SGK1 activates Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in amphibian renal epithelial cells

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Alvarez de la Rosa, Diego, Ignacio Gimenez, Biff Forbush, and Cecilia M. Canessa. SGK1 activates Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in amphibian renal epithelial cells. Am J Physiol Cell Physiol 290: C492–C498, 2006.—Serum- and glucocorticoid-induced kinase 1 (SGK1) is thought to be an important regulator of Na\textsuperscript{+} reabsorption in the kidney. It has been proposed that SGK1 mediates the effects of aldosterone on transepithelial Na\textsuperscript{+} transport. Previous studies have shown that SGK1 increases Na\textsuperscript{+} transport and epithelial Na\textsuperscript{+} channel (ENaC) activity in the apical membrane of renal epithelial cells. SGK1 has also been implicated in the modulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, the transporter responsible for basolateral Na\textsuperscript{+} efflux, although this observation has not been confirmed in renal epithelial cells. We examined Na\textsuperscript{+}-K\textsuperscript{+}-ATPase function in an A6 renal epithelial cell line that expresses SGK1 under the control of a tetracycline-inducible promoter. The results showed that expression of a constitutively active mutant of SGK1 (SGK1\textsuperscript{S425D}) increased the transport activity of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase 2.5-fold. The increase in Na\textsuperscript{+} activity was a direct consequence of activation of the pump itself. The onset of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activation was observed between 6 and 24 h after induction of SGK1 expression, a delay that is significantly longer than that required for activation of ENaC in the same cell line (1 h). SGK1 and aldosterone stimulated the Na\textsuperscript{+} pump synergistically, indicating that the pathways mediated by these molecules operate independently. This observation was confirmed by demonstrating that aldosterone, but not SGK1\textsuperscript{S425D} induced an ~2.5-fold increase in total protein and plasma membrane Na\textsuperscript{+}-K\textsuperscript{+}-ATPase α1-subunit abundance. We conclude that aldosterone increases the abundance of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, whereas SGK1 may activate existing pumps in the membrane in response to chronic or slowly acting stimuli.

sodium transport; serum- and glucocorticoid-induced kinase; A6 cells; sodium pump

REGULATION OF SODIUM REABSORPTION in the distal tubule is fundamental for electrolyte and blood pressure homeostasis. Transepithelial Na\textsuperscript{+} transport across the principal cells of the distal tubule involves luminal Na\textsuperscript{+} influx via the epithelial Na\textsuperscript{+} channel (ENaC), which represents the rate-limiting step, and basolateral Na\textsuperscript{+} efflux via Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (31). The main stimulus for Na\textsuperscript{+} reabsorption by principal cells is the hormone aldosterone, which stimulates both ENaC and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activities (31). It was recently proposed that the serum- and glucocorticoid-induced kinase 1 (SGK1) is a mediator of aldosterone regulation of Na\textsuperscript{+} transport (25, 28). Expression of SGK1 in renal cells induces a dose-dependent increase in transepithelial Na\textsuperscript{+} transport, mainly because of an increase in the density of open ENaCs in the apical plasma membrane (1, 4, 15). Moreover, mice with ablated SGK1 expression show diminished Na\textsuperscript{+} reabsorption when fed a low-Na\textsuperscript{+} diet, resulting in a 20% decrease in blood pressure (34), consistent with an inability to maximally activate ENaC.

Administration of exogenous aldosterone or glucocorticoids increases sgk1 mRNA expression in rat kidney and colon (6, 9, 10, 24). However, basal levels of SGK1 expression are high in these tissues, and changes in expression do not always correlate with aldosterone plasma variations (2, 11, 17), indicating that other stimuli play a significant role in SGK1 regulation. SGK1 has also been shown to increase Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity when coexpressed in Xenopus oocytes (27, 36), but this observation has not yet been confirmed in renal epithelial cells.

In the present study, we examined the effects of SGK1 and aldosterone on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in A6 cells derived from the Xenopus distal tubule. A6 cells endogenously express the three ENaC subunits (26) as well as the α1- and β1-subunits of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (32) and are capable of regulated vascular Na\textsuperscript{+} transport when grown on permeable supports. Furthermore, A6 cells are able to express SGK1 when stimulated with serum or steroid hormones, although kinase levels are negligible in serum-depleted cells (1). We used a tetracycline-inducible system to control the expression of a constitutively active mutant of SGK1 (SGK1\textsuperscript{S425D}) independently of any other stimuli.

MATERIALS AND METHODS

Cell culture. We previously described the generation and characterization of an A6 cell line expressing a constitutively active mutant of SGK1, SGK1\textsuperscript{S425D}, under the control of a tetracycline-inducible promoter (1). Briefly, A6 cells were transfected with two plasmids: one constitutively expresses the tetracycline repressor (tetR) under a cytomegalovirus (CMV) promoter; the other contains the full-length SGK1\textsuperscript{S425D} open-reading frame under the control of a CMV promoter coupled to a tandem repeat of the tetracycline operator (tetO). Under normal conditions, tetR binds tetO and prevents SGK1\textsuperscript{S425D} expression. Upon tetracycline stimulation, tetR is released from tetO and the CMV promoter drives SGK1\textsuperscript{S425D} expression.

Cells were grown at 27°C in amphibian 0.75× DMEM (GIBCO-BRL) buffered with NaHCO3 and supplemented with 10% FBS and antibiotics for plasmid selection (500 μg/ml zeocin and 10 μg/ml blasticidin; Invitrogen) in a humidified incubator containing 1.5% CO2. Cells were expanded in plastic dishes and subcultured onto 96-well microtiter plates 2 days before the flux experiments were begun. When indicated, cells were subcultured onto 4.7-cm² permeable supports (Transwell; Corning, Corning, NY) for at least 10 days to allow for differentiation and development of transepithelial transport properties (4). One day before the experiments were started, cells were washed twice with serum-free amphibian DMEM and kept in the same medium for 24 h. Cells were then treated with 1 μg/ml

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Na⁺–K⁺-ATPase regulation by aldosterone and SGK1

RESULTS

SGK1 effects on Na⁺-K⁺-ATPase transport activity in A6 cells. To investigate the effects of SGK1 on Na⁺-K⁺-ATPase activity, we used an inducible A6 cell line that expresses a constitutively active mutant of SGK1, SGK1<sub>542SD</sub>, under the control of tetracycline (1). A6 cells express endogenous SGK1, but the levels of the kinase are negligible when cells are grown in serum-free medium (1). Na⁺-K⁺-ATPase transport activity in A6-SGK1<sub>542SD</sub> cells was assayed as ouabain-sensitive ⁸⁶Rb⁺ uptake by the cells. Rb⁺ ions behave in the same manner as K⁺ ions and are transported from the extracellular medium into the cytosol by Na⁺-K⁺-ATPase. ⁸⁶Rb⁺ included in the flux medium initially accumulated in the cell until it reached a steady-state concentration. To ensure that the flux assay was conducted under linear uptake conditions, the time course of ⁸⁶Rb⁺ uptake was measured. ⁸⁶Rb⁺ accumulation was measured in the absence of inhibitors, in the presence of 100 µM ouabain, and with a combination of ouabain and 250 µM bumetanide (Fig. 1A). Ouabain-sensitive ⁸⁶Rb⁺ uptake accounted for ~38% of total uptake, consistent with previously reported values in A6 cells (16). The remaining ⁸⁶Rb⁺ uptake was a result of the activity of the bumetanide-sensitive Na⁺-K⁺-Cl⁻ cotransporter (NKCC) (16), as well as from K⁺ channels, which are abundantly expressed in the basolateral membrane of A6 cells (7, 13). Because ouabain-sensitive ⁸⁶Rb⁺ uptake was linear for at least 40 min (Fig. 1A), all subsequent experiments were performed with 20-min flux periods.

To study whether tetracycline per se has any effect on ⁸⁶Rb⁺ uptake in A6 cells, we conducted experiments to study fluxes
were washed and then incubated for an additional 20 min in the presence of tetracycline. At the end of tetracycline treatment, cells transport activity were studied at different times after induction and expression abrogates aldosterone effects on Na\(^{+}\)-K\(^{+}\)-ATPase activity. We next studied whether SGK1\textsubscript{T425D} expression abrogates aldosterone effects on Na\(^{+}\)-K\(^{+}\)-ATPase as expected if SGK1 mediates aldosterone action on the pump. Treatment with 100 nM aldosterone increased ouabain-sensitive \(86\text{Rb}^+\) uptake in a time-dependent manner (Fig. 3), a change greater than that induced by the two stimuli together, indicating that aldosterone and SGK1 effects are superadditive.

SGK1 and aldosterone effects on Na\(^{+}\)-K\(^{+}\)-ATPase activity are superadditive. We next studied whether SGK1\textsubscript{T425D} expression abrogates aldosterone effects on Na\(^{+}\)-K\(^{+}\)-ATPase as expected if SGK1 mediates aldosterone action on the pump. Treatment with 100 nM aldosterone increased ouabain-sensitive \(86\text{Rb}^+\) uptake in a time-dependent manner (Fig. 3), reaching a peak 2.4-fold increase after 24 h. In parallel wells, cells were treated with 1 \(\mu\text{g/ml}\) tetracycline or a combination of 100 nM aldosterone and 1 \(\mu\text{g/ml}\) tetracycline. Aldosterone plus SGK1\textsubscript{T425D} expression resulted in a 7.2-fold increase in ouabain-sensitive \(86\text{Rb}^+\) uptake (Fig. 3), a change greater than that induced by the two stimuli together, indicating that aldosterone and SGK1 effects are superadditive. We next studied whether SGK1\textsubscript{T425D} expression abrogates aldosterone effects on Na\(^{+}\)-K\(^{+}\)-ATPase as expected if SGK1 mediates aldosterone action on the pump. Treatment with 100 nM aldosterone increased ouabain-sensitive \(86\text{Rb}^+\) uptake in a time-dependent manner (Fig. 3), reaching a peak 2.4-fold increase after 24 h. In parallel wells, cells were treated with 1 \(\mu\text{g/ml}\) tetracycline or a combination of 100 nM aldosterone and 1 \(\mu\text{g/ml}\) tetracycline. Aldosterone plus SGK1\textsubscript{T425D} expression resulted in a 7.2-fold increase in ouabain-sensitive \(86\text{Rb}^+\) uptake (Fig. 3), a change greater than that induced by the two stimuli together, indicating that aldosterone and SGK1 effects are superadditive.
sterone and SGK1<sup>S425D</sup> activate Na<sup>+</sup>-K<sup>+</sup>-ATPase via independent pathways in a synergistic way.

**SGK1 effects on Na<sup>+</sup>-K<sup>+</sup>-ATPase are independent of intracellular Na<sup>+</sup> concentration.** A6 cells grown on plastic do not express ENaCs at the cell surface (22); it is possible, however, that other Na<sup>+</sup> influx pathways could be stimulated by SGK1 and thus indirectly provide a stimulus for Na<sup>+</sup> pump activity. Monensin, a Na<sup>+</sup>-specific ionophore, was used to test whether SGK1 effects on Na<sup>+</sup>-K<sup>+</sup>-ATPase transport activity are secondary to changes in intracellular Na<sup>+</sup> concentration ([Na<sup>+</sup>]). Cells grown on 96-well plates were treated with tetracycline for various time periods and then washed and incubated for 20 min with basic flux medium, which contained 96 mM Na<sup>+</sup>, in the absence or presence of 2.5 μM monensin. After being incubated with monensin, cells were washed and incubated in flux medium with 86Rb<sup>+</sup> for 20 min. Basal levels of ouabain-sensitive 86Rb<sup>+</sup> uptake in the presence of monensin, on average, were 41% higher than those observed in parallel wells without the ionophore (Fig. 4A). This finding is consistent with the notion of [Na<sup>+</sup>], modulating Na<sup>+</sup>-K<sup>+</sup>-ATPase activity and is in agreement with previously reported values (5, 14). The effects of SGK1<sup>S425D</sup> expression on Na<sup>+</sup>-K<sup>+</sup>-ATPase activity were still present, with 2.5-fold activation after 24 h of induction (Fig. 4A). The effect of SGK1 on Na<sup>+</sup> pump activity was detected earlier in cells treated with monensin, in which a 50% increase compared with control (time 0) was apparent after 6 h of SGK1<sup>S425D</sup> induction. Because monensin treatment increases [Na<sup>+</sup>], a small increase in Na<sup>+</sup> pump activity would be detected more readily under this condition. This result suggests that the increase in Na<sup>+</sup>-K<sup>+</sup>-ATPase activity induced by SGK1 is not secondary to an increase in [Na<sup>+</sup>], but is instead a direct consequence of the activation of the pump itself.

It should be noted, however, that even the use of high doses of monensin does not increase [Na<sup>+</sup>], enough to achieve V<sub>max</sub> conditions for Na<sup>+</sup>-K<sup>+</sup>-ATPase activity (14), which are in the range of 60–100 mM Na<sup>+</sup> (18). Therefore, some of the results of the monensin experiments (Fig. 4A) could still be explained by SGK1 increasing [Na<sup>+</sup>]. To confirm that SGK1<sup>S425D</sup> activated Na<sup>+</sup>-K<sup>+</sup>-ATPase independently of changes in [Na<sup>+</sup>], we examined ouabain-sensitive ATPase activity in membrane preparations of A6 cells under V<sub>max</sub> conditions. A6 cells grown on plastic dishes and maintained for 24 h in serum-free medium were treated with 100 nM aldosterone or 1 μg/ml tetracycline overnight. Membrane preparations were permeabilized with deoxycholic acid, and ouabain-sensitive ATPase activity was measured using a colorimetric assay that detects the production of P<sub>i</sub>. Preliminary experiments showed that the release of P<sub>i</sub> from ATP was linear for up to 60 min in reactions starting with up to 30 μg of total protein. Subsequent experiments were performed with 30-min incubations and 10 μg of total protein per reaction. Under these conditions, the amount of ATP consumed in the reaction varied between 4% and 7% of total ATP present in the reaction buffer. A representative experiment is shown in Fig. 4B. Ouabain-sensitive ATPase activity in untreated A6 cells was 3.54 ± 0.74 μmol P<sub>i</sub>/mg of protein·h<sup>-1</sup>. Aldosterone treatment increased ouabain-sensitive ATPase activity to 7.26 ± 0.70 μmol P<sub>i</sub>/mg of protein·h<sup>-1</sup>, a twofold increase compared with basal levels. SGK1<sup>S425D</sup> induction increased ouabain-sensitive ATPase activity to 5.15 ± 0.60 μmol P<sub>i</sub>/mg of protein·h<sup>-1</sup>, a 45% increase compared with control conditions (Fig. 4B). Therefore, SGK1 increased the turnover rate of the pump under V<sub>max</sub> conditions, indicating that the effect was independent of changes in [Na<sup>+</sup>].

**Aldosterone, but not SGK1, increases Na<sup>+</sup>-K<sup>+</sup>-ATPase expression and plasma membrane abundance.** To further study the molecular mechanisms involved in Na<sup>+</sup>-K<sup>+</sup>-ATPase activation by SGK1, we examined the total and plasma membrane abundance of Na<sup>+</sup>-K<sup>+</sup>-ATPase α-subunit in the absence or presence of SGK1<sup>S425D</sup>. We compared SGK1 effects on protein abundance with the effects of aldosterone, which was previously shown to increase Na<sup>+</sup>-K<sup>+</sup>-ATPase α<sub>1</sub>-subunit mRNA and protein abundance (21, 33). A6 cells grown on filters for at least 10 days were serum starved and treated overnight with 100 nM aldosterone or 1 μg/ml tetracycline. After treatments, plasma membrane proteins were biotinylated and recovered by streptavidin pull-down. Samples containing 40 μg of total protein from cell lysates and the products of

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**Fig. 4. SGK1<sup>S425D</sup> effects on Na<sup>+</sup>-K<sup>+</sup>-ATPase activity are direct and independent of intracellular Na<sup>+</sup> concentration.** A: cells grown on 96-well plates were treated with 1 μg/ml tetracycline for the indicated time periods. Cells were then washed and incubated in basic flux medium in the absence or presence of 2.5 μM monensin for 20 min. After incubation, cells were washed and ouabain-sensitive 86Rb<sup>+</sup> uptake was assayed for a 20-min flux time period. Black bars represent average values (n = 6 experiments) ± SE of cells not treated with monensin. Gray bars represent average values (n = 2 experiments) ± SE of cells preincubated with 2.5 μM monensin before 86Rb<sup>+</sup> uptake assay. *P < 0.05 vs. control. B: cells grown on 10-cm-diameter plastic dishes were treated overnight with 1 μg/ml tetracycline or 100 nM aldosterone. After treatment, a crude membrane preparation was obtained. Ouabain-sensitive V<sub>max</sub> ATPase activity was determined using 10 μg of proteins from a permeabilized membrane suspension incubated at 37°C for 30 min in reaction buffer containing 3 mM ATP. P<sub>i</sub> released from ATP was quantified using a colorimetric assay. Bars represent average values (n = 5 replicate samples) ± SE of ouabain-sensitive ATPase activity. *P < 0.05 vs. control.
streptavidin pull-down were analyzed in parallel using SDS-PAGE followed by Western blot analysis with anti-Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha\)-subunit antibody. Western blot analysis showed a single band migrating at \(\sim\)100 kDa, consistent with the predicted size of the \(\alpha_{1}\)-subunit of Na\(^{+}\)-K\(^{+}\)-ATPase (Fig. 5A). A control sample containing proteins from nonbiotinylated A6 cells was included to check for nonspecific protein binding to the agarose-streptavidin beads. The \(\alpha_{1}\)-subunit of Na\(^{+}\)-K\(^{+}\)-ATPase was detected in the total lysate but not in the streptavidin pull-down from nonbiotinylated cells (Fig. 5A), indicating that the signal obtained in the biotinylation experiments was specific for biotinylated proteins. A second control experiment was performed to check the possibility of intracellular biotinylation due to permeation of sulfo-NHS-SS-biotin. Blots were stripped and reprobed with anti-calnexin PAb (Fig. 5B). Calnexin is an abundant endoplasmic reticulum-resident membrane protein and should not be biotinylated by impermeant derivatives of biotin. The results show that although a strong calnexin signal was detected in the total protein lysate, only a faint signal was detected in the biotinylated samples (Fig. 5B), indicating that contamination caused by intracellular proteins was low in our experiments and did not interfere with data interpretation.

Quantification of the \(\alpha_{1}\)-subunit signals using scanning densitometry showed that aldosterone increased total \(\alpha_{1}\)-subunit abundance an average of 2.9-fold (Fig. 5C). The increase in total protein was mirrored by a 2.5-fold increase in \(\alpha_{1}\)-subunit abundance in the plasma membrane. In contrast, the expression of SGK1\(_{S425D}\) had no effect on total or plasma membrane abundance of the \(\alpha_{1}\)-subunit. Therefore, increased \(\alpha_{1}\)-subunit expression or abundance in the plasma membrane cannot account for the increase in Na\(^{+}\)-K\(^{+}\)-ATPase activity induced by SGK1\(_{S425D}\).

We also performed the same set of experiments in A6 cells grown on plastic culture dishes (data not shown). Aldosterone increased total and plasma membrane \(\alpha_{1}\)-subunit abundance 2.3- and 2.2-fold, respectively, changes that are comparable to those detected in cells grown on filters. On the other hand, SGK1\(_{S425D}\) expression did not induce any changes in total or plasma membrane \(\alpha_{1}\)-subunit abundance, confirming the results obtained in cells grown on filters.

**DISCUSSION**

The results presented herein demonstrate that SGK1 increases the activity of endogenously expressed Na\(^{+}\)-K\(^{+}\)-ATPase 2.5-fold in a Na\(^{+}\)-transporting epithelial cell line (Fig. 2). The effect of SGK1 persisted after [Na\(^{+}\)]\(i\) was increased by monensin (Fig. 4A). Most important, ouabain-sensitive ATPase activity was higher in membranes isolated from cells expressing SGK1\(_{S425D}\) than in membranes from control cells (Fig. 4B). Together, these results indicate that increased [Na\(^{+}\)]\(i\) is...

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**Fig. 5.** Effect of aldosterone stimulation or SGK1\(_{S425D}\) expression on steady-state abundance of total and plasma membrane Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_{1}\)-subunit. A: A6 cells were treated with 100 nM aldosterone or 1 \(\mu\)g/ml tetracycline for 24 h, followed by basolateral plasma membrane protein biotinylation. Aliquots of cell lysates containing 240 \(\mu\)g of protein were used for streptavidin pull-down to recover biotinylated proteins. Samples containing 40 \(\mu\)g of cell lysates (total protein) and the products of streptavidin-pull downs (plasma membrane) were run in parallel using SDS-PAGE and analyzed using Western blot analysis with an antibody against the Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_{1}\)-subunit. A Western blot representative of an experiment performed in triplicate is shown. The experiment included a sample of cells without biotin to control for nonspecific binding of the Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_{1}\)-subunit to the streptavidin-agarose beads. Migration of molecular mass markers (numbers at left indicate mass in kDa) is indicated by arrows. B: to control for specific biotinylation of plasma membrane proteins, Western blots of total protein and plasma membrane protein samples were stripped and reprobed using a PAb against calnexin, an abundant membrane protein resident in the endoplasmic reticulum. Only total protein samples showed significant signals. Migration of molecular mass markers (numbers at left indicate mass in kDa) is indicated by arrows. C: Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_{1}\)-subunit signals were quantified using scanning densitometry and normalized to control conditions. Bars represent average \(\pm\) SE of 3 independent experiments, each of which was performed in triplicate. Black bars indicate total protein, and gray bars indicate plasma membrane protein. Student’s \(t\)-test was used to compare each condition with control values. *\(P < 0.05\).
not the means by which SGK1 affects Na\(^{+}\)-K\(^{+}\)-ATPase activity. Our results are in agreement with the observations of Setiawan et al. (27), who showed that SGK1 cRNA injected into Xenopus oocytes increased the activity of endogenous Na\(^{+}\)-K\(^{+}\)-ATPase. In contrast, Zecevic et al. (36) found that SGK1 activated only exogenous Na\(^{+}\) pumps formed by coinjection of rat \(\alpha_1\)-subunits and Xenopus \(\beta_1\)-subunits, whereas endogenous pumps remained unaffected. Although the latter observation is difficult to explain, most of the results support the role of SGK1 in the regulation of endogenous Na\(^{+}\)-K\(^{+}\)-ATPase.

SGK1 and Na\(^{+}\)-K\(^{+}\)-ATPase are coexpressed in epithelial cell types other than the principal cells of the distal tubule (2). The thick ascending limb of Henle expresses both proteins together with the kidney-specific isoform of the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\)-cotransporter (NKCC2), which localizes to the apical membrane. The activity of NKCC2 is also stimulated by SGK1 when the two proteins are coinjected into Xenopus oocytes (19). Unfortunately, we could not examine the effects of SGK1 on NKCC2 directly, because the A6 cell line does not express apical NKCC2 (Alvarez de la Rosa D, Gimenez I, Forbush B, and Canessa CM, unpublished observations). The presence of SGK1 in various types of Na\(^{+}\)-transporting epithelial cells suggests that one important function of this kinase may be to coordinate the activity of the transporters that mediate entrance of Na\(^{+}\) in the apical membrane, such as ENaC and NKCC2, and the exit of Na\(^{+}\) in the basolateral membrane by the Na\(^{+}\)-K\(^{+}\)-ATPase.

The use of a tetracycline-inducible SGK1 expression system in an epithelial cell line that responds to aldosterone allowed us to test whether SGK1 mediates the early actions of aldosterone on transepithelial Na\(^{+}\) transport (25, 27). The response to aldosterone has been divided into two phases. During the early phase (i.e., the first 2 h), the increase in Na\(^{+}\) transport is thought to be mediated by the activation of preexisting ENaCs and Na\(^{+}\) pumps, whereas the late phase is mediated by the synthesis of new channels and transporters (30). Time course experiments (Fig. 2) indicated a delay of at least 6 h between SGKI\(^{T\_{425D}}\) expression and the increase in Na\(^{+}\)-K\(^{+}\)-ATPase activity. This late effect of SGK1 on Na\(^{+}\)-K\(^{+}\)-ATPase activity is in contrast to our previous observation of SGKI\(^{T\_{425D}}\) stimulating ENaC activity by 50% 1 h after SGKI\(^{T\_{425D}}\) induction in the same cell line (1). In light of these data, it is unlikely that SGK1 participates in rapid stimulation of Na\(^{+}\)-K\(^{+}\)-ATPase, although it could have an influence on the late phase of the aldosterone response. The latter possibility implies that SGK1 should reflect, at least partially, the effects of aldosterone. In contrast, the results indicate that the actions of aldosterone and SGK1 on the two key components of the transcellular pathway of Na\(^{+}\) transport, ENaC (1) and Na\(^{+}\)-K\(^{+}\)-ATPase, are additive, implying that they operate through independent mechanisms. Furthermore, cell surface biotinylation and Western blot analysis demonstrated that aldosterone increased expression and cell surface abundance of Na\(^{+}\)-K\(^{+}\)-ATPase, whereas SGK1 had no effect on protein abundance. Again, these results differ from those reported in Xenopus oocytes (36), in which SGK1 increased total and plasma membrane abundance of the injected Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_1\)-subunit but did not increase the activity or abundance of endogenous Na\(^{+}\)-K\(^{+}\)-ATPase (36). When oocytes were Na\(^{+}\) loaded via expression of ENaC, SGK1 still increased Na\(^{+}\)-K\(^{+}\)-ATPase abundance in the plasma membrane but did not have any effect on total protein expression (36). These results suggest that in oocytes, SGK1 could potentially enhance Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_1\)-subunit translation as well as trafficking to the plasma membrane. The different results obtained in A6 cells and Xenopus oocytes may be attributed to differences in the expression of proteins required for the regulation of the Na\(^{+}\)-K\(^{+}\)-ATPase by SGK1 and underscore the importance of using renal epithelial cells to study transport regulation.

Regarding the mechanism underlying the activation of Na\(^{+}\)-K\(^{+}\)-ATPase by SGK1, cell surface biotinylation and Western blot analysis demonstrated that SGK1 does not increase the synthesis or incorporation of new pumps into the plasma membrane. Instead, SGK1 increases the activity of pumps already present in the plasma membrane. Na\(^{+}\)-K\(^{+}\)-ATPase is the target of multiple regulatory mechanisms, including changes in substrate affinities, interaction with other proteins, and phosphorylation of specific residues (18, 29). The delay in the onset of SGK1 action suggests that the effect of the kinase is not direct but most likely occurs through the induction of new proteins. It has been demonstrated previously that SGK1 regulates transcriptional factors (8, 35), raising the possibility that SGK1 could increase the expression of proteins that modulate Na\(^{+}\)-K\(^{+}\)-ATPase activity. Our system, however, did not allow us to test this hypothesis, because the addition of actinomycin D or cycloheximide to block the synthesis of new proteins would have interfered with the expression of SGKI\(^{T\_{425D}}\).

In summary, our results demonstrate that SGK1 expression increases Na\(^{+}\)-K\(^{+}\)-ATPase activity in renal epithelial cells independently of changes in protein expression or abundance in the plasma membrane. At least in A6 cells, SGK1 and aldosterone modulated Na\(^{+}\) pump activity through independent processes. The precise nature of these mechanisms is yet to be identified.

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