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SGK1 increases Na,K-ATP cell-surface expression and function in *Xenopus laevis* oocytes

Received: 3 September 2003 / Accepted: 27 November 2003 / Published online: 10 January 2004
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Abstract The Na⁺-retaining hormone aldosterone increases the cell-surface expression of the luminal epithelial sodium channel (ENaC) and the basolateral Na⁺ pump (Na,K-ATPase) in aldosterone-sensitive distal nephron cells in a coordinated fashion. To address the question of whether aldosterone-induced serum and glucocorticoid-regulated kinase-1 (SGK1) might be involved in mediating this regulation of Na,K-ATPase subcellular localization, similar to that of the epithelial Na⁺ channel (ENaC), we co-expressed the Na,K-ATPase (rat α 1- and *Xenopus laevis* β 1-subunits) and *Xenopus* SGK1 in *Xenopus* oocytes. Measurements of the Na⁺ pump current showed that wild-type SGK1 increases the function of exogenous Na,K-ATPase at the surface of *Xenopus* oocytes. This appeared to be secondary to an increase in Na,K-ATPase cell-surface expression as visualized by Western blotting of surface-biotinylated proteins. In contrast, the functional surface expression of two other exogenous transporters, the heterodimeric amino acid transporter LAT1-4F2hc and the Na⁺/phosphate cotransporter NaPi-IIa, was not increased by SGK1 co-expression. The total pool of exogenous Na,K-ATPase was increased by the co-expression of SGK1, and similarly also by ENaC co-expression. This latter effect depended on the [Na⁺] of the buffer and was not additive to that of SGK1. When the total Na,K-ATPase was increased by ENaC co-expression, SGK1 still increased Na,K-ATPase cell-surface expression. These observations in *Xenopus* oocytes suggest the possibility that SGK1 induction and/or activation could participate in the coordinated regulation of Na,K-ATPase and ENaC cell-surface expression in the aldosterone-sensitive distal nephron.

Keywords Sodium pump · Kidney collecting duct · Aldosterone · Na,K-ATPase · SGK1 · *Xenopus* oocyte

Introduction

Aldosterone controls extracellular volume and blood pressure by stimulating Na⁺ reabsorption across distal nephron epithelial cells. This effect on transepithelial Na⁺ transport is mediated by coordinated activation of luminal Na⁺ influx via the epithelial Na⁺ channel ENaC (generally representing the rate-limiting step) and basolateral Na⁺ efflux via the Na,K-ATPase [31]. We have shown previously that, in the aldosterone-sensitive distal nephron (ASDN) of adrenalectomized rats, the short-term effect of aldosterone on ENaC and on Na,K-ATPase can be explained by a rapid increase in their luminal and basolateral cell-surface expression, respectively [18, 28].

Xenopus laevis oocyte expression experiments have shown that the aldosterone-induced protein serum and glucocorticoid-dependent kinase-1 (SGK1) could mediate such a stimulatory effect on the cell-surface expression of ENaC [2, 7, 18, 20, 33]. Furthermore, this effect can be explained by phosphorylation, and thus inhibition, of the ubiquitin ligase Nedd4-2 that acts on ENaC [11, 27]. Whether the effect of aldosterone on ENaC cell-surface expression is in fact mediated (in part) by the induction of SGK1 is still controversial. Recent experiments in cell culture systems and in mice support this theory only in part [1, 3, 10, 13, 16]. It must be remembered, however, the fact that the signalling network controlling ENaC cell-surface expression and activity is very complex, and that thus the rate-limiting elements within this network might differ depending on the experimental system and even on the experimental conditions.

To lead to an increase in transepithelial Na⁺ transport, the increase in apical/luminal Na⁺ influx induced by aldosterone must be paralleled by an increase in basolateral Na⁺ efflux via the Na,K-ATPase. This increase can to some extent be achieved by kinetic activation of the Na⁺ pumps already present in the basolateral membrane [22,

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28, 30]. The possibility of kinetic activation of the Na^+ pump is favoured by the fact that its intracellular Na^+ activation curve is relatively steep (positive cooperativity with a Hill coefficient of approximately 2.3) and by the fact that cytosolic $[\text{Na}^+]_i$ ($[\text{Na}^+]_i$) lies generally below that required for half-maximal Na^+ pump activation ($K_{0.5}$). Such kinetic activation would none-the-less require an increase in $[\text{Na}^+]_i$ that would activate, above a certain level, the self-inhibition mechanism of ENaC and thus limit the capability of aldosterone to stimulate transepithelial Na^+ transport.

It is thus meaningful that aldosterone rapidly increases the number of active Na^+ pumps at the cell surface in cortical collecting duct of adrenalectomized rats [28]. This does not preclude, under some circumstances, ENaC and Na,K-ATPase being regulated oppositely rather than in parallel. This is the case, for instance, when $[\text{Na}^+]_i$ rises, thus requiring that ENaC activity be decreased and Na,K-ATPase activity increased.

Interestingly, the transcription- and translation-dependent aldosterone-induced increase in Na,K-ATPase cell-surface expression is independent of the regulation of apical Na^+ influx and thus probably mediated directly by (an) aldosterone-induced regulatory protein(s). We asked now whether the aldosterone-induced regulatory protein SGK1 might play this role and thus stimulate Na,K-ATPase cell-surface expression when co-expressed in *Xenopus* oocytes, analogous to its effect on ENaC. As previously shown for the effect of constitutively active rat SGK1 on endogenous *Xenopus* oocyte Na,K-ATPase [25], we show here that wild type *Xenopus* SGK1 increases the function of exogenous Na,K-ATPase co-expressed in *Xenopus* oocytes and that this effect can be attributed to an increase in Na^+ pump cell-surface expression.

Materials and methods

Expression in *Xenopus laevis* oocytes

X. laevis ENaC α -, β - and γ -subunit cDNAs subcloned in pSD5easy [14, 19, 23], rat Na,K-ATPase $\alpha 1$ -subunit cloned in pcDNA3 [26], *X. laevis* $\beta 1$ -Na,K-ATPase subunit subcloned in pcDNA1 [29], *X. laevis* SGK in pSDeasy [7] and the XSGK kinase-dead mutant (K130A mutation in the putative ATP binding pocket) [18] were used. After linearization of the plasmids with BglII (β -XENaC, XSGK), Afl II (α - and γ -XENaC), NotI (rat $\alpha 1$ -Na,K-ATPase) and XbaI (X $\beta 1$ -Na,K-ATPase), capped cRNA was synthesized using SP6 RNA polymerase or T7 RNA polymerase (in case of Na^+ pump subunits) according to [19]. For Na^+ pump current (I_p) measurements, oocytes were injected with 8 ng rat $\alpha 1$ -Na,K-ATPase cRNA and 1 ng $\beta 1$ -subunit cRNA together with 5 ng wild type or kinase-dead mutant SGK cRNA. For controlled Na^+ loading experiments, oocytes were also co-injected with 0.05 ng of each ENaC subunit cRNA.

Pump current measurements

I_p was measured according to [17]. In brief, after cRNA injection, the oocytes were kept in ND10 solution (in mM): NaCl 10, KCl 2, CaCl_2 1.8, MgCl_2 1, *N*-methyl-D-glucamine (NMDG-Cl) 86,

HEPES 5, pH 7.4 for 2–3 days. Prior to electrophysiological measurements, oocytes were incubated in K^+ -free solution until the membrane potential had reached a stable baseline. The absence of extracellular K^+ prevents the pump from functioning and leads to a slow increase in $[\text{Na}^+]_i$. Exposure to K^+ -containing solution (in mM): Na-gluconate 80, KCl 10, MgCl_2 0.81, CaCl_2 0.41, BaCl_2 5, tetraethylammonium (TEA-Cl) 10, HEPES-NMDG 10, pH 7.4; elicits an outwards current (I_p) that can be recorded. The (50 μM) strophanthidin-sensitive portion of this current is carried by the pumps containing the endogenous *Xenopus* α -subunit and the current blocked by the subsequent addition of 3 mM ouabain corresponds to that carried by the hybrid pumps containing the ouabain-resistant rat $\alpha 1$ -subunit.

Controlled Na^+ loading of Na,K-ATPase-expressing oocytes via ENaC

cRNA-injected oocytes (including ENaC) were kept in ND10 solution [17] prior to the experiment. For Na^+ loading, oocytes were first clamped at 0 mV and washed with K^+ -free solution (in mM): Na-gluconate 100, MgCl_2 1, CaCl_2 0.5, HEPES 10, pH 7.4. Current/voltage (I/V) curves from -100 mV to +80 mV were generated in steps of 20 mV of 100 ms in Na^+ -loading solution without or with 10 μM amiloride. The I/V curve of the current carried by Na^+ via ENaC corresponds to the difference between the curve measured in the presence of amiloride and that measured just before in its absence. The reversal potential for Na^+ (E_{rev}) is thus the potential at the point of intersection of the pair of I/V curves and can be used to calculate $[\text{Na}^+]_i$ from the Nernst equation:

$$[\text{Na}^+]_i = \frac{[\text{Na}^+]_{\text{out}}}{10^{E_{\text{rev}}/58}}$$

For Na^+ loading, the membrane potential of the oocytes was clamped to negative values until E_{rev} for the amiloride-sensitive current fell into the range between 0 and +15 mV (equivalent to a mean $[\text{Na}^+]_i$ of ~70 mM) such that $[\text{Na}^+]_i$ of all oocytes was similar for I_p measurements.

Cell-surface biotinylation and streptavidin precipitation

Cell-surface proteins were labelled (20 oocytes per reaction) with Sulfo-NHS-LC-Biotin (Pierce, Rockford, Ill., USA), a reagent for which the cell membrane is impermeable and that reacts with primary amines. Oocytes injected with cRNAs and treated as those used for I_p measurements were washed 5 times for 5 min in ND96-TEA solution (in mM): 96 NaCl, 2 KCl, 1.8 CaCl_2 , 1 MgCl_2 , 5 HEPES, pH 8.8, 10 TEA and incubated for 30 min with biotinylation reagent (Sulfo-NHS-LC-Biotin, 1.5 mg/ml in ND96-TEA buffer). After washing 5 times in ND96-TEA buffer, oocytes were homogenized in a sucrose-based buffer (4 μl /oocyte) comprising (in mM) 250 sucrose, 0.5 EDTA, 5 TRIS-HCl pH 6.9, 1 phenylmethylsulphonyl fluoride (PMSF) and 10 μl /ml protease inhibitor cocktail (P8340, Sigma, St. Louis, Mo., USA) by passing them 10 times through a 25-G needle. All steps were done at 4°C. After two centrifugation steps at 100 g (4°C) for 10 min, the supernatant was collected and protein concentration determined by the Bradford assay using Biorad reagents following the manufacturer's protocol. For Western blot, 50 μg of total protein mixed with SDS-PAGE sample buffer (final concentration 50 mM TRIS-Cl, pH 6.8, 2% SDS, 10% glycerol, 2.5% ME) was loaded onto a 10% SDS-PAGE gel. For streptavidin precipitation, 500 μg protein from biotinylated oocytes was diluted in 1 ml TRIS-buffered saline (TBS, 100 mM NaCl, 50 mM TRIS-HCl pH 7.4) and 25 μl streptavidin agarose beads (Immunopure, Pierce) added. Samples were rolled at 4°C over night. Beads were then washed once with buffer A (5 mM EDTA, 50 mM NaCl, 50 mM TRIS-HCl pH 7.4), 2 times with buffer B (500 mM NaCl, 20 mM TRIS-HCl pH 7.4) and once with

buffer C (10 mM TRIS-HCl pH 7.4) with a 2-min centrifugation (1,000 g) between washes. After the final wash, buffer C was replaced with 30 μ l SDS-PAGE sample buffer. Protein samples were heated to 65°C for 15 min before separation on a 10% SDS-PAGE gel.

SDS-PAGE and Western blot

Protein samples (50 μ g total lysate or 10 μ l of the streptavidin-precipitate proteins in sample buffer) were incubated at 65°C for 10 min and separated on a 10% SDS-PAGE. Proteins were then transferred to a 0.2- μ m nitrocellulose membrane (Protran, Schleicher and Schuell) and the membranes then incubated in blocking buffer TBST (5% non-fat dry milk, TBS, and 0.2% Tween20 or Igepal C630) for 1 h. Subsequently, membranes were incubated with antibody against Na,K-ATPase holoenzyme [6] at a dilution of 1:1,000 in TBST, either overnight at 4°C or for 1 h at room temperature. After washing with TBST, the membranes were incubated in the presence of a secondary antibody at a dilution of 1:10,000 for 2 h (Goat Anti-Rabbit horse-radish peroxidase conjugated antibody, Transduction Laboratories). After washing 6 times for 5 min with TBST, immunoreactive bands were visualized by enhanced chemiluminescence (SuperSignal West Pico Chemiluminescence kit, Pierce) and exposure to film. The bands were quantified using Image QuaNT (Molecular Dynamics, Sunnyvale, Calif., USA). The values were normalized to Na,K-ATPase expression alone, and the results are expressed as protein abundance (fractional change).

Uptake rate of L-leucine and HPO_4^-

The functional rate of the heterodimeric transporter LAT2-4F2hc was tested according to [24]. Briefly, oocytes were injected with cRNAs encoding the transporter's heavy and light chains (4F2hc and LAT2, respectively) together with wild-type or kinase-dead mutant SGK1. After 24 or 48 h, they were incubated for 3 min in buffer containing 100 μ M leucine and radioactively labelled tracer. Oocytes were then washed and lysed and the amount of intracellular radioactive amino acid measured by scintillation counting. Phosphate uptake via rat NaPi-IIa, was measured as described in [34]. One experiment out of two is shown.

Statistics

Mean values (\pm SEM) of n experiments are shown. The significance of a difference between two conditions was tested using the t -test and between multiple conditions using the ANOVA followed by Tukey's multiple comparison test.

Results

SGK co-expression increases exogenous Na,K-ATPase pump current and cell-surface expression

To address the question of whether SGK1 increases the activity of the Na,K-ATPase, we injected *Xenopus* oocytes with cRNAs encoding rat α 1- and *Xenopus* β 1-Na,K-ATPase subunits alone or together with cRNA encoding *Xenopus* SGK1 in its wild-type or kinase-dead form. Using the same batches of injected oocytes, we tested the function of cell-surface pumps by measuring I_p and Na,K-ATPase α 1-subunit protein expression by Western blot-

ting. The current generated by Na⁺ pumps containing the endogenous amphibian α 1-subunit could be dissociated from that carried by the exogenous rodent one by virtue of their differential sensitivity toward cardiotonic steroids: endogenous pumps were inhibited by low strophanthidin concentrations (50 μ M) whereas 3 mM of the more potent ouabain was required subsequently to block the exogenous pumps. As shown in Fig. 1A, wild-type SGK1 increases the I_p mediated by exogenous pumps but not that carried by endogenous pumps. This effect on rat Na,K-ATPase was dependent on the kinase activity of SGK1, since kinase-dead SGK1 (K130A) did not elicit an increase in I_p .

Biotinylation of oocyte cell-surface proteins allowed us to separate cell-surface from intracellular proteins by streptavidin precipitation. Immunoblotting of total cell lysate and of cell-surface proteins revealed that both the total and surface Na,K-ATPase were increased by SGK1, an effect that depended on the kinase activity of SGK1 (Fig. 1B). It thus appears that the higher I_p in SGK1-expressing cells could be due to the higher amount of cell surface Na⁺ pumps. The fact that total exogenous Na,K-

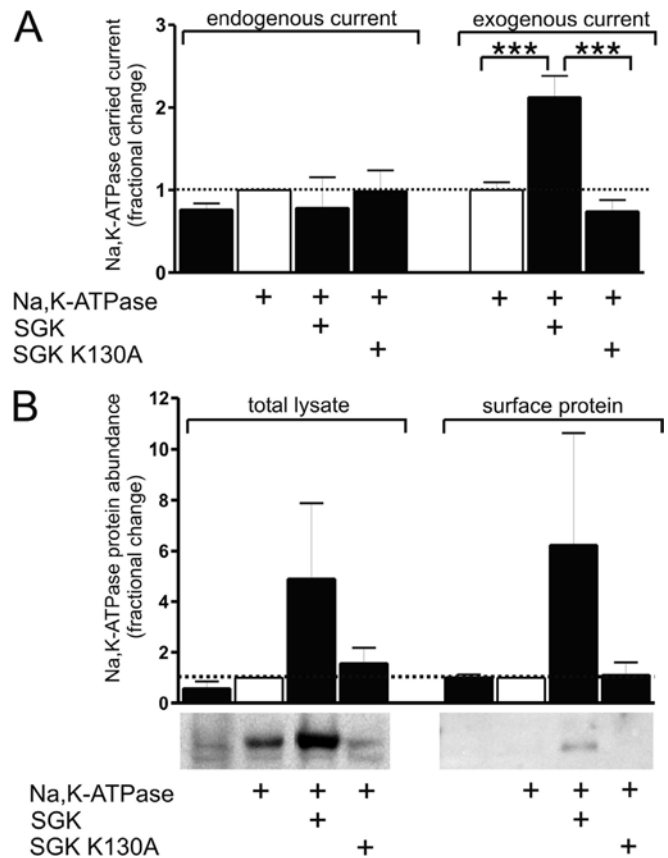


Fig. 1A, B Serum and glucocorticoid-regulated kinase-1 (SGK1) co-expression increases the amount of exogenous Na⁺ pump current and Na,K-ATPase expression in *Xenopus* oocytes. **A** SGK1 stimulates the exogenous, but not the endogenous, Na⁺ pump current in a kinase-domain dependent way. Means \pm SEM; $n=7-9$ oocytes from three different oocyte batches, *** $P<0.001$. **B** Surface expression and total protein abundance of the Na,K-ATPase was increased in the presence of SGK1 in three out of three experiments (mean fractional change shown), but not in that of the kinase-dead mutant SGK1 K130A

ATPase was increased by SGK1 indicated that, under these conditions, the surface expression of Na^+ pumps per se was not necessarily regulated by SGK1.

ENaC increases the Na,K-ATPase pool Na^+ -dependently

To address the question of whether SGK1 increases the sodium pump current and the Na^+ pump surface expression directly or indirectly via a change in $[\text{Na}^+]_i$, we co-expressed also *Xenopus* ENaC α -, β - and γ -subunits. Interestingly, this led to an increase in the total amount of Na,K-ATPase comparable to that induced by SGK1 (Fig. 2), but only when the oocytes were kept in a buffer containing 10 or 100 mM Na^+ , and thus presumably had a higher than normal $[\text{Na}^+]_i$. When oocytes expressing ENaC were kept in 1 mM Na^+ , their Na,K-ATPase level was similar to that of control oocytes, supporting the hypothesis that the effect observed on Na,K-ATPase expression was not a direct effect of ENaC on Na,K-ATPase, but was mediated by an increased $[\text{Na}^+]_i$.

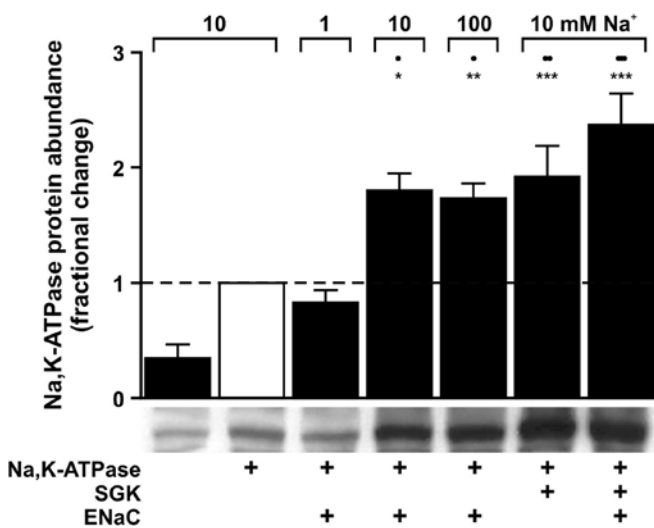


Fig. 2 ENaC co-expression in *Xenopus* oocytes leads to Na^+ -dependent increase in total Na,K-ATPase. When oocytes were kept in 10 or 100 mM Na^+ buffer, the presence of ENaC produced an increase in Na,K-ATPase protein that was similar to the effect of SGK1. The intensity of the Na,K-ATPase α subunit bands is expressed relative to that in the absence of ENaC and SGK1. Means \pm SEM; $n=6$ Western blots from four independent experiments. A representative blot is shown below. SGK1 co-expression in the presence of ENaC did not further increase the amount of Na,K-ATPase ($n=3$ blots from two independent experiments, see also Fig. 4B). The mean relative Na,K-ATPase α -subunit abundance was significantly higher under the four conditions with co-expression of ENaC (10 and 100 mM Na^+) and/or SGK1 compared with Na,K-ATPase alone (●) and Na,K-ATPase plus ENaC at 1 mM Na^+ (*). * $P<0.05$, ** $P<0.01$, *** $P<0.001$

SGK1 selectively increases cell-surface Na,K-ATPase also when ENaC is co-expressed

The co-expression of ENaC allowed us to load all oocytes similarly with Na^+ via ENaC for I_p measurements. This was achieved by placing the oocytes in a high- Na^+ solution at negative holding potentials until they reached a desired $[\text{Na}^+]_i$ calculated from the E_{rev} of the amiloride-sensitive current. According to the Nernst equation, a mean $[\text{Na}^+]_i$ of ~ 70 mM was reached for all tested conditions (Fig. 3).

Oocytes expressing exogenous Na,K-ATPase, SGK1 and ENaC were assayed in parallel for I_p and rat Na,K-ATPase $\alpha 1$ -subunit expression. SGK1 co-expression again stimulated significantly the exogenous Na^+ I_p , now measured at controlled $[\text{Na}^+]_i$, and also the Na,K-ATPase cell-surface expression (Fig. 4A, B). Under these conditions (ENaC co-expression, 10 mM Na^+), the effect of SGK1 on the cell-surface expression of Na,K-ATPase was specific, since the total Na^+ pump pool was not increased (Fig. 4B).

SGK1 does not, in contrast, increase the cell-surface activity of LAT2-4F2hc and NaPi-IIa

Since SGK1 has been implicated in the up-regulation of several membrane proteins in *X. laevis* oocytes, we asked whether it might simply cause an increase in membrane delivery and/or retention of any newly synthesized heterologous surface protein. We therefore tested the effect of SGK1 on the functional expression of two membrane proteins not related to the aldosterone-regulated sodium transport machinery, the heterodimeric amino acid transporter LAT2-4F2hc and the sodium/phosphate co-transporter NaPi-IIa.

Figure 5 shows that neither the uptake rate of L-leucine by oocytes expressing exogenous LAT2-4F2hc (Fig. 5A) nor that of phosphate by oocytes expressing exogenous NaPi-IIa (Fig. 5B) were affected by co-expression of SGK1, whereas ENaC function increased in parallel

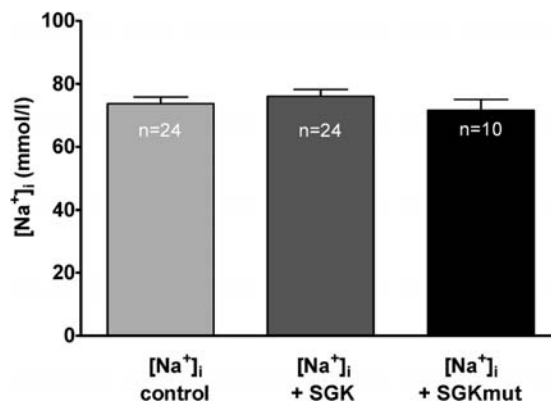


Fig. 3 Controlled and equal Na^+ loading of oocytes via ENaC. The intracellular $[\text{Na}^+]_i$ ($[\text{Na}^+]_i$) was calculated from the reversal potential of the amiloride-sensitive current (i.e. Na^+ current via ENaC). Means \pm SEM

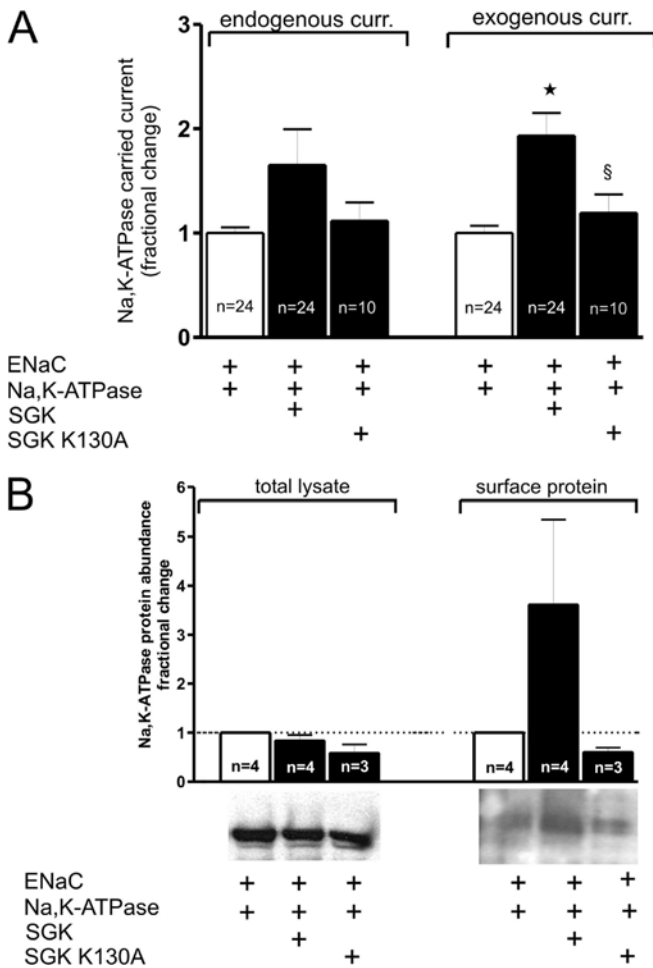
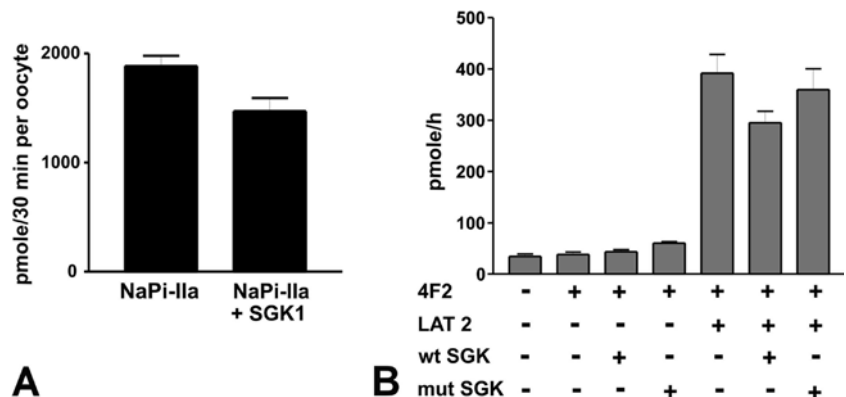


Fig. 4A, B SGK1 increases the Na^+ pump current and the surface expression of the Na,K-ATPase without modifying its total amount. Oocytes expressing Na,K-ATPase, SGK1 and ENaC were assayed in parallel for pump current and protein expression. As in the absence of ENaC (Fig. 1), SGK1 significantly stimulated the Na^+ pump current (**A**, $n=10-24$ oocytes from 3-4 different experiments, $P<0.01$) and the Na,K-ATPase cell-surface expression (**B**, $n=2-4$, cell-surface expression larger in the presence than absence of SGK1 in 4 out of 4 experiments). As in Fig. 2 and in contrast to experiments made in the absence of ENaC (Fig. 1), SGK1 did not increase the total amount of Na,K-ATPase

experiments (not shown). This indicates that the amount of

Fig. 5 Two transport proteins other than Na,K-ATPase are not affected by SGK1 co-expression. Neither L-leucine uptake rate by oocytes expressing the heterodimeric amino acid transporter *LAT2-4F2hc* nor phosphate uptake rate by oocytes expressing the Na^+ /phosphate cotransporter *NaPi-IIa* was increased when SGK1 was co-expressed



exogenous active cell-surface *LAT2-4F2hc* or *NaPi-IIa* was not increased by the action of wild-type SGK1.

Discussion

SGK1 mRNA and protein are up-regulated strongly by aldosterone within 2 h in segment-specific cells of the ASDN [18]. Expression experiments in *Xenopus* oocytes and FRT thyroid cells have shown that SGK1 increases ENaC cell-surface expression, an effect that appears to be mediated by the inhibition of Nedd4-2 [11, 27]. Besides, SGK1 acts also on the surface expression and/or function of several other transport proteins co-expressed generally in *Xenopus* oocytes, i.e. a non-selective cation channel, voltage-sensitive K^+ channels (Kv1.2-5), the slowly activating K^+ channel KCNE1/KCNQ1, the inwardly rectifying K^+ channel Kir1.1 (renal outer medullary K^+ channel ROMK), cystic fibrosis transmembrane conductance regulator (CFTR), the cardiac Na^+ channel SCN5A, Na,K-ATPase, the Na^+/H^+ exchanger NHE3, the anionic amino acid transporter EAAT1 and the system N transporter SNAT3 (SN1) [4, 12, 15, 25, 32, 33, 35, 36, 37]. In the case of NHE3, it appears that the presence of the scaffolding protein Na^+/H^+ exchanger regulatory factor 2 (NHERF2) is required to allow SGK1 to exert its stimulating effect, whereas the data are conflicting in the case of ROMK. The putative endogenous Nedd4 of *Xenopus* oocytes might play a role in mediating the SGK1 effect, at least on ENaC (see above) and SCN5A.

We asked also whether SGK1 might participate to and/or mediate the translocation of the Na,K-ATPase (Na^+ pumps) to the basolateral cell surface, because the aldosterone response of ASDN cells involves an increase in both the apical ENaC and the basolateral Na,K-ATPase, and because SGK1 is localized all over the cytoplasm of these cells. During the course of this study, a report appeared showing that the expression of constitutively active SGK1 in *Xenopus* oocytes indeed increases the pump current carried by the endogenous Na^+ pumps [25]. In the present study, we tested the action of wild-type SGK1 on co-expressed Na,K-ATPase (ouabain-resistant rat Na,K-ATPase $\alpha 1$ -subunit plus *Xenopus* $\beta 1$ -subunit) in *Xenopus* oocytes. We have shown that the I_p carried by these exogenous Na^+ pumps was increased by the co-

expression of wild-type SGK1, whether in the absence or in the presence of ENaC. This channel was co-expressed to provide a Na^+ conductance that increased the steady-state $[\text{Na}^+]_i$ and could be used to set the $[\text{Na}^+]_i$ for I_p measurements to a predetermined level. The co-expression of ENaC increased the amount of total Na,K-ATPase to a similar level in the absence and in the presence of SGK1 and thus blunted the difference in total Na,K-ATPase induced by SGK1. This observation is compatible with the hypothesis that high $[\text{Na}^+]_i$ increased the level of total cellular Na,K-ATPase in oocytes, as it does in other cells [5] and that SGK1 co-expression has a similar effect. Indeed, when ENaC was co-expressed, the total amount of Na,K-ATPase was similar in the presence or absence of SGK1. Under these conditions, SGK1 still had its stimulatory effect on the amount of cell-surface Na,K-ATPase. This demonstrates that the effect of SGK1 on Na,K-ATPase cell-surface expression is independent of its effect on the total Na,K-ATPase pool. This suggests, together with previous observations, that SGK1 might, at least in part, mediate the coordinated effect of aldosterone on the cell-surface expression of Na^+ transport proteins on both surface domains of ASDN cells.

The fact that the extent of ENaC translocation in response to aldosterone displays a variable axial gradient along the ASDN implies that SGK1 is not the sole mediator of this effect. Short-term exposure to aldosterone, for instance, induces translocation of ENaC only in initial segments of the ASDN, whereas the induction of SGK1 appears equal all along the ASDN. In that respect, it is interesting to note that the ~fivefold aldosterone-induced increase in Na,K-ATPase cell-surface expression reported earlier was observed in cortical collecting duct, a region in which ENaC translocation is quite limited under similar experimental conditions [18, 28]. Thus, it appears also that the translocation of apical channels and basolateral pumps are controlled, besides commonly by SGK1, also differentially by other regulatory pathways.

The question of the mechanism by which SGK1 co-expression leads to an increase in Na,K-ATPase cell-surface expression remains open. For instance, the absence of SGK1 phosphorylation consensus sequence on the Na,K-ATPase suggests rather an indirect mechanism [21]. Whether Nedd4-2-mediated ubiquitination might play a role remains to be established. The (poly)ubiquitination of this pump has been demonstrated as yet only in COS cells and not in relation with SGK1 expression or activity [8].

The Na,K-ATPase interacts in the ASDN at least with two different small accessory proteins of the FXYD family that have differential segmental localizations and exert opposite effects on the apparent affinity of the Na^+ pumps for Na^+ [9]. This further underlines the fact that the Na,K-ATPase is regulated in the ASDN at several different levels including that of its apparent Na^+ affinity, besides those of its transcription and surface expression.

As mentioned above, SGK1 is induced in the kidney selectively at the level of the ASDN and regulates, at least in *Xenopus* oocytes, both ENaC and Na,K-ATPase cell-surface expression. Interestingly, the apical K^+ channel

that mediates K^+ secretion in exchange for Na^+ reabsorption in these tubular segments is also regulated at the level of its cell-surface expression by SGK1 when expressed in *X. laevis* oocytes, at least in the presence of NHERF2 [35, 37]. It is thus tempting to suggest that SGK1 plays, in the ASDN, a central role in mediating a coordinated response of different transport proteins to aldosterone.

Acknowledgements The authors thank Christian Gasser for the artwork and Kathia Köhler for helping for NaPi-IIa expression. This study was supported by the Swiss NSF grant 31-59141.99 and -02 to François Verrey.

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