J. Membrane Biol. 153, 233–239 (1996)

The Journal of
Membrane
Biology

© Springer-Verlag New York Inc. 1996

Role of Protein Phosphatase in the Regulation of Na^+-K^+-ATPase by Vasopressin in the Cortical Collecting Duct

M. Blot-Chabaud, N. Coutry, M. Laplace, J.-P. Bonvalet, N. Farman
INSERM U246, Institut Fédératif de Recherches « Cellules Epithéliales « , Faculté de Médecine Xavier Bichat, B.P. 416, 75870 PARIS, Cedex 18, France

Received: 22 March 1996/Revised: 21 June 1996

Abstract. In the cortical collecting duct (CCD), arginin vasopressin (AVP) has been shown to increase the number and activity of basolateral Na^+-K^+-ATPase by recruiting or activating a latent pool of pumps. However, the precise mechanism of this phenomenon is still unknown. The aim of this study was to investigate whether this AVP-induced increase in basolateral Na^+-K^+-ATPase could depend on a dephosphorylation process. To this purpose, the effect of protein serine/threonine phosphatase (PP) inhibitors was examined on both the specific ^{3}H-ouabain binding (to evaluate the number of pumps in the basolateral membrane) and the ouabain-dependent ^{86}Rb uptake (to evaluate pump functionality) in the presence or absence of AVP. In addition, the activity of two PP, PP1 and PP2A, was measured and the influence of AVP was examined on both enzymes. Experiments have been performed on mouse CCD isolated by microdissection. Results show that inhibition of PP2A prevents the AVP-induced increase in the number and activity of Na^+-K^+-ATPases, independent of an effect on the apical cell sodium entry. In addition, AVP rapidly increased the activity of PP2A without effect on PP1. These data suggest that PP2A is implied in the regulation of Na^+-K^+-ATPase activity by AVP in the CCD and that the AVP-dependent increase in the number of Na^+-K^+-ATPases is mediated by a PP2A-dependent dephosphorylation process.

Key words: Isolated tubules — Sodium pump — Phosphorylation

Introduction

In the cortical collecting duct (CCD), aldosterone is involved in the constitution of a latent pool of Na^+-K^+-ATPases [1, 7]. The recruitment and/or activation of these pumps is induced by an increase in intracellular sodium concentration [1, 7, 15] or in cellular volume [16]. Recently, we have shown that arginin vasopressin (AVP) could induce the phenomenon, leading to a synergy of action of AVP and aldosterone on the basolateral Na^+-K^+-ATPase [17]. However, the precise mechanism leading to the increased Na^+-K^+-ATPase activity is still unknown.

Recent studies have demonstrated that Na^+-K^+-ATPase can be present under several phosphorylation states. Serine and/or threonine residues of the Na^+-K^+-ATPase can be phosphorylated by protein kinase A (PKA) or C (PKC) leading to an inactivated enzyme [4, 5, 9, 18]. Phosphorylation/dephosphorylation processes could thus represent a new regulation pathway of the Na^+-K^+-ATPase activity [10, 11]. The role of protein serine/threonine phosphatases (PP) such as PP1 or PP2A that could dephosphorylate Na^+-K^+-ATPase is still unknown. It has been shown that dephosphorylation by PP is involved in the regulation of ion channel [2, 3, 22, 23, 25, 27] or Na^+-K^+-$2Cl^-$ [21]. Several PP inhibitors such as okadaic acid, calyculin A and tautomycin have been found to be potent and selective inhibitors of PP1 and PP2A [6, 12, 20, 24, 30] and have been commonly used to examine the effects of dephosphorylation processes [21, 22, 28]. In the present study, we have examined the role of PP in the AVP-induced increase in the number and activity of Na^+-K^+-ATPase pumps and searched for the type of PP involved. Results have shown that (i) AVP-induced pump recruitment or activation is blunted in the presence of protein phosphatases inhibitors; (ii) AVP activates PP2A, not PP1, in the CCD. It is concluded that the AVP-induced recruitment or activation of the latent pool of Na^+-K^+-ATPase is due to a PP2A-dependent dephosphorylation process and that this phenomenon is mediated by a selective increase in PP2A activity by AVP.

Correspondence to: M. Blot-Chabaud

Material and Methods

Animals

Experiments have been performed on female Swiss mice (20–30 g body wt; $n = 24$). Each animal was bilaterally adrenalectomized and an osmotic minipump (Alzet 2001, Alza, Palo Alto, CA) delivering 1 μg. 100 g body wt^{-1}. day^{-1} dexamethasone and 30 μg. 100 g body wt^{-1}. day^{-1} aldosterone was immediately placed subcutaneously. Animals were fed a standard laboratory diet and had free access to tap water. Experiments were performed 5–7 days after surgery. Plasma aldosterone concentration (radioimmunoassay, Oris Industrie, Saclay, France) was 136 ± 4 ng/dl.

Microdissection of CCD

Mice were injected i.p. with 50 U heparin sodium 5 min before the beginning of the experiment. They were anesthetized with ether, and 1 ml blood was collected from the vena cava after abdominal incision (to determine plasma aldosterone concentration). Kidneys were perfused via the aorta with an ice-cold rinsing solution containing (in mM): 137 NaCl, 5 KCl, 0.8 $MgSO_4$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 1 $MgCl_2$, 1 $CaCl_2$, 5 D-glucose, and 10 TRIS (hydroxymethyl) aminomethane HCl, pH 7.4. Subsequently, they were perfused with a collagenase solution, which was identical to the rinsing solution except that 0.1% collagenase (0.85 U/mg, Serva, Heidelberg, Germany) was added. Thin slices of kidney were cut and incubated in collagenase solution at 30°C for 1 hr under air bubbling. Dissection of CCD was then performed at 4°C under stereomicroscope in a Na+K solution containing (in mM): 139 NaCl, 10 $NaHCO_3$, 5 KCl, 0.8 $MgSO_4$, 5 glucose, 1 $MgCl_2$, 1 $CaCl_2$, 1 alanine, 20 HEPES/TRIS and 1 mg/ml bovine serum albumin (BSA), pH 7.4.

Determination of Tubular Volume

After microdissection at 4°C, CCD were incubated at 37°C for 10 min in the Na+K solution to restore electrochemical gradients. CCD were then photographed using a camera placed on a stereomicroscope. This photograph served to determine the tubular volume of each CCD, using an image analyzer (Biocom, France). Tubular surface area and tubular length were determined and tubular volume was calculated assuming a cylindrical shape since it has been shown that the lumen of nonperfused tubules represents < 5% of tubular volume [29]. Results were expressed per nanoliter tubular volume.

Ouabain Binding

The number of pumps present in the basolateral membrane of the tubular cells was determined using ^{3}H-ouabain binding technique [7, 17]. Briefly, CCD were microdissected at 4°C, then incubated for 10 min at 37°C in the Na+K solution, and the tubular volume of each CCD was determined (*see above*). After a preincubation step in the Na+K solution in the presence or absence of protein phosphatase inhibitor for 5 min, CCD were transferred into the cavity of a microscope slide containing 15 μl of a Na solution (identical to the Na+K solution but without KCl) and 5×10^{-5} M ^{3}H-ouabain (15-30 Ci/mmol; Du Pont-New England Nuclear), with or without AVP and in the presence or absence of protein phosphatase inhibitor (okadaic acid or calyculin A). This was performed both in the absence and in the presence of a 100-fold excess of unlabeled ouabain (to determine total and non specific ^{3}H-ouabain binding). Slides were covered with a microscope slide to prevent evaporation and placed on a thermoregulated plate at 37°C. Incubation was performed for 5 min, an elapse of time sufficient to reach equilibrium of the specific 3H-ouabain binding [16]. At the end of the incubation time, tubules were rinsed at 4°C in a choline solution for 10–15 min to reduce non specific ^{3}H-ouabain binding [7, 16]. This rinsing solution contained (in mM): 149 chloride choline, 0.80 $MgSO_4$, 5 glucose, 1 $MgCl_2$, 1 $CaCl_2$, 1 alanine, 20 HEPES/TRIS and 10 mg/ml BSA, pH 7.4. The radioactivity of each CCD was counted with a liquid scintillation beta counter (model 1217; LKB). Using ^{3}H counts and tubular volume of each CCD, specific ^{3}H-ouabain binding was calculated and expressed as femtomoles per nanoliter tubular volume.

Rubidium Uptake

The functional activity of pumps present in the membrane was examined using ^{86}Rb uptake technique [8, 16, 17]. Briefly, after CCD microdissection and tubular volume determination, CCD were preincubated for 5 min in the cavity of a microscope slide containing 15 μl of the Na+K solution to which protein phosphatase inhibitor was added or not. Then, CCD were incubated for 4 min in 15 μl of the Na+K solution or 15 μl of the Na solution plus 10^{-4} M ouabain (to measure total and ouabain-insensitive ^{86}Rb uptake) in the presence or absence of protein phosphatase inhibitor. This was performed in the presence and absence of AVP. Slides were covered and placed at 37°C. After these preincubations, CCD were rapidly transferred into another slide for 1 min at 37°C containing 15 μl of the Na solution with 5 mM RbCl and a tracer amount of ^{86}RbCl (1–35 Ci/g; Du Pont-New England Nuclear), with or without protein phosphatase inhibitor in the presence or absence of AVP. Incubation was stopped by the addition of 100 μl of ice-cold rinsing solution (*see above*) with 3 mM $BaCl_2$, and slides were transferred onto ice. CCD were briefly rinsed (<4 min) at 4°C in the rinsing solution to eliminate extracellular ^{86}Rb contamination (intracellular ^{86}Rb loss was <5% after a 4-min rinse). The radioactivity of each CCD was counted using a liquid scintillation beta counter. Using ^{86}Rb counts and tubular volume of each CCD, ouabain-sensitive ^{86}Rb uptake was calculated and expressed as picomoles per nanoliter tubular volume per minute.

Determination of Protein Phosphatase (PP) Activity

PP activity was measured using a kit (GIBCO-BRL, France) [13, 28]. Briefly, this kit uses as the substrate 32p-labeled glycogen phosphorylase a, which is a common substrate for PP1 and PP2A. To determine the type of phosphatase implied, we took advantage of the differential sensitivity of PP1 and PP2A to okadaic acid (OK) and tautomycin (TAUT). OK 1nM preferentially inhibits PP2A, whereas 3nM TAUT inhibits mainly PP1 [14, 24]. After CCD microdissection and tubular volume determination, CCD were preincubated for 5 min with or without protein phosphatase inhibitor in the presence or absence of AVP. Afterwards, CCD were pelleted and cell extract was prepared as follows. The pellet was homogenized in hypotonic buffer containing (in mM): 50 Tris.HCl, pH 7.5, 0.1 EDTA, 0.1 ethylene glycol-bis(β-aminoethyl ether)-N, N, N′, N′-tetraacetic acid, 0.1% (vol/vol) β-mercaptoethanol, 25 μg/ml leupeptin, 25 μg/ml aprotinin, and 0.1% (vol/vol) Triton X-100, and incubated for 30 min at 4°C. PP inhibitor and AVP were still present in this solution when required. The homogenate was

centrifuged at 12,000× *g* for 25 min. Preliminary studies were performed so that PP activity was not > 0.02 nmol/min in the assay and activity was linear with time and protein concentration. The reaction was started by adding the (^{32}P) phosphorylase a to the supernatant and incubating in a water bath at 30°C for 10 min. The reaction was stopped by the addition of 20% trichloroacetic acid and refrigeration on ice for 20 min. The tubes were centrifuged at 12,000× *g* for 5 min at 4°C, and the clear supernatant was used to determine the amount of radioactivity released in the assay as ^{32}Pi. The results were expressed as nanomoles ^{32}Pi released per nanoliter tubular volume per minute.

Drugs

AVP was from Sigma. Okadaic acid and calyculin A were from ICN. Tautomycin was from Calbiochem.

Statistical Analysis

Data were expressed as means ± SE (*n*, number of CCD). Statistical analyses were performed using a two tailed student's *t* test for independent means.

Results

Effect of Okadaic Acid and Calyculin on Specific ^{3}H-ouabain Binding and on Ouabain-sensitive ^{86}Rb Uptake in the Presence or Absence of AVP

Specific ^{3}H-ouabain binding was determined in CCD incubated in the presence or absence of AVP with or without addition of okadaic acid 1 μM or calyculin A 50 nM which inhibits both PP1 and PP2A. Incubation time was 5 min with or without the inhibitor followed by 5 min incubation in the presence or absence of AVP. Figure 1 illustrates the results. Okadaic acid (*A*) as well as calyculin A (*B*) prevented the AVP-induced increase in specific ^{3}H-ouabain binding.

Ouabain-sensitive ^{86}Rb uptake was also determined in the same conditions. Figure 2 shows that whereas AVP induced a significant increase in ouabain-sensitive ^{86}Rb uptake in the absence of inhibitor, no effect of AVP could be observed when CCD were incubated with 1 μM okadaic acid or 50 nM calyculin A.

Influence of Nystatin on the Effect of Calyculin A on Specific ^{3}H-ouabain Binding in the Presence of AVP

To determine whether PP inhibitors act directly on Na^+-K^+-ATPase or exert their effects by affecting primarily the apical Na^+ channel, the effect of calyculin A on the AVP-induced increase in specific ^{3}H-ouabain binding was determined in the presence or absence of the sodium ionophore nystatin 120 μg/ml. In a previous study, we have shown that nystatin rapidly equilibrated intra- and extracellular Na^+ concentrations, leading to an increase in the specific ^{3}H-ouabain binding in the absence of apical sodium entry through the Na^+ channel [16]. Results are shown in Fig. 3. Calyculin A was able to prevent the AVP-induced increase in specific ^{3}H-ouabain binding with or without nystatin treatment, suggesting a direct action of the inhibitor on Na^+-K^+-ATPase.

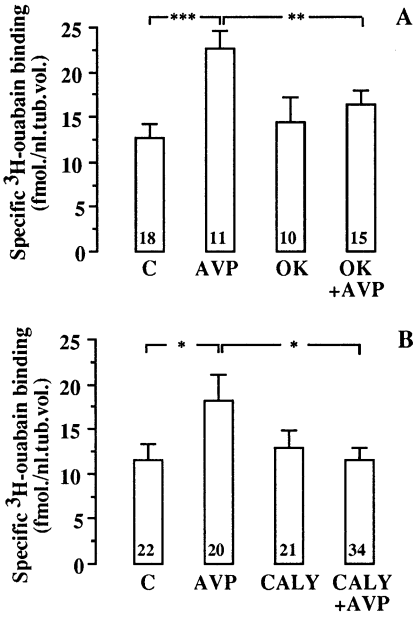

Fig. 1. Effect of 1 μM okadaic acid (OK) and 50 nM calyculin A (CALY) on specific ^{3}H-ouabain binding in the presence or absence of 10^{-8} M AVP. Experiments have been performed on CCD preincubated for 5 min in the presence or absence of 1 μM OK (panel *A*) or 50 nM CALY (panel *B*). Specific ^{3}H-ouabain binding was measured by incubation of CCD for 5 min with 5×10^{-5} M ^{3}H-ouabain binding ± 100-fold excess of unlabeled ouabain, in the presence (AVP) or absence (C) of 10^{-8} M AVP with or without drug. The increase in specific ^{3}H-ouabain binding induced by AVP was prevented in the presence of each inhibitor. Numbers in bars are the number of CCD tested. Bars are mean values ± SE. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Concentration Dependence of the Effect of Okadaic Acid and Calyculin A on Specific ^{3}H-ouabain Binding in the Presence of AVP

The effect of increasing doses of okadaic acid or calyculin A was determined on the specific ^{3}H-ouabain binding of CCD incubated in the presence of AVP. Results are shown in Fig. 4. Okadaic acid produced a dose-dependent decrease in the AVP-induced specific ^{3}H-ouabain binding (Fig. 4*A*). The apparent $K_{1/2}$ of inhibition (as determined by the reverse plot analysis, $n = 5$, $r = 0.98$) was about 2nM. Calyculin A also produced a dose-dependent decrease in the AVP-induced specific ^{3}H-ouabain binding (Fig. 4*B*) with a $K_{1/2}$ of inhibition <6 nM.

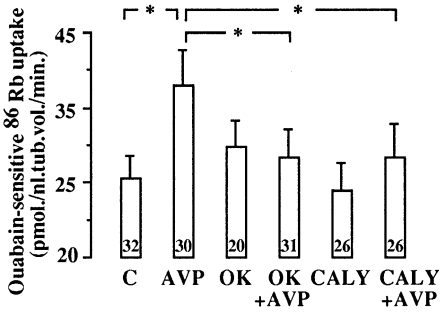

Fig. 2. Effect of 1 μM okadaic acid (OK) and 50 nM calyculin A (CALY) on the ouabain-sensitive ^{86}Rb uptake in the presence or absence of 10^{-8} M AVP. Experiments have been performed on CCD preincubated for 5 min in the presence or absence of 1 μM OK or 50 nM CALY. ^{86}Rb uptake was measured after 4-min incubation with 5 mM Rb by addition of a tracer amount of ^{86}Rb during 1 min, in the presence or absence of 10^{-4} M ouabain. AVP 10^{-8} M was added (AVP) or not (C) to the medium for the last 5 min in the presence or absence of each inhibitor. The increase in ouabain-sensitive ^{86}Rb uptake induced by AVP was prevented in the presence of each inhibitor. Numbers in bars are the number of CCD tested. Bars are mean values ± SE. *$P < 0.05$.

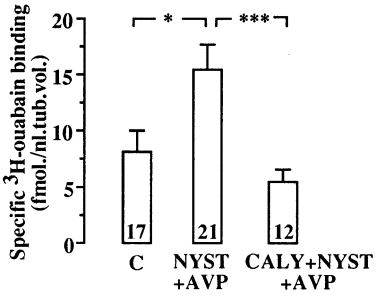

Fig. 3. Effect of nystatin (NYST) on the AVP-induced increase in specific ^{3}H-ouabain binding in the absence or presence of 50 nM calyculin A (CALY). CCD were preincubated for 5 min in the absence or presence of CALY. Afterwards, specific ^{3}H-ouabain binding was measured by incubation for 5 min with 5×10^{-5} M ^{3}H-ouabain ± 100-fold excess of unlabeled ouabain in the absence (*C*) or presence of 120 μg/ml NYST and 10^{-8} M AVP (NYST+AVP). In the presence of nystatin, the effect of AVP on the specific ^{3}H-ouabain binding was also abolished. Numbers in bars are the number of CCD tested. Bars are mean values + SE: *$P < 0.05$, ***$P < 0.001$.

Effect of AVP on PP activity

In a first series of experiments, the effects of 1nM OK and 3nM TAUT (which mainly inhibit PP2A and PP1 activity respectively [14, 24]) were tested on PP activity. Results are given in Fig. 5*A*. The total PP activity in isolated CCD was about 1.4×10^{-3} nmole ^{32}P released per nanoliter tubular volume and per minute. One nM OK decreased this activity by about 40% and 3nM TAUT by about 60%. When both inhibitors were added, the whole PP activity was blocked suggesting that 1nM OK

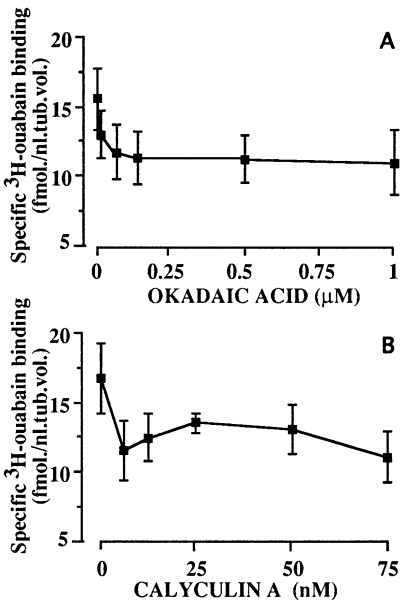

Fig. 4. Concentration-dependence of okadaic acid (OK) and calyculin A (CALY) effects on specific ^{3}H-ouabain binding. Specific ^{3}H-ouabain binding was determined in CCD incubated for 5 min with 10^{-8} M AVP in the presence of various concentrations of OK (panel *A*) or CALY (panel *B*). In each condition, CCD were first preincubated for 5 min with the drug. ^{3}H-oubain concentration was $5- \times 10^{-5}$ M ± 100-fold excess of unlabeled ouabain. Bars are mean values ± SE of 4–11 measurements.

and 3nM TAUT were able to inhibit specifically PP1 and PP2A. The effect of 10^{-8} M AVP was examined on PP activity in control condition and in the presence of each inhibitor, 1nM OK or 3nM TAUT. Results (Fig. 5*B*) show that, in control condition, AVP significantly increased the total PP activity by about 15–20%. This effect was due to an increase of PP2A activity since the effect of AVP was unaltered in the presence of 3nM TAUT whereas the increase was prevented in the presence of 1 nM OK.

Discussion

Na^+-K^+-ATPase is a major enzyme implied in the regulation of intracellular Na^+ and K^+. In epithelia, this enzyme is located at the basolateral membrane and participates in sodium reabsorption and potassium secretion. In the kidney, the cortical collecting duct is an important site of control of sodium reabsorption. We and others [1, 7] have shown that, in this epithelium, aldosterone is responsible for the constitution of a latent pool of Na^+-K^+-ATPase. In a recent study, we have demonstrated that arginin vasopressin (AVP) could induce the recruitment or the activation of this latent pool of Na^+-K^+-ATPases and that the phenomenon was mediated by an

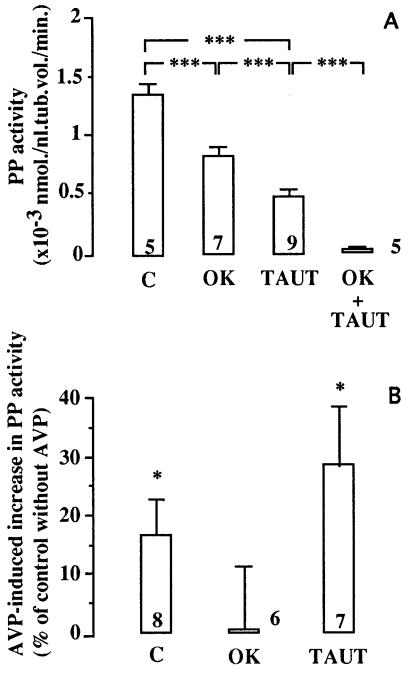

Fig. 5. Effect of 10^{-8} M AVP on the serine/threonine protein phosphatase (PP) activity in the absence or presence of 1 nM okadaic acid (OK) or 3 nM tautomycin (TAUT). The effects of OK, TAUT or OK+TAUT were examined on PP activity as described in Material and Methods (panel *A*). OK decreased PP activity by about 40% whereas TAUT decreased it by about 60%. When both OK and TAUT were added, PP activity was about zero. Bars are mean values ± SE. Numbers in bars give the number of CCD tested. ***$P < 0.001$. The effect of AVP 10^{-8} M was examined on PP activity (panel *B*) in the presence of OK or TAUT, or in the absence of drug (*C*). PP activity was increased by AVP either in the absence of drug or in the presence of TAUT but was not modified in the presence of OK. Bars are mean values ± SE. Figures in bars give the number of CCD tested. *$P < 0.05$, AVP-treated *vs.* control CCD.

apical Na^+ entry via the amiloride-sensitive sodium channel [17]. However, the precise mechanism leading to the functional activation or insertion of Na^+-K^+-ATPase is still unknown. Recently, a new regulation pathway of Na^+-K^+-ATPase has been described. This pathway implies phosphorylation and dephosphorylation steps, leading to inactivated or activated form of the enzyme [9, 18]. In vitro, it has been documented that both PKA and PKC can phosphorylate the α subunit of Na^+-K^+-ATPase with different phosphorylation sites on serine or threonine residues [4]. In another study [5], it was shown that this phosphorylation step was accompanied with an inhibition of Na^+-K^+-ATPase activity as measured by the rate of ^{32}P-ATP hydrolysis. In the kidney, several studies have attempted to evaluate the role of this phenomenon in the regulation of Na^+-K^+-ATPase. In the proximal tubule, dopamine inhibits Na^+-K^+-ATPase activity via the PKA pathway with a regulation pathway implying the DARPP system [28]. Phosphorylation/dephosphorylation processes are not only involved in the regulation of Na^+-K^+-ATPase but have also been described for the regulation of different transport systems. In particular, $Na^+/K^+/2Cl^-$ cotransport and K^+ channels have been shown to be regulated by a pathway implying phosphatases [21, 22].

The goal of our study was to examine whether an AVP-induced dephosphorylation of latent Na^+-K^+-ATPase could be responsible for the AVP-induced increase in the number of active Na^+-K^+-ATPases present in the basolateral membrane. To this purpose, common inhibitors of serine-threonine phosphatase (PP) 1 and 2A like okadaic acid, calyculin A and tautomycin have been used. We have first demonstrated that, when CCD are first preincubated with PP inhibitors at a concentration where both PP1 and PP2A activity was blocked, the AVP-induced increase in specific 3H-ouabain binding and the AVP-induced increase in ouabain-sensitive ^{86}Rb uptake are prevented. These experiments suggest that serine-threonine PP might be involved in the phenomenon.

Since PP effect could primarily act on the sodium channel (which controls cell sodium entry) rather than on the Na^+-K^+-ATPase, we checked whether the same effect could be observed in the presence of the sodium ionophore nystatin. In a previous study, we had shown that this ionophore could induce the recruitment or activation of latent Na^+-K^+-ATPases [16]. Results (Fig. 3) indicate that, in the presence of nystatin, the PP inhibitor calyculin A still prevented the AVP-induced recruitment of the latent pool of pumps. This suggests that the PP-induced increase in the number of basolateral Na^+-K^+-ATPases was not due to an effect on the Na^+ channel. Such hypothesis was indeed unlikely since an increase in the activity of the Na^+ channel has been shown to depend on channel phosphorylation, not dephosphorylation [26].

To determine whether PP1 or PP2A was implied in the phenomenon, we analyzed the dose dependency of the effect of calyculin A and okadaic acid and we directly measured the activity of both PP. Although specificity of inhibitors is always a concern, okadaic acid and calyculin A have been found to be without effect on other signaling processes [19, 21, 30, 31]. Okadaic acid is a potent inhibitor of PP2A with a weaker activity against PP1 (IC50 about 1nM and 60–500 nM respectively) [6, 20, 21]. Calyculin A is equipotent to okadaic acid in inhibiting PP2A but is a more potent inhibitor of PP1 (IC50 about 0.3–2.0 nM) [20, 21]. The fact that the $K_{1/2}$ of the effect of okadaic acid on the AVP-induced increase in 3H-ouabain binding was about 2 nM indirectly suggested that PP2A rather than PP1 could be implied in the Na^+-K^+-ATPase activation process. This was confirmed by experiments designed to determine directly PP activity. In these experiments, we took advantage of the fact that tautomycin 3 nM inhibits mainly PP1 activity

[14, 24, 28]. Results showed that in mouse CCD, both PP1 and PP2A are present, representing respectively about 60% and 40% of the total activity. However, whereas PP1 activity is not modulated by AVP (experiments performed in the presence of a low dose of okadaic acid to block selectively PP2A activity), PP2A activity is significantly increased by 5 min treatment with the hormone (experiments performed in the presence of 3 nM tautomycin to block selectively PP1 activity). Taken together, these results suggest that the activation of PP2A is important in the regulation of Na^+-K^+-ATPase number and activity by AVP. Interestingly, it is to be noticed that in principal cells of rat CCD, Kubokawa et al. [22] have described a PP2A-dependent regulation of the ATP-sensitive K^+ channel, whose activity is closely linked to Na^+-K^+-ATPase activity.

In summary, we have shown that, in the mouse CCD, AVP increases the number of functional Na^+-K^+-ATPase present in the basolateral membrane by enhancing the activity of PP2A. This effect is rapid and has to be integrated in the recently described pathways of Na^+-K^+-ATPase regulation by phosphorylation/dephosphorylation processes. Whether Na^+-K^+-ATPase activation by this PP2A-dependent dephosphorylation process directly affects the enzyme or implies an intermediate regulatory protein rather than Na^+-K^+-ATPase itself will have to be determined.

References

1. Barlet-Bas, C., Khadouri, C., Marsy, S., Doucet, A. 1990. Enhanced intracellular sodium concentration in kidney cells recruits a latent pool of Na^+-K^+-ATPase whose size is modulated by corticosteroids. *J. Biol. Chem.* **265:**7799–7809
2. Becq, F., Fanjul, M., Merten, M., Figarella, C., Hollande, E., Gola, M. 1993. Possible regulation of CFTR-chloride channels by membrane-bound phosphatases in pancreatic duct cells. *FEBS Lett.* **327:**337–342
3. Becq, F., Jensen, T.J., Chang, X.B., Savoia, A., Rommens, J.M., Tsui, L.C., Buchwald, M., Riordan, J.R., Hanrahan, J.W. 1994. Phosphatase inhibitors activate normal and defective CFTR chloride channels. *Proc. Natl. Acad. Sci. USA* **91:**9160–9164
4. Beguin, P., Beggah, A.T., Chibalin, A.V., Burgener-Kairuz, P., Jaisser, F., Mathews, P.M., Rossier, B.C., Cotecchia, S., Geering, K. 1994. Phosphorylation of the Na,K-ATPase α-subunit by protein kinase A and C in vitro and in intact cells. *J. Biol. Chem.* **269:**24437–24445
5. Bertorello, A.M., Aperia, A., Walaas, I., Nairn, A.C., Greengard, P. 1991. Phosphorylation of the catalytic subunit of Na^+-K^+-ATPase inhibits the activity of the enzyme. *Proc. Natl. Acad. Sci. USA* **88:**11359–11362
6. Biajolan, C., Takai, A. 1988. Inhibitory effect of marine-sponge toxin, okadaic acid, on protein phosphatases. *Biochem. J.* **256:**283–290
7. Blot-Chabaud, M., Wanstock, F., Bonvalet, J.P., Farman, N. 1990. Cell sodium-induced recruitment of Na^+-K^+-ATPase pumps in rabbit cortical collecting tubules is aldosterone dependent. *J. Biol. Chem.* **265:**11676–11681
8. Cheval, L., Doucet, A. 1990. Measurement of Na^+-K^+-ATPase-mediated rubidium influx in single segments of rat nephron. *Am. J. Physiol.* **259:**F111–F121
9. Chibalin, A.V., Lopina, O.D., Petukhov, S.P., Vasilets, L.A. 1993. Phosphorylation of the Na,K-ATPase by Ca, phospholipid-dependent and cAMP-dependent protein kinases. Mapping of the region phosphorylated by Ca, phospholipid-dependent protein kinase. *Journal of Bioenergetics and Biomembranes* **25:**61–66
10. Cohen, P. 1985. The role of protein phosphorylation in the hormonal control of enzyme activity. *Eur. J. Biochem.* **151:**439–448
11. Cohen, P., Cohen, P.T.W. 1989. Protein phosphatases come of age. *J. Biol. Chem.* **264:**21435–21438
12. Cohen, P., Holmes, C.F.B., Tsukitani, Y. 1990. Okadaic acid: a new probe for the study of cellular regulation. *TIBS* **15:**98–102
13. Cohen, P., Klumpp, S., Schelling, D.L. 1989. An improved procedure for identifying and quantitating protein phosphatases in mammalian tissues. *FEBS Lett.* **250:**596–600
14. Cohen, P., Schelling, D.L., Spark, M.J.R. 1989. Remarkable similarities between yeast and mammalian protein phosphatases. *FEBS Lett.* **250:**601–606
15. Coutry, N., Blot-Chabaud, M., Mateo, P., Bonvalet, J.P., Farman, N. 1992. Time course of sodium-induced Na^+-K^+-ATPase recruitment in rabbit cortical collecting tubule. *Am. J. Physiol.* **263:**C61–C68
16. Coutry, N., Farman, N., Bonvalet, J.P., Blot-Chabaud, M. 1994. Role of cell volume variations in Na^+-K^+-ATPase recruitment and/or activation in cortical collecting duct. *Am. J. Physiol.* **266:**C1342–C1349
17. Coutry, N., Farman, N., Bonvalet, J.P., Blot-Chabaud, M. 1995. Synergistic action of vasopressin and aldosterone on basolateral Na^+-K^+-ATPase in the cortical collecting duct. *J. Membrane Biol.* **145:**99–106
18. Ewart, H.S., Klip, A. 1995. Hormonal regulation of the Na^+-K^+-ATPase: mechanisms underlying rapid and sustained changes in pump activity. *Am. J. Physiol.* **269:**C295–C311
19. Haystead, T.A.J., Sim, A.T.R., Carling, D., Honnor, R.C., Tsukitani, Y., Cohen, P., Hardie, D.G. 1989. Effects of the tumor promoter okadaic acid on intracellular protein phosphorylation and metabolism. *Nature* **337:**78–81
20. Ishihara, H., Martin, B.L., Brautignan, D.L., Katraki, H., Osaki, H., Kato, Y., Fustani, N., Hashimoto, K., Uemura, D., Hartshorne, D.J. 1989. Calyculin A and okadaic acid: inhibitors of protein phosphatase activity. *Biochem. Biophys. Res. Comm.* **159:**871–877
21. Klein, J.D., Perry, P.B., O'Neil, W.C. 1993. Regulation by cell volume of Na^+-K^+-$2Cl^-$ cotransport in vascular endothelial cells: role of protein phosphorylation. *J. Membrane Biol.* **132:**243–252
22. Kubokawa, M., Mc Nicholas, C.M., Higgins, M.A., Wang, W., Giebisch, G. 1995. Regulation of ATP-sensitive K^+ channel by membrane-bound protein phosphatases in rat principal tubule cell. *Am. J. Physiol.* **269:**F355–F362
23. Kume, H.A., Takai, A., Tokuno, H., Tomita, T. 1989. Regulation of Ca^{2+}-dependent K^+ channel activity in tracheal myocytes by phosphorylation. *Nature* **341:**152–154
24. MacKintoch, C., Klumpp, S. 1990. Tautomycin from the bacterium streptomyces verticillatus, another potent inhibitor of protein phosphatases 1 and 2A. *FEBS Lett.* **277:**137–140
25. McNicholas, C.M., Wang, W., Ho, K., Hebert, S.C., Giebisch, G. 1994. Regulation of ROMK K^+ channel activity involves phosphorylation processes. *Proc. Natl. Acad. Sci. USA* **91:**8077–8081
26. Oh, Y., Smith, P.R., Bradford, A.L., Keeton, D., Benos, D. 1993. Regulation by phosphorylation of purified epithelial Na^+ channels in planar lipid bilayers. *Am. J. Physiol.* **265:**C85–C91

27. Shenolikar, S., Nairn, A.C. 1991. Protein phosphatases: recent progress. *Adv. Second Messenger Phosphoprotein Res.* **23:** 1–121
28. Slobodyansky, E., Aoki, Y., Gaznabi, A.K.M., Aviles, D.H., Fildes, R.D., Jose, P.A. 1995. Dopamine and protein phosphatase activity in renal proximal tubules. *Am. J. Physiol.* **268:**F279–F284
29. Sudo, J.I., Morel, F. 1984. Na^+ and K^+ cell concentrations in collagenase-treated rat kidney tubules incubated at various temperatures. *Am. J. Physiol.* **246:**C407–C414
30. Suganuma, M., Fujiki, H., Suguri, H., Yoshizawa, S., Hirota, M., Nakayasu, M., Ojika, M., Wakamatsu, K., Yamada, K., Sugimura, T. 1988. Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. *Proc. Natl. Acad. Sci. USA* **85:**1768–1771
31. Takai, A., Bialojan, C., Troschka, M., Ruegg, J.C. 1987. Smooth muscle myosin phosphate inhibition and force enhancement by black sponge toxin. *FEBS Lett.* **217:**81–84