Contrary to Rat-Type, Human-Type Na,K-ATPase Is Phosphorylated at the Same Amino Acid by Hormones that Produce Opposite Effects on Enzyme Activity

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Renal sodium homeostasis is a major determinant of BP and is regulated by several natriuretic and antinatriuretic hormones. These hormones, acting through intracellular secondary messengers, either activate or inhibit proximal tubule Na,K-ATPase. It was shown previously that phorbol esters and angiotensin II and serotonin induce the phosphorylation of both Ser-11 and Ser-18 of the Na,K-ATPase α-subunit. This results in the recruitment of Na,K-ATPase molecules to the plasma membrane and an increased capacity to transport sodium ions. Treatment of the same cells with dopamine leads to phosphorylation of the Na,K-ATPase α-subunit Ser-18. The subsequent internalization of Na,K-ATPase molecules results in a reduced capacity to transport sodium ions. These effects are observed in cells that express the rat-type Na,K-ATPase. However, the Na,K-ATPase α1-subunit of several species, such as human, pig, and mouse, does not have a Ser-18 in their N-terminal region. Therefore, the possibility exists that, in those species, the Na,K-ATPase is not regulated by the hormones that regulate natriuresis. This study presents evidence that in cells that express the human-type Na,K-ATPase, dopamine inhibits and phorbol esters activate the Na,K-ATPase-mediated transport. These opposite effects are mediated by the phosphorylation of the same amino acid residue, Ser-11 of Na,K-ATPase α1, and the presence of α1 Ser-18 is not essential for the hormonal regulation of Na,K-ATPase activity in LLC-PK1 cells. It was observed that, whereas the regulatory stimulation of Na,K-ATPase is mediated by protein kinase Cβ, the regulatory inhibition is mediated by protein kinase Cζ. This is similar to what was demonstrated previously in cells that express the rat-type Na,K-ATPase.

phosphorylated Na,K-ATPase molecules are either retrieved from the plasma membrane to intracellular compartments or recruited to the plasma membrane. The presence of Ser-18 is essential for both stimulation and inhibition of Na,K-ATPase-mediated transport. Most of the studies that are related to the hormonal regulation of the Na,K-ATPase have been performed in rat tissue or cells transfected with the rat α1, which contains Ser-18. However, as shown in Figure 1, human α1 has a glycine residue at position 18 (15). The mouse, the animal of choice to produce transgenics, also has a Gly at position 18 (Figure 1).

Because of the lack of Ser-18, it was suggested that hormones may not regulate the human proximal tubule Na,K-ATPase (16). If this is the case, then the results obtained in rat tissues may not reflect a similar human mechanism, in whose understanding we are mostly interested. Furthermore, we are involved in the production of transgenic mice to test the hypothesis that the hormonal mechanisms described above are physiologically relevant. Therefore, it is critical to determine whether the renal human-type Na,K-ATPase (no Ser-18 in α1) is regulated by hormones following a mechanism similar to that described in rat. LLC1 is a cell culture model of proximal tubule epithelia of pig origin (17). Some of the studies of hormonal regulation of the Na,K-ATPase have been performed in LLC1 cells that were transfected with the rat α1 (16,18). However, the endogenous LLC1 α1 does not contain a serine at position 18 (Figure 1). We tested in LLC1 cells the hypotheses (i) that the renal human-type Na,K-ATPase is regulated by hormones following a mechanism similar to that described for the rat-type Na,K-ATPase and (2) that the hormonal regulation is mediated by the exclusive phosphorylation of Ser-11.

Materials and Methods
The Na,K-ATPase α1 antibodies that were used for immunoprecipitation or Western blot analysis were gifts from Drs. R.W. Mercer (Washington University, St. Louis, MO) and M.J. Caplan (Yale University, New Haven, CT), respectively. Ouabain, phorbol 12-myristate 13-acetate (PMA), dopamine, anti-phosphoserine antibody, staurosporine, and chelerythrine chloride were obtained from Sigma Chemical Co. (St. Louis, MO). LY335351 was a gift from Eli Lilly (Indianapolis, IN). [86Rb]-RbCl was purchased from Perkin Elmer (Norwalk, CT). Other reagents were of the highest quality available.

Cell Culture and Transfection
OK or pig kidney (LLCPK1) cells were grown in DMEM that contained 10% calf serum and antibiotics. Before treatment with hormones, cells were incubated for 30 min in the same culture medium without serum and buffered with 50 mM HEPES (DMEM-HEPES). All treatments and assays were performed with cells attached to cell culture dishes and in the DMEM-HEPES medium (19,20). In some experiments, cells that stably expressed the rat α1 or mutants of this protein were used. Plasmid preparation, site-directed mutagenesis, and stable expression of exogenous Na,K-ATPase α1 were performed as described previously (19,20). Unlike the Na,K-ATPase from other species, the enzyme from rat has relatively high resistance to ouabain; therefore, we inhibited the endogenous Na,K-ATPase activity of transfected cells by growing the cells and performing the experiments in the presence of 3 μM ouabain. Accordingly, any Na,K-ATPase activity that we observed in these cells must originate with the Na,K-ATPase that contains the introduced exogenous α1 (19,20).

Determination of Protein Concentration
Cells were solubilized with SDS, and aliquots of the solubilized material were used for protein determination. Protein concentration was determined by the bicinchoninic acid method (Pierce, Rockford, IL) using BSA as standard.

Determination of Na,K-ATPase–Mediated Transport
All assays and treatments were performed at room temperature (22 to 24°C) as described previously in our publications (19,20). Briefly, Na,K-ATPase–mediated transport was determined from the ouabain-inhibitable 86Rb+ uptake. For determining the effect of DA on Rb+ uptake, cells were incubated with 5 μM monensin for 30 min (9,10,21). When PKC inhibitors were used, the cells were incubated with the indicated concentration of the inhibitor for 30 min before treatment with PMA (1 μM, 10 min) or DA (1 μM, 5 min). Na,K-ATPase–mediated transport is expressed as nanomoles of Rb+ transported per milligram of total protein per minute.

Determination of Na,K-ATPase α1 Phosphorylation
Immunoprecipitation of α1 was performed as described previously (11). Briefly, after treatment of the cells with hormone or phorbol ester, the cell medium was replaced with a medium that contained 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2 mM EGTA, 1% Triton X-100, and a cocktail of protease inhibitors. Cells were detached by scraping with a rubber policeman, and cell suspensions were transferred to assay tubes. Solubilization of the cells was helped by freeze-thawing. The Na,K-ATPase α1 was precipitated with an anti-α1 antibody (Mercer antibody) and Protein A/G Plus Agarose beads (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The immunoprecipitated protein was separated by SDS/PAGE using the Laemmli buffer system (22) and transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Phosphorylated protein was determined using an anti-phosphoserine antibody. Then, the PVDF membrane was stripped and the Na,K-ATPase α1 was performed as described previously (19,20). Briefly, after treatment of the cells with hormone or phorbol ester, the cell medium was replaced with a medium that contained 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2 mM EGTA, 1% Triton X-100, and a cocktail of protease inhibitors. Cells were detached by scraping with a rubber policeman, and cell suspensions were transferred to assay tubes. Solubilization of the cells was helped by freeze-thawing. The Na,K-ATPase α1 was precipitated with an anti-α1 antibody (Mercer antibody) and Protein A/G Plus Agarose beads (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The immunoprecipitated protein was separated by SDS/PAGE using the Laemmli buffer system (22) and transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Phosphorylated protein was determined using an anti-phosphoserine antibody. Then, the PVDF membrane was stripped and the Na,K-ATPase α1 was performed as described previously (19,20). Briefly, after treatment of the cells with hormone or phorbol ester, the cell medium was replaced with a medium that contained 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2 mM EGTA, 1% Triton X-100, and a cocktail of protease inhibitors. Cells were detached by scraping with a rubber policeman, and cell suspensions were transferred to assay tubes. Solubilization of the cells was helped by freeze-thawing. The Na,K-ATPase α1 was precipitated with an anti-α1 antibody (Mercer antibody) and Protein A/G Plus Agarose beads (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The immunoprecipitated protein was separated by SDS/PAGE using the Laemmli buffer system (22) and transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Phosphorylated protein was determined using an anti-phosphoserine antibody. Then, the PVDF membrane was stripped and the Na,K-ATPase α1 was performed as described previously (19,20).

Figure 1.

Statistical Analyses
Comparison between two experimental groups was made with the nonpaired t test. P < 0.05 was considered significant and is indicated with “*” in the figures.

Table 1. Sequence of the first 30 amino acids of Na,K-ATPase α1 in human, pig, mouse, and rat. Gaps were allowed for maximal homology of the alignment between the different species. Arrows indicate sites of phosphorylation.
Results

The experiments were performed in OK and LLCPK1 cells, which are cell culture models of proximal tubule epithelia (17,23). For obtaining results that are comparable between the two cell lines, OK and LLCPK1 cells were transfected with the same plasmid, which contained the rat Na,K-ATPase α1 subunit cDNA, and selected with 3 μM ouabain. As we have previously demonstrated, this concentration of ouabain inhibits the endogenous Na,K-ATPase but does not affect the Na,K-ATPase that contains the rat 1\(^\alpha\) (19,20). In this way, the exogenously expressed 1\(\alpha\) replaces the endogenous 1\(\alpha\) (19,20). Therefore, in transfected cells, there is no interference of the endogenous 1\(\alpha\).

Cells were transfected with a plasmid that contained the wild-type rat 1\(\alpha\) cDNA, which has a Ser-18 (rat-type 1\(\alpha\)), or the S18A mutant (human-type 1\(\alpha\)), or the S11A/S18A mutants. The basic (nonhormone treatment) level of Na,K-ATPase–mediated transport in cells that were transfected with these plasmids is the same as the transport measured in nontransfected cells; therefore, there was no overexpression of the Na,K-ATPase (19,20).

Although it is known that Ang II stimulates other mechanisms in LLCPK1 cells (24), we could not find any reference in the literature that this hormone regulates the endogenous Na,K-ATPase–mediated transport. Indeed, we could not determine an Ang II–dependent effect (stimulatory or inhibitory) on endogenous Na,K-ATPase–mediated transport in LLCPK1 cells (data not shown). We previously determined that in OK cells, phorbol esters activate the same intracellular signaling mechanism as hormones that stimulate the Na,K-ATPase activity (7,10). Then, we assumed that PMA may also stimulate a hormonal pathway linked to Na,K-ATPase regulation in LLCPK1 cells.

Effects of DA and PMA on Rb\(^{+}\) Uptake Mediated by Na,K-ATPase

The effect of DA and PMA on the Na,K-ATPase–mediated transport first was determined in nontransfected cells (NT in Figure 2) and in cells that were transfected with either S18A or S11A/S18A mutants of rat 1\(\alpha\). As previously demonstrated (19,20), DA inhibits and PMA activates the Na,K-ATPase of native (nontransfected) OK cells. When these cells expressed the S18A or S11A/S18A mutants of rat 1\(\alpha\), DA and PMA had no effect on the Na,K-ATPase–mediated transport (Figure 2, OK). Whereas Ser-18 is essential for DA-dependent inhibition of Na,K-ATPase, both Ser-11 and Ser-18 are essential for the activation of Na,K-ATPase by PMA and hormones like Ang II and 5-HT (7,11,10).

In nontransfected LLCPK1 cells, which contain the Na,K-ATPase 1\(\alpha\) lacking Ser-18, PMA produced a 59% activation and DA a 30% inhibition of Na,K-ATPase–mediated transport (Figure 2, LLCPK1). Similar results were observed in LLCPK1 cells that were transfected with the rat Na,K-ATPase 1\(\alpha\) that contained the S18A mutation. On the contrary, no effect of either PMA or DA on the Na,K-ATPase–mediated transport was observed in LLCPK1 cells that were transfected with the 1\(\alpha\) S11A/S18A mutant (Figure 2, LLCPK1). It should be noted that when the cells are transfected with the rat 1\(\alpha\) S18A mutant, the cells express an 1\(\alpha\) that is essentially the same as the LLCPK1 endogenous 1\(\alpha\) (the one that we call human-type 1\(\alpha\)). When the cells are transfected with the rat 1\(\alpha\) S11A/S18A mutant, the cells express an 1\(\alpha\) that is essentially the same as the LLCPK1 endogenous 1\(\alpha\) with a mutation in Ser-11. The results illustrated in Figure 2 suggest that, contrary to what was observed in OK cells, the presence of 1\(\alpha\) Ser-18 is not essential for the hormonal regulation of Na,K-ATPase–mediated transport in

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**Figure 2.** Effects of phorbol 12-myristate 13-acetate (PMA) and dopamine (DA) on the Na,K-ATPase–mediated Rb\(^{+}\) uptake in LLCPK1 and opossum kidney (OK) cells. Na,K-ATPase activity (ouabain-sensitive Rb\(^{+}\) uptake) was determined in nontransfected cells (NT) or cells that expressed stably either S18A or S11A/S18A mutants of rat 1\(\alpha\). Cells were treated with 1 μM of either PMA or DA before assay, as described in Materials and Methods. Bars represent mean ± SEM of % activity change [(activity in PMA or dopamine treated cells – activity in nontreated cells) × 100/activity in nontreated cells] of three independent experiments. Each experiment was performed in triplicate. *P < 0.05 with respect to NT of the same cell line (LLCPK1 or OK).
LLCPK1 cells. However, the presence of α1 Ser-11 is required for the regulatory effects that either activate or inhibit the Na,K-ATPase-mediated transport in LLCPK1 cells.

Effects of DA and PMA on Na,K-ATPase α1 Phosphorylation

We previously demonstrated that whereas PMA treatment of OK cells that express the rat α1 leads to the phosphorylation of α1 Ser-11 and Ser-18, DA treatment results in α1 Ser-18 phosphorylation (11,12). Figure 3 shows that elimination of Ser-18 completely abolishes the PMA- or DA-dependent Na,K-ATPase α1 phosphorylation in OK cells. To characterize the amino acids that are phosphorylated in LLCPK1 cells, we treated with either PMA or DA cells that expressed the rat Na,K-ATPase α1 with either the S18A or S11A/S18A substitutions. Then, α1 was immunoprecipitated with a specific antibody, separated by SDS-PAGE, and transferred to a piece of PVDF membrane. The phosphorylation status of α1 was determined by Western blot analysis using an anti-phosphoserine antibody. As described previously with OK cells (11,12), there was no significant change in the basal (no PMA or DA) small level of anti-phosphoserine antibody binding to the Na,K-ATPase α1 in LLCPK1 cells that were transfected with the S18A and S11A/S18A mutants of α1-subunit (data not shown). Treatment of nontransfected LLCPK1 cells with either PMA or DA promoted a significant increase in the level of α1 phosphorylation (Figure 3). Contrary to what was observed in OK cells, in LLCPK1 cells, there was a significant increase in the level of anti-phosphoserine antibody binding to the Na,K-ATPase α1 S18A after treatment with either PMA or DA. Similar to OK cells, PMA and DA treatments did not increase the level of anti-phosphoserine antibody binding to the Na,K-ATPase α1 in LLCPK1 cells that expressed the α1 S11A/S18A mutant. These results are consistent with those described in Figure 2 suggesting that, in LLCPK1 cells, PMA- and DA-dependent regulation of Na,K-ATPase activity requires only the phosphorylation of α1 Ser-11.

Opposite Effects on Regulation of Na,K-ATPase Activity by DA and PMA Are Mediated by Different PKC Isoforms

There is no doubt that the effect of PMA on the Na,K-ATPase activity is mediated by PKC, because PMA is a specific activator of PKC. Chelerythrine chloride is a potent, cell-permeable inhibitor of PKC (IC_{50} = 660 nM) that binds to the catalytic domain of PKC. Chelerythrine chloride is at least 100-fold more selective for PKC isoforms than for other kinases (26). Chelerythrine chloride (2 μM) totally blocked the inhibitory effect of DA on the Na,K-ATPase–mediated transport in both OK and LLCPK1 cells (Figure 4, Chel. 2), which demonstrates that the DA effect is indeed mediated by PKC.

To determine which isoforms of PKC are involved in the hormonal regulation of Na,K-ATPase in LLCPK1 cells, we measured the effect of PMA and DA on the Na,K-ATPase–mediated Rb\(^+\) uptake in cells that had been treated with PKC inhibitors. LY333531, at 10 nM concentration, is a PKCβ-specific inhibitor (IC_{50} of 4.7 and 5.9 nM for isoforms β1 and β2, respectively) (27). As illustrated in Figure 4 (LY 2 and LY 10), 10 nM LY333531 totally impaired the stimulatory effect of PMA on Na,K-ATPase–mediated Rb\(^+\) uptake in both OK and LLCPK1 cells. Staurosporine inhibits all of the PKC isoforms, except PKCζ, and the PKA activities with an IC_{50} < 200 nM (27). PKCζ is inhibited by staurosporine with an IC_{50} of 1.5 μM (27). Figure 4 (St. 0.5 and St. 5) illustrates that 0.5 μM staurosporine had no effect on the DA inhibition of the ouabain-dependent Rb\(^+\) uptake; however, 5 μM of staurosporine totally abolished the

Figure 3. Effects of PMA and DA on α1 phosphorylation in LLCPK1 and OK cells. The level of Na,K-ATPase α1 phosphorylation was determined in nontransfected cells (NT) or cells that expressed stably either S18A or S11A/S18A mutants of rat α1. Cells were treated with 1 μM of either PMA or DA, the Na,K-ATPase α1 was immunoprecipitated using a specific antibody, and the level of α1 phosphorylation was determined by Western blot analysis using an anti-phosphoserine antibody (representative blots are shown). More details are presented in Materials and Methods. Bars represent mean ± SEM of % phosphorylation [(phosphorylation level in PMA- or DA-treated cells – phosphorylation level in nontreated cells) × 100 / phosphorylation level in nontreated cells] of three independent experiments. The phosphorylation level was normalized to the protein concentration as described in Materials and Methods. *P < 0.05 with respect to NT of the same cell line (LLCPK1 or OK).
DA-dependent regulation in both cell lines. These results indicate that the same PKC isoforms are involved in the hormone-dependent regulation of Na,K-ATPase-mediated transport in both cell lines, PKCβ for PMA-dependent activation and PKCζ for DA-dependent inhibition.

Figure 4E contains an additional piece of information. This plot illustrates the level of Na,K-ATPase-mediated transport in LLCPK1 cells that expressed the wild-type rat Na,K-ATPase α1, which contains an additional Ser-18 that is not present in the endogenous LLCPK1 Na,K-ATPase α1. The level of PMA-dependent activation and DA-dependent inhibition of Rb⁺ transport is the same as that observed in native (nontransfected) LLCPK1 cells (Figure 2, LLCPK1 NT) and in LLCPK1 cells that express the rat Na,K-ATPase α1 S18A mutant (Figure 2, LLCPK1 S18A). These results indicate that the presence of the additional Ser-18 in the Na,K-ATPase α1 does not affect in any way the regulation of the PMA- and DA-dependent regulation of Na,K-ATPase in LLCPK1 cells.
Discussion

Our results demonstrate that DA inhibits and PMA activates the Na,K-ATPase-mediated transport in LLCPK1 cells. However, these opposite effects are mediated by the phosphorylation of the same amino acid residue, Ser-11, of Na,K-ATPase α1. Contrary to what was observed in OK cells, the presence of α1 Ser-18 is not essential for the hormonal regulation of Na,K-ATPase-mediated transport in LLCPK1 cells. These results seem to be due to cell-specific characteristics of the DA and PMA intracellular signaling and not to the structure of the Na,K-ATPase, because the different results were observed when both cell lines were expressing the same Na,K-ATPase α1 plasmid. In OK cells, both α1 Ser-11 and Ser-18 are essential for the regulation of Na,K-ATPase-mediated transport by PMA, and these amino acids are phosphorylated during this process (11). In the same cells, it is α1 Ser-18 that is essential for the DA-dependent inhibition of Na,K-ATPase-mediated transport, and this is the amino acid that is phosphorylated. On the contrary, in LLCPK1 cells, phosphorylation of α1 Ser-11 alone seems to be enough to mediate the opposite effects produced by PMA and DA on the Na,K-ATPase-dependent Rb⁺ transport. In these cells, introduction of an additional α1 Ser-18 does not produce any modification on the hormonal regulation of Na,K-ATPase activity. Besides the specific amino acid that is phosphorylated, the PMA- and DA-dependent protein phosphorylation step in the mechanism of hormonal regulation seems to be similar in both OK and LLCPK1 cells because (1) it involves α1 N-terminal serine phosphorylation and (2) the Na,K-ATPase α1 phosphorylation induced by either DA or PMA α1 is mediated by the same PKC isoforms in both cell lines.

Most of the studies on the hormonal regulation of Na,K-ATPase have been performed in rat tissues or in cells that express the rat-type Na,K-ATPase (1,3,5,28). Although human and rat kidneys express only the α1 isoform of the Na,K-ATPase, there are some amino acids that are different between α1 of these two species. N-terminal cytoplasmic domains (amino acids 1 to 89) of the mature α1 of rat, mouse, and pig are different only in the 18th and 19th amino acids (SK in rat, GK in mouse, absent in pig). Human α1 has two more conservative changes (A21G and E24D) and the H13Q substitution. Critical is the presence of Ser-18, which plays a crucial role in the hormonal regulation of the rat-type Na,K-ATPase. The presence of this amino acid is essential for hormones that either activate or inhibit the rat-type Na,K-ATPase (7,10,12). Hormone-dependent phosphorylation of serine residues that are present in the Na,K-ATPase α1 N-terminal segment is the triggering mechanism that leads to either internalization of plasma membrane Na,K-ATPase molecules (e.g., dopamine-induced inhibition) or plasma membrane recruitment of Na,K-ATPase from intracellular compartments (e.g., Ang II- or 5-HT-dependent activation of Na,K-ATPase). If Ser-18 is not present in the kidney Na,K-ATPase α1 of human, pig, and mouse, then which amino acids of Na,K-ATPase α1 are phosphorylated during the hormonal regulation of this activity? LLCPK1 is a cell culture model of proximal tubule epithelia and contains the human-type Na,K-ATPase α1, which does not have a serine residue at the position 18. In this report, we have demonstrated that hormones may produce opposite effects by phosphorylation of α1 Ser-11 in LLCPK1 cells. We previously established that in OK cells, the regulation of Na,K-ATPase-mediated transport is produced by modification of the size of the plasma membrane pool of Na,K-ATPase molecules, and phosphorylation by itself
does not point to the direction of the Na,K-ATPase molecules’ translocation but rather labels molecules that should be translocated. The scheme of Figure 5 illustrates a model that is consistent with our results. Hormones that produce stimulation of Na,K-ATPase-mediated transport (increase in plasma membrane pool of Na,K-ATPase molecules) induce the phosphorylation of Na,K-ATPase molecules that are located in intracellular compartments (28). Hormones that produce inhibition of Na,K-ATPase-mediated transport (decrease in plasma membrane pool of Na,K-ATPase molecules) induce the phosphorylation of Na,K-ATPase molecules that are located in the plasma membrane. It is likely that in LLCPK1 cells, regulation of the Na,K-ATPase-mediated transport is also achieved by translocation of sodium pump molecules between the plasma membrane and intracellular compartments; then in these cells (and likely in other cells that contain the human-type Na,K-ATPase α1), hormones that produce opposite effects may induce the phosphorylation of the same α1 Ser-11, but the phosphorylation occurs in different compartments of the cell.

The findings described in this report are important in several ways. The hypothesis that the hormonal regulation of proximal tubule Na,K-ATPase is a major contributor to the regulation of BP has been developed on the basis of experiments performed in animal (mainly rat) cells or in cultured cell lines. To determine whether this hypothesis is correct would require experiments in live animals. The best option is the production of transgenic mice that contain a mutation in α1 so that the Na,K-ATPase-mediated transport is not responsive to hormones. Mouse α1 is a human-type isoform, which does not contain a serine residue at position 18. Actually, besides three other conservative mutations, the presence of Ser-18 is the only major difference between rat and mouse α1. However, because of the lack of Ser-18, it was suggested that human kidney Na,K-ATPase may not be regulated by dopamine (29). Researchers who tested the effects of phorbol esters or DA on human cells did not observe any effect because they were looking for changes in the Na,K-ATPase intrinsic activity (30,31). As we have demonstrated (10–13), the hormonal regulation of Na,K-ATPase does not occur by changes in the intrinsic enzyme activity but by changes in the size of the Na,K-ATPase molecule pool at the plasma membrane. Our results demonstrate that the lack of Ser-18 in the Na,K-ATPase α1 is not a problem for the hormonal regulation of Na,K-ATPase in cells that express the human-type α1 and that Ser-11 in the human-type α1 may substitute for the rat-type α1 Ser-18.

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