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Protein Kinase C Activates the Renal Apical Membrane Na^+/H^+ Exchanger

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Summary. Studies were performed on purified brush-border membranes from the kidney of the rabbit to examine the relation between protein kinase C and the Na^+/H^+ exchanger in these membranes. The brush-border membranes were transiently opened by exposure to hypotonic media and the membrane proteins phosphorylated by exposure to ATP and phorbol esters or partially purified protein kinase C. The membranes were resealed and the intravesicular space acidified by incubation in a sodium-free isotonic solution (pH 5.5). The rate of uptake of 1 mM $^{22}Na^+$ (pH 7.5), with and without amiloride (1 mM), was assayed and the proton gradient-stimulated, amiloride-inhibitable component of $^{22}Na^+$ taken as a measure of the activity of the Na^+/H^+ exchanger. 12-0-tetradecanoyl phorbol-13-acetate (TPA) increased the amiloride-sensitive component of $^{22}Na^+$ uptake. TPA did not affect the amiloride-insensitive component of $^{22}Na^+$ uptake or the equilibrium concentration of sodium. TPA also did not affect the rate of dissipation of the proton gradient in the absence of sodium or the rate of sodium-dependent or -independent uptake of D-glucose. Other "active" phorbol esters stimulated the rate of Na^+/H^+ exchange, but phorbol esters of the 4 α configuration did not. Incubation of the opened membranes in partially purified protein kinase C increased the rate of proton gradient-stimulated, amiloride-inhibitable sodium uptake. The stimulatory effect of TPA and protein kinase C was not additive. In the absence of ATP, neither TPA nor protein kinase C affected Na^+/H^+ exchange transport. To determine the membrane-bound protein substrates, parallel experiments were conducted with γ-[^{32}P] ATP in the phosphorylating solutions. The reaction was stopped by SDS and the phosphoproteins resolved by PAGE and autoradiography. TPA stimulation of protein kinase C resulted in phosphorylation of approximately 13 membrane-bound proteins ranging in apparent molecule from 15,000 to 140,000 daltons. These studies indicate that activation of endogenous renal brush-border protein kinase C by phorbol esters or exposure of these membranes to exogenous protein kinase C increases the rate of proton gradient-stimulated, amiloride-inhibitable sodium transport. Protein kinase C activation also results in phosphorylation of a finite number of membrane-bound proteins.

Key Words protein phosphorylation · acid/base regulation · protein kinase C · cAMP-dependent protein kinase · sodium transport · renal proximal tubule

Introduction

Considerable recent investigate interest has focused around the role of protein kinase C in cell function. These relationships have been reviewed in detail in recent publications [6]. In brief, calcium-phospholipid-dependent protein kinase (protein kinase C) is present both in the cytosol and in plasma membranes. It is activated by calcium, phospholipids, and diacylglycerol and may be linked to agonist-stimulated alterations in the metabolism of phosphatidylinositol [1, 15]. Protein kinase C is also the major binding protein for the tumor-promoting phorbol esters [13]. Of particular interest has been the observation in several cell systems that activation of protein kinase C results in intracellular alkalinization by an amiloride-sensitive mechanism [2, 3, 7, 8, 16, 17, 21]. In these nonrenal tissues, the evidence to date would suggest that activation of protein kinase C results in stimulation of a Na^+/H^+ exchange transport. The effect of activation of protein kinase C on the well-defined Na^+/H^+ exchanger in renal tissue has not yet been studied in detail.

In renal tissue, evidence has been advanced that protein kinase C is present in both the cytosol and in the plasma membranes [9, 12]. Specific phorbol binding to renal membranes has also been reported [8]. The present studies were designed to examine the effect of protein kinase C on Na^+/H^+ exchange transport in the brush-border membrane of the rabbit kidney. In addition, the membrane-bound protein substrates of this kinase were determined.

Materials and Methods

Brush-border membrane vesicles were prepared from the kidney cortices of female New Zealand white rabbits by a modification of a magnesium aggregation method as previously described by this laboratory [10]. The enrichment of the membrane preparation was determined by measurement of the alkaline phosphatase activity in the brush-border membranes and in the homogenates of the kidney cortex.

The general protocol, except where otherwise indicated, was to open the vesicles by exposure to hypotonic media and

incubate the opened membranes in a phosphorylating solution containing ATP and the test reagent. For electrolyte transport studies, the membranes were resealed by exposure to an isotonic solution appropriate to the transport system under study. For the analysis of the phosphoproteins, the phosphorylating solution contained radioactive γ-[^{32}P]ATP. The reaction was stopped by sodium dodecyl sulfate (SDS) and the phosphoproteins resolved by polyacrylamide gel electrophoresis (PAGE) and autoradiography.

The membrane vesicles were opened by incubation in a hypotonic solution containing 10 mM $MgCl_2$, 10 mM KF, and 5 mM MES/Tris buffer (pH 6.8). ATP (10 to 500 μM) was added. All studies were carried out in the presence of calcium (1 to 10 mM). 12-0-tetradecanoyl phorbol-13-acetate (TPA), phorbol 12,13 didecanoate (PDD), phorbol 12,13 diacetate (PDA), 4 α phorbol (4αP), and 4 α phorbol 12,13 diacetate (4αPDA) were individually added to the phosphorylating solution to examine their effect on Na^+/H^+ exchange. The concentration of TPA ranged from 10^{-9} to 10^{-4} M. All the other phorbol esters were studied at a concentration of 10^{-6} M. Partially purified protein kinase C was prepared from rat kidney or brain by the method of Parker et al. [19]. The activity of the protein kinase C in a histone H1 phosphorylation assay was 40 units per ml. When studied, protein kinase C was added to the phosphorylating solution. In these experiments 1-oleoyl-2-acetyl-glycerol (OAG) (100 μg/ml) was also added to the incubation media. Incubations were carried out at 30°C.

For the study of Na^+/H^+ exchange, the membranes were resealed and the intravesicular space acidified by exposure to a solution containing (in mM): 227.8 mannitol, 42 MES, 8.6 Tris, 13.7 HEPES, 0.4 $MgSO_4$, 1.8 $MgCl_2$, 0.18, 1.8 $CaCl_2$, 1.8 KF and 1.8 ATP (pH 5.5) for 45 min at room temperature. The vesicles were then centrifuged at 24,000 × g for 30 min and resuspended in the same solution to a volume of 10 μl. This volume of vesicles was then mixed with 40 μl of a solution containing (in mM): 1.25 $^{22}Na^+$, 157.5 mannitol, 10 MES, 50 Tris, and 80 HEPES (pH 7.4), with and without amiloride (1 mM). Sodium uptake was assayed in triplicate at 22°C by a rapid Millipore filtration technique as previously described [10]. In separate experiments, the above protocols were performed in the absence of ATP. To determine if ATP and TPA required access to the inside of the vesicular space, incubations in phosphorylating solution containing ATP and TPA were performed in unopened vesicles using an isotonic solution containing (in mM): 254 mannitol, 10 Tris, 16 HEPES, 10 $MgCl_2$, 1 $CaCl_2$, 50 μM ATP (pH 5.5) with and without TPA (10^{-6} M).

Assessment of proton leak was determined by measurement of the change in fluorescence of acridine orange in the presence of an outwardly directed proton gradient. Brush-border vesicles were phosphorylated and resealed to an intravesicular pH of 5.5 as described above. The membranes were then diluted into a sodium-free medium containing 6 μM of acridine orange at pH 7.5. The calculation of the rate constant for hydrogen ion gradient dissipation was as previously described from this laboratory [11].

The rates of D-glucose transport were determined in vesicles which were resealed in a solution containing (in mM): 200 mannitol, 10 $MgSO_4$, 50 Tris, 80 HEPES (pH 7.5). The uptake of 0.2 μM D-[^{3}H]-glucose was assayed at 22°C in the presence of (in mM): 120 mannitol, 2 $MgSO_4$, 10 Tris, 16 HEPES (pH 7.5) and 100 NaCl or KCl.

To determine the proteins phosphorylated by protein kinase C, opened membranes were incubated in the phosphorylating solution containing γ-[^{32}P]-ATP and TPA. In some experiments, membranes were incubated in γ-[^{32}P] ATP and the catalytic subunit of cAMP-dependent protein kinase (15 mU/ml). After *in vitro* phosphorylation, the reaction was stopped by the addition of 60 mM Tris-glycine buffer (pH 8.3) containing 3% (wt/vol) sodium dodecyl sulfate (SDS) and 30% (wt/vol) glycerol. Samples were placed in a boiling water bath for 3 min and then cooled on ice. 80 μg of total protein was loaded on 7 to 16% gradient polyacrylamide gels. Electrophoresis was performed for 4 hr at 40 mA/gel. The gels were stained with Coomassie Blue, dried, and exposed to X-ray film (Kodak XAR-5) using cassettes containing tungsten phosphate intensifier screens for 2 to 14 days at −70°C. The autoradiograms were scanned with a soft laser densitometer to quantitate the phosphorylated protein bands.

Alkaline phosphatase activity was measured as described in Sigma Technical Bulletin No. 104. Protein concentrations were determined by the method of Lowry et al. [14]. Results of transport experiments are expressed as the mean of means of triplicate determinations ± SEM. The experiments were designed to permit comparison of control and experimental observations by the paired t-test.

Results

The brush-border membranes were enriched in the membrane marker alkaline phosphatase 10-fold or more above that of the whole homogenate. The Table indicates the rates of sodium uptake in vesicles opened, exposed to ATP and calcium, and resealed. In control membranes not exposed to TPA, total sodium uptake into an acidified intravesicular space at 10 sec was 2.68 ± 0.23 nmol/mg protein. The equilibrium concentration of sodium was 1.50 ± 0.08 nmol/mg protein. Thus, at 10 sec there was an overshoot of the equilibrium concentration of sodium of 178%. Sodium uptake at 10 sec in the presence of amiloride was inhibited 87% to 0.35 ± 0.05 nmol/mg protein.

The addition of TPA to the phosphorylating solution increased the total and amiloride-sensitive component of $^{22}Na^+$ uptake. In preliminary studies, incubations were carried out with TPA (10^{-6} M) for 1 to 5 min and with concentrations of ATP of 10, 50, 100 and 500 μM. The results were identical for all time points and with all concentrations of ATP tested. Accordingly, all the remaining transport studies were conducted with an ATP concentration of 50 μM and with 5 min of incubation. All transport studies were performed in the presence of calcium in the phosphorylating solution. Concentrations ranging from 1 to 10 mM yielded identical results. The dose-response relation between the amiloride-sensitive component of $^{22}Na^+$ uptake and the concentration of TPA was examined. Concentrations of TPA of 10^{-6} M and greater yielded a maximum response. TPA in a concentration of 10^{-9} M did not affect the proton gradient-stimulated uptake of sodium. In the remaining studies, TPA was used in a concentration of 10^{-6} M. As shown in the Table,

Table. Effect of TPA on $^{22}Na^-$ uptake[a]

Sodium uptake (nmol/mg protein)	Control	TPA (10^{-6} M)	Difference	*P*
Total (10 sec)	2.68 ± 0.16	2.92 ± 0.17	0.24 ± 0.04	≤0.1
Amiloride sensitive (10 sec)	2.33 ± 0.13	2.59 ± 0.15	0.26 ± 0.05	≤0.1
Amiloride insensitive (10 sec)	0.34 ± 0.04	0.32 ± 0.03	0.02 ± 0.01	NS
Equilibrium (90 min)	1.50 ± 0.06	1.54 ± 0.07	0.04 ± 0.02	NS

[a] Results are expressed as the mean ± SEM. NS = not significant.

TPA (10^{-6} M) significantly increased the total and amiloride-sensitive component of sodium uptake as compared to the same vesicles not exposed to TPA ($n = 6$). The amiloride-insensitive component and the equilibrium concentration of sodium were not significantly different between control and experimental vesicles. In vesicles exposed to ATP but not opened, the amiloride-sensitive component of sodium uptake at 10 sec was 2.40 ± 0.24 nmol/mg protein in the absence of TPA and 2.21 ± 0.16 ($n = 3$; p = NS) in the presence of TPA. In vesicles opened and resealed but not exposed to ATP, the 10-sec uptake of $^{22}Na^+$ was 2.39 ± 0.09 nmol/mg protein in the absence of TPA and 2.22 ± 0.22 ($n = 3$; p = NS) in the presence of TPA.

To exclude the possibility that the change in $^{22}Na^+$ uptake when membranes were exposed to TPA was the secondary consequence of altered permeability of the vesicles to protons, the rate constant for dissipation of the proton gradient was determined using acridine orange fluorescence. The rate constant for proton dissipation averaged 0.023 ± 0.006 sec^{-1} and 0.024 ± 0.006 in control and TPA-treated vesicles, respectively (p = NS). The specificity of the effect of TPA was examined by measurement of the rates of sodium-dependent and -independent uptake of D-glucose. In control vesicles, the 10-sec uptake of glucose in the presence of a sodium gradient was 1.7 ± 0.4 pmol/mg protein in controls and 1.6 ± 0.2 ($n = 3$; p = NS) in TPA-treated vesicles. Glucose uptake at 10 sec in the presence of a potassium gradient averaged 0.07 ± 0.02 pmol/mg protein and 0.07 ± 0.004 (p = NS) in control and experimental vesicles, respectively. Equilibrium concentrations of glucose averaged 1.1 ± 0.1 pmol/mg protein in controls and 1.0 ± 0.1 (p = NS) in TPA-treated vesicles.

Additional studies were performed using other phorbol esters. The phorbol esters were classified as active or inactive based on their ability to activate protein kinase C and/or specifically displace radioactive phorbol esters in other tissue systems. As shown in Fig. 1, the active phorbol esters PDD and PDA, in addition to TPA, all significantly stimulated the amiloride-sensitive component of $^{22}Na^+$ uptake as compared to controls. The degree of stimulation was approximately equal for all three of the active phorbol esters. Phorbol esters containing the 4 α configuration did not stimulate sodium uptake.

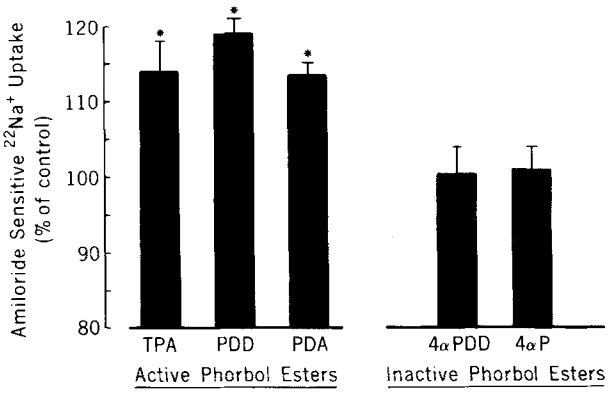

Fig. 1. Effect of active and inactive phorbol esters on proton gradient-stimulated, amiloride-inhibitable $^{22}Na^+$ uptake. Results are expressed as percent of control ± SEM. * indicates $P \leq 0.05$. TPA = 12-0-tetradecanoyl phorbol-13-acetate; PDD = phorbol 12,13 didecanoate; PDA = phorbol 12,13 diacetate, 4αPDD = 4αphorbol 12,13 diacetate; 4αP = 4αphorbol

Phorbol esters activate endogenous protein kinase C. To determine if the amount of endogenous protein kinase C was rate limiting for affecting the Na^+/H^+ exchanger, membranes were incubated in OAG and partially purified protein kinase C from either the kidney or brain of the rat. Both preparations of protein kinase C yielded comparable results. The results are summarized in Fig. 2. Protein kinase C stimulated amiloride-sensitive uptake of sodium from 2.26 ± 0.12 nmol/min in controls to 2.65 ± 0.17 ($n = 5$; $p < 0.01$); an increase of 17.3 ± 2.7%. In the same vesicles, TPA increased the proton gradient-stimulated amiloride-sensitive uptake of $^{22}Na^+$ at 10 sec 17.3 ± 2.6%. Incubation of the membranes in both protein kinase C and TPA resulted in $^{22}Na^+$ uptake that was 18.5 ± 3.2% above controls. The rates of Na^+/H^+ exchange transport were significantly increased by protein kinase C, TPA, and both protein kinase C and TPA together. There were no differences, however, between the

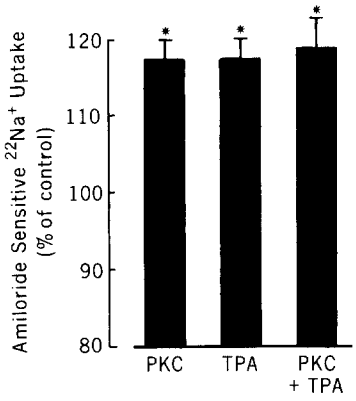

Fig. 2. Effect of exogenous protein kinase C (PKC), TPA, and PKC and TPA on proton gradient-stimulated, amiloride-inhibitable $^{22}Na^+$ uptake. Results are expressed as percent of control ± SEM. OAG was added to the phosphorylating solution in the experiments utilizing protein kinase C. indicates $p \leq 0.05$

percent stimulation in any of these three experimental protocols. Incubation of the membranes with protein kinase C in the absence of ATP did not significantly affect $^{22}Na^+$ uptake (percent change = $-1.3 \pm 1.4\%$).

Both TPA and the catalytic subunit of cAMP-dependent protein kinase stimulated phosphorylation of membrane-bound proteins. Time-course studies, in the presence of fluoride, indicated phosphorylation was linear for up to 5 min of incubation. Significant dephosphorylation was evident by 10 min of incubation. Figure 3 is a representative SDS-PAGE autoradiogram of membranes incubated in radioactive ATP and either TPA (10^{-6} M) (PKC), or catalytic subunit of cAMP-dependent protein kinase (cAMP PK). Control vesicles were exposed to neither TPA nor catalytic subunit. The gel shown is the phosphoprotein pattern after 3 min of incubation of membranes in the phosphorylating solutions. The molecular weights are shown. The arrows indicate the membrane substrate proteins whose phosphorylation is stimulated by protein kinase C or cAMP-dependent protein kinase. Figure 4 is a tabular representation of Fig. 3. Approximately 13 phosphoproteins ranging in molecular weight from 16,000 to 140,000 daltons are stimulated by incubation of membranes with ATP and TPA. All of the proteins indicated were stimulated in a time frame consonant with the observed physiologic response. There are approximately 18 proteins ranging in molecular weights from 16,000 to 140,000 daltons stimulated by the catalytic subunit of cAMP-dependent protein kinase.

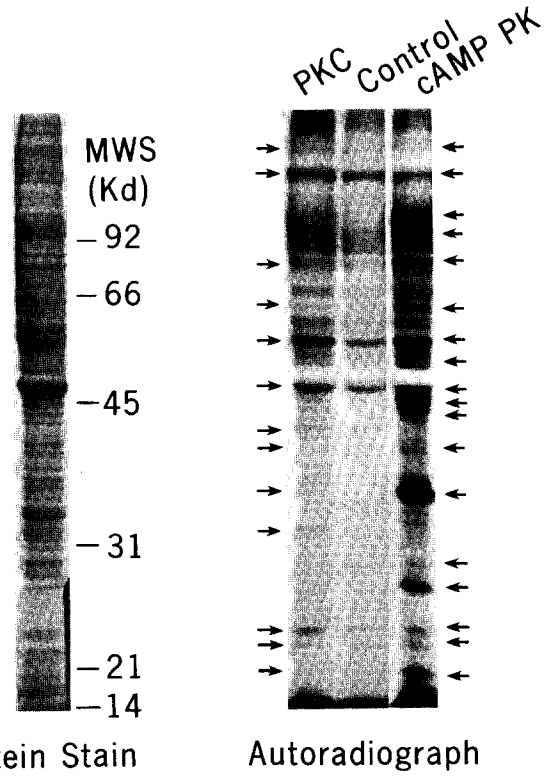

Fig. 3. Autoradiogram of brush-border membranes incubated in γ-^{32}[P] ATP and TPA (PKC), catalytic subunit of cAMP-dependent protein kinase (cAMP PK), or neither TPA or catalytic subunit (Control). The Coomassie Blue protein stain is shown and apparent molecular weights are indicated. The arrows indicate membrane-bound proteins whose phosphorylation is stimulated by TPA or the catalytic subunit of cAMP-dependent protein kinase

Discussion

Several recent review articles have detailed the growing interest in the role of protein kinase C on cell function [6]. The activity of this kinase appears to be linked to agonist-induced changes in phosphatidylinositol metabolism [1, 15]. In addition, it appears that the tumor-promoting phorbol esters bind specifically to and directly activate protein kinase C [13, 18, 22]. The use of the phorbol esters has provided an experimental approach to study the biologic effects of protein kinase C activation. Protein kinase C is present in both the cytosol and the plasma membranes of cells [12]. Studies by others, as well as recent studies in our laboratory have indicated that this kinase is present in the cytosol and in the brush-border membrane of the cells of the kidney [9]. Studies by Hammerman et al. have indicated specific phorbol binding to renal plasma mem-

branes [8]. In other cell systems, evidence has been advanced to indicate that phorbol esters result in intracellular alkalinization by a sodium-dependent mechanism that is sensitive to inhibition by amiloride [2, 3, 7, 16, 17, 21]. It is suggested from such studies that protein kinase C activation results in stimulation of a sodium-proton exchanger. The brush-border membrane of the kidney contains an electroneutral transport system which exchanges sodium for protons and is inhibited by amiloride. The effect of protein kinase C on the renal Na^+/H^+ exchanger has not yet been studied in detail. The present studies were designed to examine the effect of activation of protein kinase C with phorbol esters on the Na^+/H^+ exchanger of the apical membrane of the renal proximal tubule. The effect of exogenous protein kinase C on this transport system was also evaluated. Finally, in the brush-border membrane, the membrane-bound protein substrates of protein kinase C were studied.

As previously detailed from this laboratory, the methadologic approach was to incubate opened brush-border membrane vesicles in a phosphorylating solution [23]. After *in vitro* phosphorylation, the membranes were either resealed to permit study of the rates of transport or processed by SDS-PAGE and autoradiography to study the protein substrates of protein kinase C. As shown in the Table, TPA resulted in an increase in the amiloride-sensitive component of proton gradient-stimulated $^{22}Na^+$ uptake. TPA did not affect the amiloride-insensitive component of $^{22}Na^+$ uptake, an estimate of membrane permeability to sodium, the equilibrium concentration of sodium, an estimate of the intravesicular volume, or the rate constant for dissipation of the proton gradient. The sodium-dependent and -independent uptake of D-glucose and the equilibrium concentration of glucose were also not affected by TPA. The glucose uptake experiments indicate that there is specificity to the effect of TPA and given the electrogenic nature of sodium-glucose cotransport, support the conclusion that TPA did not affect the passive movement of sodium or protons. In the absence of ATP, TPA did not affect sodium uptake. In addition, exposure of membranes which had not been opened to both ATP and TPA failed to affect proton-stimulated $^{22}Na^+$ uptake. Taken together, it would appear that TPA-stimulated the sodium-proton exchanger in the brush-border membrane of the rabbit kidney in an ATP-dependent manner.

To extend the above observations, studies were performed using other phorbol esters which have to be classified as active or inactive based on their

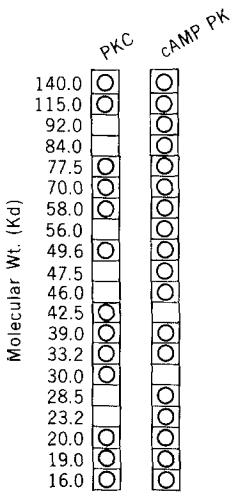

Fig. 4. Tabular representation of membrane-bound protein substrates for protein kinase C and cAMP-dependent protein kinase derived from Fig. 3. The circles indicate detection of a stimulated phosphoprotein band on SDS-PAGE autoradiography

ability to directly stimulate protein kinase C and/or specifically displace phorbol esters [7, 13]. As summarized in Fig. 1, there was an excellent correlation between the studies in other tissues and the effect of the phorbol esters on the Na^+/H^+ exchanger in the renal apical membrane. In addition to TPA, PDD and PDA significantly increased proton gradient-stimulated, amiloride-inhibitable sodium uptake. Phorbol esters of the 4α configuration (inactive phorbol estes) failed to affect renal Na^+/H^+ exchange.

The effect of exogenous protein kinase C on Na^+/H^+ exchange was examined to determine if the amount or activity of endogenous protein kinase C was rate limiting. Partially purified protein kinase C, in the presence of OAG, stimulated $^{22}Na^+$ uptake to a degree comparable to that observed with TPA. Incubation of the membranes with both protein kinase C and maximum concentrations of TPA resulted in no further stimulation of $^{22}Na^+$ uptake. As was the case for TPA, protein kinase C was inactive in the absence of added ATP.

The magnitude of the stimulation by either active phorbol esters or protein kinase C was between 15 and 20%. It seems unlikely that aspects of experimental design influenced the magnitude of the response. In the majority of the studies, the ATP concentration was 50 μM. Stimulation of transport was maximal at 10 μM and increases in the ATP concentration to 500 μM resulted in no further stimulation of transport. In the studies reported, the time of phosphorylation was 5 min. Time-course studies in-

dicated, however, that the response was evident by 1 min. In addition, studies performed with and without fluoride, an inhibitor of protein phosphatase activity, yielded identical results. It would appear that neither the time of phosphorylation nor dephosphorylation of membrane phosphoproteins was affecting the magnitude of the change in Na^+/H^+ exchange. The finding that incubation of the membranes with both exogenous protein kinase C and TPA resulted in a stimulation of Na^+/H^+ countertransport, which was no greater than that observed with either agent alone, suggests that the activation or amount of protein kinase C was not rate limiting. The above findings would also suggest that exogenous protein kinase C and endogenous protein kinase C affect the same substrate proteins involved in modulation of Na^+/H^+ exchange activity.

Besterman and co-workers have recently reported that amiloride directly inhibits protein kinase C and caution against the use of amiloride as a specific inhibitor of Na^+/H^+ countertransport in experimental circumstances where the activity of the transporter is being correlated with phosphorylation of proteins [2]. In these studies, the presence of amiloride at the time of phosphorylation significantly inhibited protein phosphorylation. In the present experiments, amiloride was not present when the membranes were exposed to the phosphorylating solution. Amiloride, in the concentration employed, probably does not affect protein phosphatase activity [2]. Under the conditions of study in the present experiments, therefore, amiloride should still provide a valid estimate of Na^+/H^+ exchange activity.

The membrane-bound proteins which are substrates for protein kinase C were studied by incubating membranes in γ-[^{32}P] ATP and TPA. As shown in Fig. 2, there are approximately 13 proteins which are phosphorylated in a protein kinase C-dependent fashion. All of the substrate proteins are phosphorylated within a time frame consonant with the physiologic response measured, namely Na^+/H^+ exchange transport.

It is of interest to contrast and compare the effect of activation of two membrane-bound protein kinases on the activity of the Na^+/H^+ exchanger. Prior studies from this and other laboratories have indicated that cAMP, by activating cAMP-dependent protein kinase, inhibits the renal apical membrane Na^+/H^+ antiporter [4, 5, 11, 20, 23]. As demonstrated in the present studies, protein kinase C activation stimulates Na^+/H^+ exchange. For each of these kinases, we have determined the apparent molecular weights of substrate proteins in the brush-border membrane. Each kinase reproducibly phosphorylates specific apical membrane-bound proteins. Some of the phosphoproteins identified are common to the two kinases. While it is premature to speculate beyond the existing data, it is of considerable interest that two kinases with opposing effects on a transport system have some proteins as common substrates. Figure 3 summarizes the proteins in the brush-border membranes phosphorylated by protein kinase C- and cAMP-dependent protein kinase. This analysis indicates the presence of at least 11 proteins which are substrates for both kinases. While the nature of these proteins is unknown at the present time, it is possible that one or more of the common substrate proteins is a regulator of the Na^+/H^+ exchanger or perhaps the exchanger itself. Further work is necessary to explore this issue.

In summary, the present studies indicate that protein kinase C phosphorylation membrane-bound protein substrates and stimulates the rate of proton gradient-stimulated, amiloride-inhibitable sodium uptake in rabbit renal brush-border membranes. In conjunction with other studies, it would seem that the apical membrane Na^+/H^+ exchanger is modulated by at least two membrane-bound kinases, cAMP-dependent protein kinase and protein kinase C. The demonstration of protein substrates which are common to these two kinases raises the interesting possibility that one of the common proteins in a regulatory protein for Na^+/H^+ countertransport or the transporter itself.

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