subsequently received 3×10^{-9} mol/kg/min or 3×10^{-10} mol/kg/min PDBu was not significant. Unlike ethoxyresorufin, ibuprofen (3 μmol/kg/min) infused before PDBu did not prevent Na^+, K^+-ATPase inhibition by this phorbol ester (data not presented). These results suggest that P450-monooxygenase products mediate the inhibitory effect of PKC and that this inhibitory effect after high doses of PDBu is superimposed on preexisting Na^+, K^+-ATPase stimulation.

DISCUSSION

Our results indicate that PKC activating phorbol esters — PDBu and PMA have dose-dependent effect on renal cortical Na^+, K^+-ATPase. Low doses of these esters stimulate this enzyme whereas higher doses decrease its activity. Several lines of evidence indicate that the effect of phorbol esters involves the activation of PKC: 1) 4 α PDD, phorbol ester which is not able to stimulate protein kinase C did not change Na^+, K^+-ATPase activity in any of the tested doses; 2) PDBu did not alter Na^+, K^+-ATPase activity directly when it was added to the incubation medium during enzyme assay; 3) specific inhibitor of PKC, staurosporine administered before PDBu blocked its both stimulatory and inhibitory effect. Because more than 90% of renal cortical Na^+, K^+-ATPase is contained in proximal convoluted tubules (PCT) (3), our findings should apply to this part of the nephron. Contrary to the renal cortex, phorbol esters had no influence on Na^+, K^+-ATPase activity in the renal medulla which is contained mainly in medullary thick ascending limbs (mTAL) and medullary collecting ducts (MCD). This is in agreement with the results obtained by Satoh *et al.* (10) who observed no effect of PKC on Na^+, K^+-ATPase in mTAL.

Protein kinase C exists in several isoforms which can be divided into three groups: classical, new and atypical PKC (19). Only classical and new PKC isoenzymes are activated by phorbol esters, so our results should apply only to these two groups. These results can not clarify the role of atypical PKC isoforms in the regulation of renal Na^+, K^+-ATPase. Rat proximal tubular cells contain not only clasical PKC α and new PKC δ and ε but also atypical PKC ζ (20—22). Unlike phorbol esters, mediators which activate PKC through the receptor mechanism can selectively regulate the activity of different PKC isoforms, among them also atypical, insensitive to phorbol esters. So the results obtained from our experimental model can not be directly extrapolated to *in vivo* conditions when PKC is activated through the receptor-operating mechanism.

It is well documented that prolonged action of phorbol esters down-regulates PKC activity. Therefore one could speculate that a high dose of PDBu causes accelerated stimulation of PKC followed by its inhibition and that decrease in Na^+, K^+-ATPase activity induced by high doses of PDBu results from inhibition not stimulation of PKC. However, this seems unlikely because staurosporine administered in rats not treated with PDBu did not change Na^+, K^+-ATPase activity. If inhibition of PKC resulted in decrease in Na^+, K^+-ATPase activity, staurosporine would reproduce this effect. Moreover, staurosporine blocked Na^+, K^+-ATPase inhibition by a high dose of PDBu indicating that this effect resulted from stimulation, not inhibition of PKC.

The results of our study suggest that inhibition of Na^+, K^+-ATPase induced by high doses of phorbol esters is mediated by cytochrome P450-dependent arachidonate metabolites. Ethoxyresorufin, the specific inhibitor of this part of arachidonate cascade blocked PDBu-induced Na^+, K^+-ATPase inhibition. Ethoxyresorufin did not influence Na^+, K^+-ATPase stimulation by a low dose of PDBu but it significantly augmented stimulation induced by a higher dose of this PKC activator and converted inhibition induced by the highest dose of PDBu to stimulation. Thus the influence of PKC activated by higher doses of phorbol esters on Na^+, K^+-ATPase seems to be the result of the two apposite mechanisms: stimulation and inhibition. The latter dominates after strong stimulation of PKC but is superimposed on stimulation, which can be unmasked when cytochrome P450-dependent arachidonate metabolism is inhibited.

The inhibitory action of PKC-stimulating phorbol esters on renal Na^+, K^+-ATPase was demonstrated in several studies (6, 8, 9, 10, 23). The role of cytochrome P450 dependent arachidonate metabolites in the regulation of the sodium pump is also well documented (24). Some of these compounds like 5, 6-epoxyeicosatrienoic acid (5, 6-EET), 11, 12-dihydroxyeicosatrienoic acid (11, (12-DHT) and 20-hydroxyarachidonic acid (20-HETE) inhibit Na^+, K^+-ATPase in the proximal tubule (18, 25, 26). These compounds were also shown to mediate the inhibitory effect of PKC in this nephron segment (3, 10, 18). Protein kinase C and cytochrome P450 dependent arachidonate metabolites are involved in Na^+, K^+-ATPase inhibition by dopamine (10, 18, 27, 28, 29) and parathyroid hormone (18, 25). However, in none of these studies performed on isolated native or cultured renal epithelial cells any stimulation of Na^+, K^+-ATPase by any dose of phorbol esters was observed. The mechanism through which PKC triggers arachidonate cascade is unclear, but it was demonstrated that PKC could activate phospholipase A_2 in cultured kidney cells (30).

The mechanism of Na^+, K^+-ATPase stimulation observed by us after the administration of low doses of PKC activators (and after higher doses combined with ethoxyresorufin) is less clear. Some mediators whose receptors are coupled to phospholipase C-PKC signal transduction, such as angiotensin and norepinephrine were shown to stimulate proximal tubular sodium and water reabsorption (31, 32). Stimulation of Na^+, K^+-ATPase by phorbol esters was demonstrated in rat PCT cells (3). Interestingly, this effect appeared only when cells were incubated in a well-oxygenated medium (which may better represent *in vivo* conditions); in usual *in vitro* conditions PKC inhibited Na^+, K^+-ATPase (3). Unlike in our experiments, these authors observed an increase in Na^+, K^+-ATPase activity only when intracellular Na^+ was rate limiting and not at saturating Na^+ concentration. Bertorello (11) described activation of Na^+, K^+-ATPase in PCT cells after stimulation of PKC but this effect was secondary to increased sodium entry. PKC mediators increase in Na^+,

K^+-ATPase activity induced by β2-adrenergic receptor agonists in rat PCT cells, however, this also results from increased apical sodium entry (23). Stimulatory effect of PKC activation on Na^+, K^+ATPase-dependent $^{86}Rb^+$ uptake was reported in cultured opossum kidney (OK) cells (the experimental model of the proximal tubule) transfected with the rodent Na^+, K^+-ATPase α1-subunit (5).

The stimulatory effect of PKC on Na^+, K^+-ATPase can be mediated, at least in part, by direct phosphorylation of the enzyme. Na^+, K^+-ATPase can be phosphorylated by PKC *in vitro* (7, 12, 33, 34) and in intact cells (4, 9, 35, 36). Phosphorylated residues are localized in the N-terminus of the α subunit (35, 36) and Pedemonte *et al.* (5) reported that NH_2 terminus of the α1 subunit is necessary for Na^+, K^+-ATPase stimulation by PKC in OK cells. Phosphorylation of the Na^+, K^+-ATPase α subunit after stimulation of PKC correlates with an increase in its transport activity in rat PCT cells (4). On the other hand, Feschenko and Sweadner (12, '35) did not observe any effect of PKC-mediated phosphorylation on Na^+, K^+-ATPase activity. Thus it is possible that PKC phosphorylates another protein which activates Na^+, K^+-ATPase or that phosphorylated Na^+, K^+-ATPase becomes sensitive to stimulation by other activators.

In conclusion, our study demonstrates that stimulation of protein kinase C by *in vivo* administration of phorbol esters has bi-directional effect on Na^+, K^+-ATPase activity (measured in the presence of saturating Na^+ concentration) in the rat renal cortex. Low doses of phorbol esters stimulate this enzyme whereas higher doses cause its inhibition. Both these effects do not result from altered sodium entry into the cell. The inhibitory effect is probably mediated by cytochrome P450-dependent arachidonate metabolites whereas the mechanism of the stimulatory effect remains to be elucidated.

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Author's address: Jerzy Bełtowski, Department of Pathophysiology, University School of Medicine, ul. Jaczewskiego 8, 20-090 Lublin, Poland.