The Serum and Glucocorticoid Kinase sggk Increases the Abundance of Epithelial Sodium Channels in the Plasma Membrane of Xenopus Oocytes*

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The serum- and glucocorticoid-induced kinase (sggk) is a serine and threonine kinase that stimulates amiloride-sensitive sodium transport in Xenopus oocytes. Because aldosterone induces phosphorylation on serine/threonine (Ser/Thr) residues in the carboxyl termini of β and γ subunits of epithelial sodium channels (ENaCs) and causes an increase in the sggk transcript in mammalian and amphibian renal epithelial cells, it seems likely that sggk mediates the action of aldosterone to stimulate sodium transport. Experiments were performed in Xenopus oocytes to determine the mechanism by which sggk increases sodium conductance by examining its effect on phosphorylation, kinetics, and membrane abundance of ENaC. Our results demonstrate that deletions of the carboxyl termini of the three subunits do not inhibit sggk-induced sodium current, indicating that the effect of sggk is not mediated via phosphorylation through the carboxyl termini of ENaC. They also show no evidence that sggk reduces the removal of ENaC from the plasma membrane because mutations of tyrosine residues in the sequences necessary for endocytosis and degradation did not affect the response to sggk. Further studies performed with the patch-clamp technique indicated that sggk did not increase the open probability or changed the kinetics of ENaC. These studies, however, showed a 3-fold increase in the abundance of ENaC in the plasma membrane in the presence of sggk compared with control. Together, the experiments indicate that sggk stimulates electrogenic sodium transport by increasing the number of ENaCs at the cell surface and suggest that sggk may mediate the early increase in aldosterone-induced sodium current.

The cortical collecting tubule of the kidney exhibits a rapid increase in sodium permeability after stimulation with aldosterone (1). The initial increase in sodium permeability occurs prior to the synthesis of new channels (2, 3). In contrast, chronic aldosterone treatment results in the synthesis of many proteins involved in sodium reabsorption, including the subunits of ENaC,1 Na+/K+-ATPase, and mitochondrial enzymes (1, 4, 5). Therefore, the increase in sodium permeability seen in the early phase might be mediated by activation of silent channels or by insertion of preformed channels. Recent findings suggest that sggk may be involved in the early phase of the action of aldosterone. sggk (serum- and glucocorticoid-induced kinase) is a novel member of the serine and threonine kinase gene family (6). The activity of sggk is regulated by rapid induction of transcription of its mRNA. Depending on the cell type, sggk expression is induced by glucocorticoids (6), follicle-stimulating hormone (7), cell volume changes (8, 9), and aldosterone (10, 11). In the cortical collecting tubule of the rat and rabbit (10) and in the A6 cell line derived from the distal tubule of the kidney of Xenopus laevis (11), aldosterone increases the levels of sggk mRNA and protein within 30 min after addition of the hormone. This rapid induction suggests that sggk may mediate the early increase of sodium permeability characteristically observed in the cortical collecting tubule after stimulation with aldosterone.

Because sggk is a Ser/Thr kinase, the first possibility to consider is whether sggk phosphorylates ENaC. This possibility was raised by previous studies demonstrating that the carboxyl terminus of β and γ subunits of ENaC are phosphorylated when the channels are expressed in Madin-Darby canine kidney cells (a cell line derived from canine distal tubule of the kidney) (12). Phosphorylation of ENaC could increase the open probability or the insertion of preformed channels and/or decrease the retrieval. Alternatively, the effect of sggk could involve phosphorylation of other proteins that modify the activity of ENaC or its abundance in the plasma membrane.

In this study, we examined the basic mechanism by which sggk increases sodium permeability. We used Xenopus oocytes co-injected with cRNAs encoding ENaC and sggk as our experimental model. This strategy provides an approach to examine the activity of channels with electrophysiological techniques and also to measure expression of channel proteins with biochemical methods. The validity of this model system is based on the observation that sggk increases the magnitude of amiloride-sensitive currents in oocytes (10, 11), indicating that these cells have the machinery necessary to mediate the sggk response.

MATERIALS AND METHODS

cDNA Constructs—cDNAs from rat ENaC subunits were used in all experiments. Deletions of the carboxyl termini to generate truncated subunits αb, βb, and γb, and replacement of tyrosine for alanine residues to generate Y673A, Y618A, and Y628A have been previously de-

1 The abbreviations used are: ENaC, epithelial sodium channel; BFA, brefeldin A.
scribed (13). Substitutions of six serine and threonine residues (T570A/ T575A/T584A/T613A/S620A/S631A) in the carboxyl terminus of the β subunit for alanines (βPO4), and of five in the amino terminus of the α subunit (S80A/T92A/T93A/S103A/T110A) were made by polymerase chain reaction. Presence of the mutations in the cDNAs was confirmed by DNA sequencing. The FLAG epitope was introduced in the α subunit by substituting residues Thr194 to Arg211 with the sequence DYKD-DDDK (α FLAG) (14). The mouse sgk/pSP64poly(A) construct was generated as described earlier (10). cRNAs were made from linearized plasmids using the mMessagemMachine kit (Ambion, Austin, TX) according to the manufacturer’s instructions.

Oocyte and cRNA Injection—Xenopus laevis oocytes were surgically removed from adult female frogs using standard procedures. Stage V–VI oocytes were injected with 1 ng of cRNA from each of the wild-type or mutant subunits in the following combinations: α, β, and γ; α and β; α and γ; or α alone. When indicated, 1 ng of sgk cRNA was also injected. Oocytes were incubated at 19 °C for 1–3 days in amphibian Ringer supplemented with 10 μM amiloride.

Electrophysiology and Data Evaluation—Electrophysiological recordings were performed using two-electrode voltage clamp and patch clamp techniques. Current and voltage electrodes were pulled from borosilicate glass and filled with 3 M KCl. Electrode resistances were <1 MΩ. Epithelial sodium channel currents were calculated as the difference in whole-cell current before and after the addition of 50 μM amiloride at the back of the bathing solution. Currents were recorded with an OC-725B oocyte voltage clamp (Warner Instrument Corp., Hamden, CT) and digitized at 0.1 kHz (ITC-16), and the values were stored on the hard drive of a personal computer. Membrane potential was held at −60 mV. Current-voltage relations were generated by changing the membrane potential from −180 to 80 mV in 20 mV incremental steps of 200 ms duration using Pulse (HEKA, Lambrecht, Germany). I–V curves were fitted to the constant field equation. The composition of the standard bath solution was 100 mM sodium gluconate, 4 mM KCl, 2 mM CaCl2, 10 mM HEPES, pH adjusted to 7.4 with KOH. Single channel recordings were made from cell-attached and inside-out patches. For patch-clamp recordings, pipette-to-membrane seals with resistances of 9–15 GΩ were formed with pipettes made from borosilicate capillary glass by a two-stage pulling and fire-polishing process. The composition of the pipette solution was 150 mM LiCl, 1 mM CaCl2, 10 mM Tris buffered to pH 7.4, and the composition of the bath was 150 mM KCl, 5 mM EDTA, 10 mM HEPES, pH adjusted to 7.4 with KOH. An Axopatch 200B amplifier and Digidata 1200B (Axon Instruments, Forster City, CA) interfaced to a personal computer were used to acquire data at 5 kHz. The data were filtered at 100 Hz during acquisition using an eight-pole Bessel filter (Frequency Devices, Inc., Haverford, PA) and stored directly on the hard drive of a personal computer. Open probability was calculated from single- and multichannel patches using pClamp7. Lists of open- and closed-current intervals were generated via a half-amplitude threshold crossing criterion using Fetchan. Only patches containing one channel were used to determine open and closed times, and all of open- and closed-current intervals were generated via a half-amplitude fitting method was simplex-least squares. Results are expressed as mean ± S.E. Differences between groups were assessed using Student’s test, and p < 0.05 was considered to be statistically significant.

Metabolic Labeling of Oocytes—Oocytes injected with ENaC alone or with ENaC and sgk were labeled for 8 h with 0.5 μCi/ml of a mixture of [35S]methionine and cysteine (in vitro cell labeling mix, Amersham Pharmacia Biotech). After labeling, cells were homogenized in 1% Triton X-100 and evaluated for total protein and specific activity. An equal number of counts were immunoprecipitated with anti-α, α-β, and γ specific antibodies. A 50-μl aliquot of protein A slurry was added to each sample to isolate the immune complexes. After washes with homogenization buffer, the products were resolved by 10% SDS-polyacrylamide gel electrophoresis. Gels were treated with 1 N sodium salicylate, dried, and exposed to x-ray film for fluorography.

Labeling of Surface Channels from Oocytes—Oocytes were injected with a FLAG-cRNA and wild-type β and γ cRNAs, with or without sgk cRNA. After 36 h, cells were incubated on ice with 10% bovine serum albumin for 30 min. 25 nM/100 μl anti-FLAG M2 monoclonal antibody (Kodak, Rochester, NY) was added, and the incubation continued for 1 h. After 10 washes with ice-cold amphibian Ringer solution, cells were incubated with 125I-protein G (1 μCi/100 μl, 1 nCi/100 μl) (ICN Biomedical, Irvine, CA). Oocytes were washed with 1 ml of ice-cold amphibian Ringer 10 times. Three oocytes were placed in each tube for measurements of gamma counts. Water-injected oocytes served as controls in these experiments. Results are expressed as mean ± S.E.

RESULTS

Role of Serine and Threonine Residues in the Carboxyl Terminus of β Subunits in the Response to sgk.—We have previously shown that ENaCs are phosphorylated in the carboxyl termini of the β and γ subunits under basal conditions and that aldosterone induces additional phosphorylation mainly in the β subunit in transfected Madin-Darby canine kidney cells (12). Because sgk activates Xenopus and rat ENaCs, the most likely candidates for sgk phosphorylation are serine and threonine residues conserved in the β subunits from both species. We identified six conserved residues in the sequence of the rat β subunit: Thr-570, Thr-575, Thr-584, Thr-613, Ser-620, and Ser-631. These six Ser/Thr residues were replaced by alanines to generate the mutant βPO4. Oocytes were injected with wild-type α and γ subunits and mutant βPO4 with and without sgk cRNA. 36 h after injection, the activity of channels was examined by measuring amiloride-sensitive whole-cell currents with the two-electrode voltage clamp. Fig. 1 shows the I–V curves from oocytes expressing wild-type αβγ channels with or without sgk and mutated αβPO4γ channels with or without sgk. The mean currents in channels and αβPO4γ channels were of the same magnitude: 2.53 ± 1.17 μA/oocyte in the wild-type and 3.35 ± 0.65 μA/oocyte in the mutant group (values taken at a membrane potential of −100 mV). Coexpression with sgk increased the current of wild-type channels to 9.45 ± 1.35 and of mutant channels to 6.42 ± 1.17 μA/oocyte, which represents a 3.7- and 2.9-fold increase in current, respec-
whether by associating with the carboxyl termini of the subunits. To test these proteins by phosphorylation of residues in this domain or plasma membrane, we treated oocytes with 5 mM BFA and measured whole-cell currents at 3-hour intervals with the two-electrode voltage clamp. Arrows indicate the times of addition and removal of BFA. Each symbol represents the mean of 8–10 oocytes; error bars are S.E.

tively. Although the response of mutant channels was slightly smaller, the difference was not statistically significant. These results indicate that sgk-induced sodium permeability is not mediated by phosphorylation of conserved serine and threonine residues in the carboxyl terminus of the β subunit. In addition, these six Ser/Thr residues are not required to maintain basal channel activity because wild-type and mutant channels exhibited the same level of current.

sgk and Retrieval of Channels from the Plasma Membrane—In addition to the six Ser/Thr residues mutated in the β subunit, the carboxyl termini of the three subunits contain other potential phosphorylation sites, as well as PY sequences involved in interactions with the ubiquitin ligase Nedd4 (15, 16) and with components of the endocytic machinery (13). Ubiquitination of the subunits by Nedd4 and clathrin-mediated endocytosis both promote retrieval of channels from the plasma membrane. sgk could disrupt interaction of the channel with these proteins by phosphorylation of residues in this domain or by associating with the carboxyl termini of the subunits. To test whether sgk slows the rate of endocytosis of channels from the plasma membrane, we treated oocytes with 5 mM brefeldin A (BFA) and followed the levels of amiloride-sensitive currents over a period of several hours with the two-electrode voltage clamp (13). BFA inhibits the secretory pathway by blocking anterograde vesicular transport from the endoplasmic reticulum and delivery of newly synthesized channels to the plasma membrane. In the presence of BFA, the decrease of amiloride-sensitive currents reflects the rate of removal of channels from the plasma membrane. We added BFA to the incubation medium of oocytes expressing ENaC ± sgk and measured whole-cell currents at 3-hour intervals in both groups. Currents decreased at a similar rate in oocytes expressing sgk (Fig. 2), suggesting that endocytosis of ENaC is not affected by sgk. Furthermore, because the PY motifs have been shown to be necessary for removal of channels from the cell surface (13, 14), we also examined whether mutations or deletions of these motifs from α, β, and γ subunits could affect the response to sgk. Channels with mutations in all three tyrosine residues (αY618AβY628AγY629A) and with deletions of the carboxyl termini (αβγγγγγγ) were expressed with and without sgk. As expected, oocytes injected with αY618AβY628AγY629A (5.66 ± 0.4 μA/oocyte) and αβγγγγγγ (14 ± 1.26 μA/oocyte) expressed larger currents than oocytes injected with wild-type channels. Most significantly, αY618AβY628AγY629A and αβγγγγγγ channels responded to sgk with a further increment in current: 14.6 ± 2.9 and 34.2 ± 4.9 μA/oocyte, respectively (Fig. 3).

Effect of sgk on ENaCs with Different Subunit Compositions—Amiloride-sensitive Na⁺ channels with distinct functional properties are generated by various combinations of the α, β, and γ subunits: αβγ, αβ, αγ, and α alone (17). To determine which subunits are required for the response to sgk, we expressed α, αβ, and αγ channels with and without sgk. As previously reported, α, αβ, and αγ channels expressed current smaller than that of αβγ (18) but large enough for accurate measurements. All these three types of channels responded to sgk with an increase in current. In oocytes expressing α channels, the current increased from 20 ± 3 to 180 ± 25 nA/oocyte, in αγ channels from 0.5 ± 0.12 to 1.37 ± 0.19 μA/oocyte, and in αβ channels from 2 ± 0.22 to 10 ± 2.2 μA/oocyte. These mean values were taken at –60 mV; currents at different voltages are shown in the I–V curves in Fig. 4.

These results provide important information regarding the mechanism of channel activation by sgk. According to one of our previous reports (18), the mean open probability (Pₒ) of αγ channels is ~0.5, whereas the Pₒ of αβ channels is close to 1. Because the Pₒ of αβ channels is near unity, the increase in current induced by sgk could be accounted for only by an increase in the number of active channels in the plasma membrane and not by changes in Pₒ.

In addition, the data indicate that β and γ subunits are not required for the response to sgk because oocytes expressing

![Fig. 2](image1.png) Rate of retrieval of channels from the plasma membrane. Oocytes were injected with αβγ alone or with sgk cRNAs 36 h later, 5 μM BFA was added to the incubation medium, and whole-cell currents were measured at 3-hour intervals with the two-electrode voltage clamp. Arrows indicate the times of addition and removal of BFA. Each symbol represents the mean of 8–10 oocytes; error bars are S.E.

![Fig. 3](image2.png) Stimulation of amiloride-sensitive currents by sgk in oocytes injected with channels mutated in the PY motifs or with deletions of the carboxyl termini from all three subunits. A, I–V curves of oocytes injected with αY618AβY628AγY629A channels alone or with sgk. B, I–V curves of oocytes injected with αβγγγγγγ channels alone or with sgk. Each data point represents the mean of 20–30 oocytes. Error bars are S.E. p < 0.01.
only α subunits exhibited a similar increase in currents. Within the α subunits, the carboxyl terminus is also not necessary, as we showed in the experiments in Fig. 3B. To investigate whether the amino terminus from α subunits could be phosphorylated by sgk, we made a construct in which the first 77 residues were deleted. However, deletion of most of the amino terminus from α completely inactivated the channels. Mutations of five Ser and Thr residues (S80A/T92A/T93A/S103A/T110A) common to the rat and Xenopus α sequences also produced inactive channels.

Measurements of \( P_o \) and Characterization of the Kinetics of αβγ ENaCs Coexpressed with sgk—The previous experiments strongly suggest that sgk does not increase the \( P_o \) of αβ channels. To further investigate the effect of sgk on the kinetics of ENaC, we examined the \( P_o \) and kinetics of αβγ channels alone and in the presence of sgk with the patch clamp technique. The mean \( P_o \) was calculated from at least 16–18 patches for each condition. Single- and multichannel patches were included in this analysis. Data were collected under identical experimental conditions for both groups: the same batch of oocytes was injected with αβγ or with αβγ and sgk, and recordings were made the same day from both groups.

The mean \( P_o \) of αβγ channels was 0.83 ± 0.031, and in the presence of sgk, the \( P_o \) was 0.87 ± 0.04 (Table I). The mean open (\( \tau_o \)) and closed (\( \tau_c \)) times were determined with data collected from patches containing only single channels. Histograms were constructed with data from several patches in order to accumulate enough number of events. Fig. 5 shows the frequency distributions of the open and closed dwell times of αβγ channels expressed alone and with sgk. Histograms of the open dwell times were well fitted with a single exponential probability density function. The time constants of the open states were 1309 ms for αβγ channels and 1865 ms for channels coexpressed with sgk. In contrast, the closed dwell time histograms were best fitted with two exponentials. The \( \tau_c \) of the short closed state of αβγ channels was 5.8 ms, and the \( \tau_o \) of the long close estate was 266 ms. The two \( \tau_o \) of αβγ channels with sgk were 5.2 and 356 ms, respectively (Fig. 5 and Table I). The closed times with and without sgk were not statistically different for the two groups.

On several occasions, channels were initially recorded in the cell-attached configuration for 5–10 min, and then they were excised and the recording continued for an additional 5–10 min. Finally, the patches were reintroduced into the cytoplasm of the oocyte and recorded for an additional 5–10 min. In these experiments, we did not detect significant changes in the \( P_o \) or in the kinetics of channels when the patches were excised or after reestablishing contact with the cytoplasm. These observations strengthen the notion that sgk does not modify the \( P_o \) of ENaC.

Abundance of the Subunits of ENaC in Oocytes Expressing sgk—Results of the previous experiments are consistent with sgk increasing the number of channels at the cell surface without changing the \( P_o \). Such an effect could be produced by enhancing the synthesis and insertion of subunits, thereby increasing the number of channels in the cell. To investigate this possibility, we estimated the amount of ENaC subunits by immunoprecipitation with specific anti-ENaC antibodies in oocytes expressing channels only or with sgk. Oocytes were labeled with \(^{35}\)S)methionine and cysteine for 8 h. After determining the specific activity of the labeled proteins, equal amounts of counts were immunoprecipitated from each condition, and the products resolved by SDS-polyacrylamide gel electrophoresis. The results of these experiments are illustrated in the gels shown in Fig. 6. Quantification of the intensity of the bands revealed similar levels of expression of each of the subunits in the two groups.

Expression of Channels at the Cell Surface—Although sgk does not change the amount of subunits in oocytes, we cannot
exclude a selective increase in the number of channels at the cell surface, because only a small fraction of the total number of channels is expressed in the plasma membrane of oocytes (19).

In order to assess the number of channels in the plasma membrane, we introduced a FLAG epitope in the extracellular domain of the $\alpha$ subunit in the same manner as reported previously by Firsov et al. (14). Oocytes were injected with $\alpha$ FLAG bg alone or in combination with sgk, and after 36 h, intact cells were incubated with an anti-FLAG monoclonal antibody. Antibodies reacting with $\alpha$ FLAG on the surface of oocytes were detected with $^{125}$I-protein G. Groups of three oocytes were measured as a single data point. The radioactivity from the three experimental conditions, $\alpha$ bg, $\alpha$ bg with sgk, and water-injected oocytes, is shown in Fig. 7. Oocytes expressing sgk had 3-fold more counts than oocytes injected with channels alone, reflecting a larger number of $\alpha$ subunits expressed at the cell surface.

**DISCUSSION**

The results of this work demonstrate that sgk, an aldosterone-induced Ser/Thr kinase in collecting duct cells (10), activates ENaC by increasing the number of channels in the plasma membrane. The effect is not achieved by reducing the rate of endocytosis, as is the case for mutations causing Liddle’s syndrome, but rather by insertion of channels into the plasma membrane. sgk did not change the $P_o$ or kinetics of channels. This conclusion was reached from several different experiments. With the patch clamp technique, we determined that the $P_o$ of wild-type channels was high in oocytes, and the kinetics were characterized by long openings of several seconds’ duration and two closed states, a short one of less than 10 ms and a longer one in the range of 200–300 ms. Expression with sgk did not change the $P_o$ or the kinetics. The only difference was a larger number of channels in patches from oocytes injected with sgk. Another line of evidence that argues against
sgk affecting the \( P_o \) was the finding that \( \alpha \beta \) channels responded to \( \text{sgk} \) with a 3-fold increase in whole-cell currents. Such an increment could only be accounted for by the presence of more functional channels at the cell surface, because the \( P_o \) of \( \alpha \beta \) channels is already \( \sim 1 \).

Previous studies performed in the rat cortical collecting tubule (20) and in the cell line A6 (21) have shown a great variability in the \( P_o \) of ENaC from 0.01 to 0.9 with a mean of 0.5. We consistently found a much higher \( P_o \) (0.83–0.87) in oocytes. We do not think that the discrepancy is due to selection for channels with high \( P_o \). After formation of the seal we kept patches for at least 5 min, a period long enough to detect activity even if channels had a very low \( P_o \). If any openings were detected, data from that patch were included in the calculations. Most likely, channels exhibit high \( P_o \) because the process or factor that down-regulates ENaC activity in native tissues does not operate in oocytes.

The lack of effect on \( P_o \) suggested that \( \text{sgk} \) mainly increases number of channels at the cell surface. However, we also considered an alternative explanation, i.e. that \( \text{sgk} \) activates silent channels already present in the plasma membrane. Firsov et al. (14) have reported that a large number of channels in the plasma membrane of oocytes are indeed inactive. However, labeling of surface channels with anti-FLAG antibodies and radioactive protein G gave more counts in the oocytes expressing \( \text{sgk} \). The simplest interpretation of these results is that oocytes injected with \( \text{sgk} \) express more channels on the surface.

Lastly, our results suggest that ENaC is not the direct substrate for \( \text{sgk} \) phosphorylation. We have previously reported that \( \beta \) and \( \gamma \) but not \( \alpha \) are phosphorylated and that the phospho-residues are located in the carboxyl termini of the subunits (12). Here, we showed that the effect of \( \text{sgk} \) remained after deletion of the carboxyl termini from the three subunits. Moreover, channels lacking \( \beta \) or \( \gamma \) responded to \( \text{sgk} \), indicating that these two subunits do not mediate the effects of \( \text{sgk} \). Therefore, the only functional significant phosphorylation may take place in the amino terminus of \( \alpha \). We could not completely disprove this possibility because channels with deletions of the first 77 amino-terminal residues or with mutations in five conserved Ser/Thr were not functional. Because phosphorylation studies with \(^{32}\)P are not feasible in \( \text{Xenopus} \) oocytes, a definitive answer awaits experiments in a cell line.

Taken together, the data suggest that \( \text{sgk} \) phosphorylates a yet unidentified protein that in turn mediates translocation of ENaC from an intracellular compartment to the plasma membrane and thus increases sodium permeability.

REFERENCES

1. Verrey, F. (1995) J. Membr. Biol. 144, 93–110
2. May, A., Pouti, A., Gaeggeler, H.-P., Horisberger, J.-D., and Rossier, B. C. (1997) J. Am. Soc. Nephrol. 1813–1822
3. Asher, C., Wbild, H., Rossier, B. C., and Garty, H. (1996) Am J. Physiol. 271, C605–C611
4. Rossier, B. C., and Palmer, L. G. (1992) in The Kidney: Physiology and Pathophysiology (Seldin, D. W., and Giebisch, G., eds) Vol. 1, pp. 1373–1409, Raven Press, New York.
5. Garty, H., and Palmer, L. G. (1997) Physiol. Rev. 77, 359–396
6. Webster, M. K., Goya, L., Ge, Y., Maiyar, A. C., and Firestone, G. L. (1993) Mol. Cell. Biol. 13, 2081–2090
7. Alliston, T. N., Maiyar, A. C., Buse, P., Firestone, G. L., and Richards, J. S. (1997) Mol. Endocrinol. 11, 934–949
8. Imaiuzumi, K., Tsuda, M., Wanaka, A., Tobiyama, M., and Takagi, T. (1994) Brain Res. Mol. Brain Res. 26, 189–196
9. Waldegger, S., Barth, P., Raber, G., and Lang, F. (1997) Proc. Natl. Acad. Sci. USA 94, 4440–4445
10. Náray-Fejes-Tóth, A., Canessa, C., Cleaveland, E. S., Aldrich, G., and Fejes-Tóth, G. (1999) J. Biol. Chem. 274, 16973–16978
11. Chen, S.-Y., Bhargava, A., Mastroberardino, L., Meijer, O., Wang, J., Buse, P., Firestones, G., Verrey, F., and Pearce, D. (1999) Proc. Natl. Acad. Sci. USA 96, 2514–2519
12. Shimkets, R., Lifton, R. P., and Canessa, C. M. (1997) Proc. Natl. Acad. Sci. USA 95, 3301–3305
13. Shimkets, R. A., Lifton, R. P., and Canessa, C. M. (1997) J. Biol. Chem. 272, 25537–25541
14. Firsov, D., Schild, L., Gautschi, I., Merillat, A.-M., Schneeberger, E., and Rossier, B. C. (1996) Proc. Natl. Acad. Sci. USA 93, 15370–15375
15. Staub, O., Dho, S., Henry, P. C., Correa, J., Ishikawa, T., McGlade, J., and Rotin, D. (1986) EMBO J. 15, 2371–2380
16. Staub, O., Gautschi, I., Ishikawa, T., Breitschopf, K., Ciechanover, A., Schild, L., and Rotin, D. (1997) EMBO J. 16, 6325–6336
17. McNicholas, C., and Canessa, C. M. (1997) J. Gen. Physiol. 109, 681–692
18. Fyfe, G. K., and Canessa, C. M. (1996) J. Gen. Physiol. 112, 423–432
19. Valentijn, J. A., Fyfe, G. K., and Canessa, C. M. (1998) J. Biol. Chem. 273, 30344–30351
20. Pacha, J., Frindt, G., Antonian, L., and Palmer, L. (1993) J. Gen Physiol. 102, 25–42
21. Kemendy, A. E., Kleyman, T. R., and Eaton, D. C. (1992) Am. J. Physiol. 263, C825–C837