Pflügers Arch - Eur J Physiol (2002) 443:617–624
DOI 10.1007/s00424-001-0737-1

ORIGINAL ARTICLE

S. Wärntges · B. Friedrich · G. Henke · C. Duranton
P. A. Lang · S. Waldegger · R. Meyermann · D. Kuhl
E. J. Speckmann · N. Obermüller · R. Witzgall
A. F. Mack · H. J. Wagner · C. A. Wagner · S. Bröer
F. Lang

Cerebral localization and regulation of the cell volume-sensitive serum- and glucocorticoid-dependent kinase SGK1

Received: 9 May 2001 / Revised: 21 August 2001 / Accepted: 13 September 2001 / Published online: 9 November 2001

Abstract The serum- and glucocorticoid-dependent kinase SGK1 is regulated by alterations of cell volume, whereby cell shrinkage increases and cell swelling decreases the transcription, expression and activity of SGK1. The kinase is expressed in all human tissues studied including the brain. The present study was performed to localize the sites of SGK1 transcription in the brain, to elucidate the influence of the hydration status on SGK1 transcription and to explore the functional significance of altered SGK1 expression. Northern blot analysis of human brain showed SGK1 to be expressed in all cerebral structures examined: amygdala, caudate nucleus, corpus callosum, hippocampus, substantia nigra, subthalamic nucleus and thalamus. In situ hybridization and immunohistochemistry in the rat revealed increased expression of SGK1 in neurons of the hippocampal area CA3 after dehydration, compared with similar slices from brains of euvolaemic rats. Additionally, several oligodendrocytes, a few microglial cells, but no astrocytes, were positive for SGK1. The abundance of SGK1 mRNA in the temporal lobe, including hippocampus, was increased by dehydration and SGK1 transcription in neuroblastoma cells was stimulated by an increase of extracellular osmolarity. Co-expression studies in *Xenopus laevis* oocytes revealed that SGK1 markedly increased the activity of the neuronal K^+ channel Kv1.3. As activation of K^+ channels modifies excitation of neuronal cells, SGK1 may participate in the regulation of neuronal excitability.

Keywords K^+ channels · Kv1.3 · Neuroblastoma · Dehydration · Glial cells · Ca^{2+} · Epilepsy · Brain

S. Wärntges and B. Friedrich contributed equally to this work and thus share first authorship

S. Wärntges · B. Friedrich · G. Henke · C. Duranton · P.A. Lang
S. Waldegger · C.A. Wagner · S. Bröer · F. Lang (✉)
Physiologisches Institut der Universität Tübingen,
Gmelinstrassse 5, 72076 Tübingen, Germany
e-mail: florian.lang@uni-tuebingen.de
Tel.: +49-7071-2972194, Fax: +49-7071-295618

R. Meyermann
Department of Brain Research, University of Tübingen, Germany

D. Kuhl
Zentrum für Molekulare Neurobiologie, Universität Hamburg

E.J. Speckmann
Department of Physiology, University of Münster, Germany

N. Obermüller · R. Witzgall
Department of Anatomy, University of Heidelberg, Germany

A.F. Mack · H.J. Wagner
Department of Anatomy, University of Tübingen, Germany

C.A. Wagner
Department of Cellular and Molecular Physiology,
Yale University, New Haven, Conn. USA

Introduction

Many genes are modulated by alterations of osmolarity and/or cell volume [5, 23]. A gene particularly sensitive to even minor alterations of osmolarity is the *serum- and glucocorticoid-dependent kinase-1* (*SGK1*) originally cloned from rat mammary tumour cells [41, 42]. Human *SGK1* (*hSGK1*) has been cloned as a cell volume-sensitive gene from human hepatoma cells [37] and is expressed in all human tissues hitherto tested, including the brain [37]. In situ hybridization has shown SGK1 kinase mRNA in the rat brain to be expressed mainly in oligodendrocytes and hippocampus neurons [13].

The present study was performed to define the cerebral localization of SGK1 at both mRNA and protein levels, and to test for an influence of dehydration on the expression of cerebral SGK1.

The study also addresses a functional role of SGK1. SGK1 is known to up-regulate the renal epithelial Na^+ channel (ENaC) [4, 6, 8, 24, 26, 36], the $Na^+/K^+/2Cl^-$ co-transporter (NKCC or BSC1) [24] the cystic fibrosis

transmembrane conductance regulator (CFTR)-dependent Cl^- channel [36] and the cation channel induced by 4F2/LAT1 [35]. The present study explores the possible influence of SGK1 on the function of Kv1.3, with respect to participation in the regulation of neuroexcitability.

Materials and methods

Cell culture

SKNSH human neuroblastoma cells (about 0.5×10^6 per sample) were maintained in RPMI 1640 supplemented with 10% (vol/vol) fetal calf serum (FCS) and 100 units/ml penicillin/100 µg/ml streptomycin containing 10.1 mM glucose at 37 °C, pH 7.4 (5% CO_2). Cells were grown to subconfluency, then deprived of FCS for 24 h followed by substitution of complete medium without FCS prior to stimulation.

Acquisition of rat brain tissue

Male Sprague-Dawley rats (200–250 g) were maintained on standard chow and either allowed free access to water (control, *n*=6) or were deprived of water for 3 days (*n*=6). The animals were killed by inhalation of CO_2 for 5 min. Brain tissue was removed carefully, immediately snap-frozen in liquid nitrogen and stored at –70 °C until analysis.

Northern blotting

For preparation of mRNA, approximately 200 mg frozen rat brain (temporal lobe including hippocampus) or SKNSH cells were homogenized in 2 ml Trizol (Life Technologies, Karlsruhe, Germany) or in 0.4 ml RNA lysis buffer (Macherey and Nagel, Düren, Germany), respectively. Tissue samples were minced using a mechanical homogenizer (Miccra D-8, Roth, Karlsruhe, Germany), followed by centrifugation at 275 *g* for 10 min (Sorvall RT 6000 B, Kendro, Hanau, Germany). The supernatant was collected, centrifuged (12,000 *g*, 15 min, 4 °C, Heraeus Biofuge 22R, Kendro) and again collected. Total RNA was isolated as indicated in the protocol provided by the distributors. For SGK1 detection in mammalian brain the Human Brain Multiple Northern Blot III (Clontech, Heidelberg, Germany) was hybridized. Alternatively Northern blots were prepared with 15 (SKNSH) or 20 µg (tissue) total RNA that had been separated by electrophoresis through 10 g/l agarose gels (Life Technologies) in the presence of 2.2 M formaldehyde (Roth, Karlsruhe, Germany). Vacuum blotting (Oncor Trans DNA Express Vacuum Blotter, Appligene, Heidelberg, Germany) was used to transfer the RNA onto positively charged nylon membranes (Roche, Mannheim, Germany). The RNA was then cross-linked to the membrane under 120 mJ ultraviolet light (UV Stratalinker 1800, Stratagene Europe, Amsterdam, Netherlands). Hybridization overnight was performed in Dig-Easy-Hyb (Roche) at a probe concentration of 25 µg/l at 50 °C. The digoxigenin (Dig)-labelled SGK1 probe was generated by PCR as described in [37]. For generation of Dig-labelled β-actin probes, the sense primer 5′-AAC CGC GAG AAG ATG ACC CAG ATC ATG TTT-3′ and the antisense primer 5′-AGC AGC CGT GGC CAT CTC TTG CTC GAA GTC-3′ were chosen from the coding sequence of accession no. X00351 and obtained from Life Technologies. cDNA was reverse transcribed from total RNA isolated from HEK 293 cells as described above. Using an oligo$(dT)_{18}$ primer (Advantage RT-for-PCR Kit, Clontech) RT-PCR was performed in accordance with the manufacturer's protocol. The PCR reaction mix contained 4 µl cDNA, 82.5 µl distilled H_2O, 1 µl digoxigenin-labelled or non-labelled nucleotide mix (10 mM each), respectively, 1 µl sense primer, 1 µl antisense primer (10 µM each), 0.5 µl Taq polymerase and 10 µl 10×reaction buffer (15 mM $MgCl_2$). PCR of samples was performed by incubation for 3 min at 94 °C and subsequent amplification by 30 cycles (denaturation: 45 s at 94 °C, annealing: 1 min at 56 °C, extension: 1 min at 72 °C) followed by a final extension at 72 °C for 7 min. PCR products (15 µl) were separated on a 1.5% agarose gel and checked by ethidium bromide staining. Nucleotide mixes, Taq polymerase and reaction buffer were obtained from Roche. The Northern blot signals were quantified by scanning densitometry, using the PC version of the public domain NIH Image program (Image PC, Scion, Frederick, Md., USA available on the internet at http://rsb.info.nih.gov/nih-image) and the density of SGK1 signals compared to the β-actin signals.

In situ hybridization

Rats were anaesthetized deeply with pentobarbitone sodium (40 mg/kg body weight) and perfused retrogradely through the distal abdominal aorta with 2% freshly depolymerized paraformaldehyde in PBS, pH 7.4, at a pressure of 180–200 mmHg for 3 min. Thereafter the brain was carefully dissected free and cut in half mid-sagittally. One half was immersion-fixed overnight in the same fixative and embedded in paraffin, the other half was incubated in 18% sucrose/1×PBS for 5 h at room temperature before being snap-frozen in liquid nitrogen-cooled isopentane. For in situ hybridization a 1.35 kb rat SGK-1 cDNA fragment was cloned into pBluescript (Stratagene). To generate sense and antisense riboprobes, the plasmid was digested with *Sal* I and *Not* I and then transcribed with T7 and T3 RNA polymerase, respectively. In vitro transcription was carried out according to the manufacturer's protocol (Roche). To improve the penetration of the probes during the in situ hybridization experiments, transcripts were subjected to partial alkaline hydrolysis to obtain fragments of a calculated average length of 250 nt. In situ hybridization using cryostat sections was carried out essentially as described previously [27]. Complete sagittal rat brain cryosections (5–7 µm thick) were transferred onto silane-coated glass slides, post-fixed in 4% paraformaldehyde/PBS for 15 min and washed in diethylpyrocarbonate (DEPC)-treated double-distilled water. Alternatively, deparaffinized brain sections (4 µm thick) were post-fixed in 4% paraformaldehyde/PBS for 10 min, rinsed 3 times in PBS and then treated with proteinase K (8 µg/ml in PBS) for 30 min at 37 °C. Following the digest with proteinase K, sections were washed briefly in PBS, fixed again for 1 min in 4% paraformaldehyde/PBS and washed in PBS. Thereafter, both cryostat and deparaffinized sections were processed similarly. To reduce background, slides were acetylated in triethanolamine-containing acetic anhydride, rinsed in PBS, dehydrated in 70, 80 and 95% ethanol and air-dried. Prehybridization and hybridization was performed as described previously [27]. Rat SGK1 sense and antisense probes were used at a concentration of 10 ng/µl in the hybridization mixture. Hybridization was carried out overnight at a temperature of 46 °C and followed by several stringent post-hybridization washes (the most stringent wash was in 0.2×SCC/50% formamide at 54 °C for 2 h). Immunological detection of the hybridized polyclonal alkaline phosphatase-coupled anti-Dig-antibody was performed by incubation with a polyclonal alkaline phosphatase-antibody (diluted 1:500 in blocking medium) for 2 h at room temperature followed by an overnight-incubation at 4 °C. The washed slices were covered with large amounts of a freshly prepared substrate solution containing 0.417 mM nitro blue tetrazolium chloride (NBT) pre-dissolved in 70% dimethylformamide; 0.406 mM 5-bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt (BCIP) pre-dissolved in 100% dimethylformamide and 1 mM levamisol. Subsequently, slides were kept at 4 °C in a moist chamber in the dark for usually 12–24 h. Colour reactions were monitored under the microscope and terminated by immersing the slides twice for 5 min in 100 mM TRIS-HCl, 1 mM EDTA, pH 8.0. After rinsing in PBS, sections were mounted in bicarbonate-buffered glycerol, pH 8.6 [27]. Control experiments included incubations with sense probes on alternate sections. These controls yielded completely negative results.

The cellular staining pattern of the hybridization signals for rSGK1 antisense probe in the cryostat sections was virtually identical to that in the respective paraffin sections. Because of the better histomorphological quality of paraffin sections, however, the latter were used for the photographical documentation of the in situ hybridization experiments.

Immunohistochemistry

Dehydrated and control rats were anaesthetized, the brains removed quickly, and small brain pieces immersion-fixed in 4% paraformaldehyde for at least 12 h. The tissue was subsequently cryoprotected in 30% sucrose. Sections (approx. 20 µm) were collected on gelatinized slides and stored at –40 °C until further processing. Before applying specific reagents, sections were rehydrated in PBS and pre-incubated for 1 h in 4% normal goat serum in PBS containing 0.3% Triton-X. Anti-SGK1 antibodies (diluted 1:500, [12]) were then applied alone or as a cocktail with one of the following cell-specific antibodies: anti-glial fibrilliary acidic protein [GFAP; monoclonal antibody (MAB)360, Chemicon, Hofheim, Germany 1:400] specific for astrocytes, anti-oligodendrocyte MAB1580 (Chemicon, clone RIP, 1:200) and anti-rat integrin αM[CD11b] (MAB1405, clone OX-42, Chemicon, 1:50) to label microglial cells. Sections were incubated with primary antibodies in a moist chamber overnight at 4 °C, all other reactions proceeded at room temperature. Antibodies were diluted in PBS with 1% DMSO and 0.3% Triton-X 100. Binding of anti-SGK1 antibodies was visualized with anti-rabbit antibodies either conjugated to CY-2 in single stains, or conjugated to Texas Red when combined with other stains. The cell type-specific monoclonal antibodies were detected with anti-mouse antibodies coupled to AlexaGreen (Molecular Probes, Eugene, Ore., USA). Secondary antibodies were applied as cocktails for 1.5 h. After several rinses, sections were incubated with Neurotrace 640 (fluorescent Nissl stain, Molecular Probes), rinsed again and cover-slipped. With each staining process we performed control stains by omitting the primary antibodies. In addition, specific staining was abolished by incubating the primary antibody in the presence of SGK1-peptide (10 µg/ml). Sections were analysed on a confocal laser-scanning microscope (Zeiss LSM 410) using an Ar laser at 488 nm and two He/Ne lasers at 543 and 633 nm excitation wavelengths with the appropriate filter sets. To avoid cross-talk between the emission signals only one laser was used at a time. Images were later merged in three different color channels. Control slides were analysed with the same gain and brightness settings to test for unspecific and background staining.

Two-electrode voltage clamp

Plasmid DNA of hSGK1 and mouse Kv1.3 were linearized with Not I and transcribed in vitro with T7 RNA polymerase in the presence of the cap analogue $m^7G(5')ppp(5')G$ at a concentration of 1 mM. Template cDNA was removed by digestion with RNase-free DNase I. The complementary RNA (cRNA) was purified by phenol/chloroform extraction followed by precipitation with 0.5 vol 7.5 M ammonium acetate and 2.5 vol ethanol to remove unincorporated nucleotides. The integrity of the transcripts was checked by denaturing agarose gel electrophoresis. Dissection of *Xenopus laevis* ovaries, collection and handling of the oocytes (stages V and VI) have been described in detail elsewhere [34]. Oocytes were injected with 0.125 ng cRNA of Kv1.3 plus H_2O or with 0.125 ng cRNA of Kv1.3 plus 7.5 ng cRNA of hSGK1 in 50 nl water per oocyte. Experiments were performed at room temperature 1–4 days after injection. Two-electrode voltage-clamp recordings were made at room temperature with voltage jumps from a holding potential of –80 mV to –60, –40, –20, 0, +20 and +40 mV for 15 s each with an interval of 45 s. Measurements were only started when there was no run-down of peak currents for at least 5 min. The data were filtered at 10 Hz and recorded with MacLab digital-to-analogue converter and software for data acquisition and analysis (AD Instruments, Castle Hill, Australia). The external control solution (superfusate/ND96) contained (in mM) 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES, pH 7.4. Where applicable, currents are given as means±SEM for *n* oocytes.

Statistical analysis

Data are expressed as arithmetic means±SEM. Data were analysed using Student's *t*-test or ANOVA, where applicable. $P<0.05$ was considered significant.

Results

Cerebral localization of SGK1

Northern blots of human brain (Fig. 1) showed hSGK1 to be expressed in all structures analysed. The most abundant expression was in the corpus callosum.

In situ hybridization of brain sections from dehydrated rats showed SGK1 mRNA mainly in the neurons of the hippocampus region (Fig. 2a, b higher magnification). Positive signals were also detected in the periventricular area (Fig. 2c, d). A small number of signals was observed scattered throughout all layers of the cortex (Fig. 2e). No hybridization signal was detected with a sense SGK1 probe (Fig. 2f). Immunohistochemical analysis showed that a few neurons in the hippocampal area CA3 of control brains (Fig. 3Aa, c) expressed SGK1 whereas in a similar section of dehydrated hippocampus (Fig. 3Ab, d) SGK1 expression was particularly high in large projection neurons. Additionally, the number of these SGK1 expressing cells seemed to be increased, as became apparent after counterstaining with a fluorescent Nissl stain (Fig. 3, compare blue and red color channels). Staining was strongest in the cytoplasm of the cell bodies and detectable in some dendrites of large neurons.

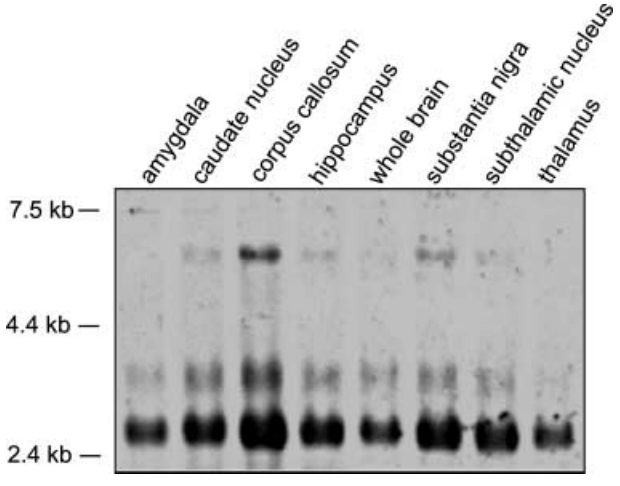

Fig. 1 Distribution of human serum- and glucocorticoid-dependent kinase (hSGK1) transcripts (2.5 kb) in human brain. PolyA$^+$RNA of distinct brain areas, available as a blotted nylon membrane, was hybridized with a digoxigenin (Dig)-labelled probe of hSGK1. A Dig-labelled RNA molecular weight marker determined the position of the bands (*kb* kilobases)

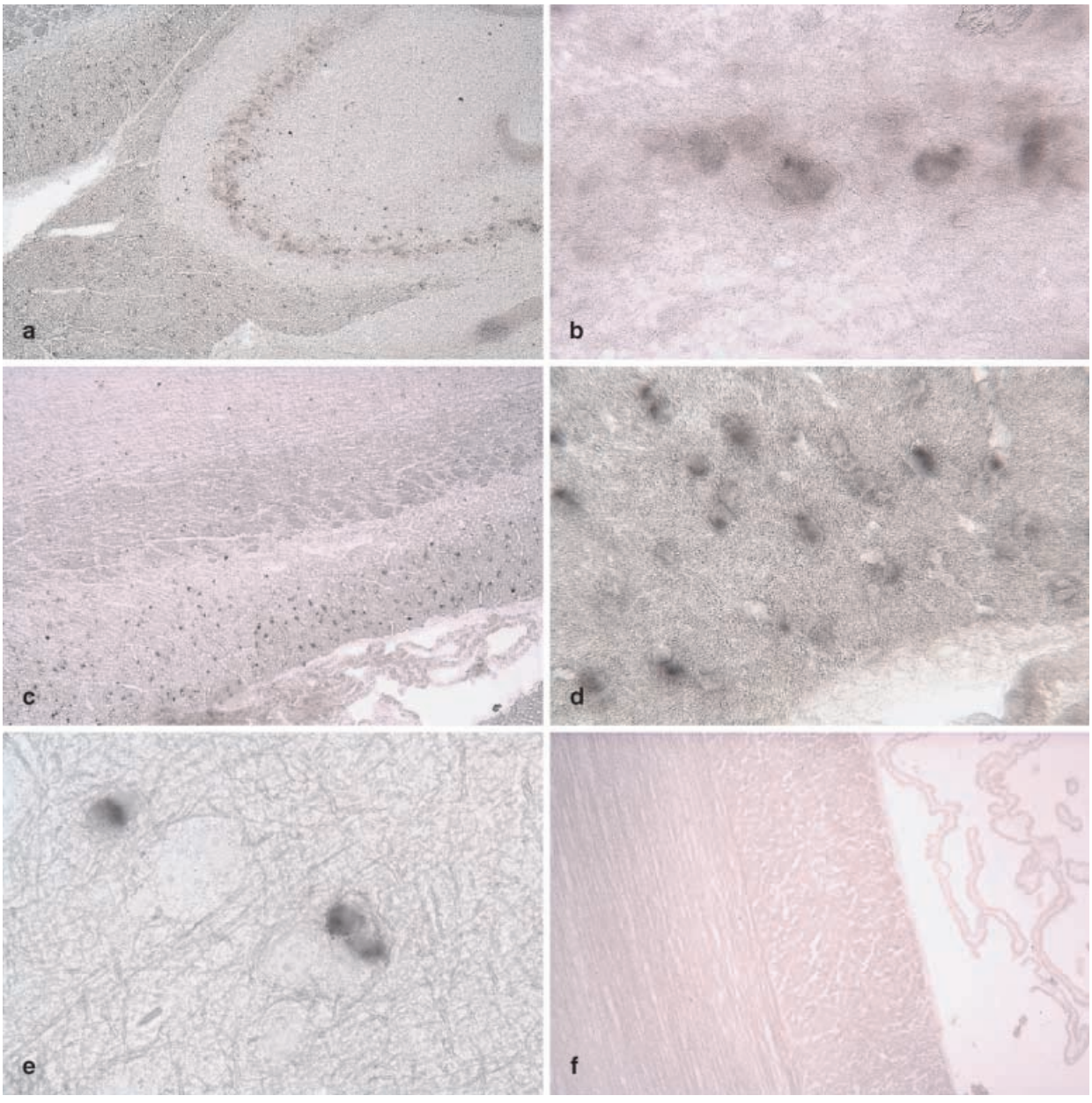

Fig. 2a–f Expression of SGK1 mRNA in the brain of dehydrated rats localized by in situ hybridization with an anti-sense SGK1 probe. Positive hybridization signals occurred mainly in neurons of the hippocampus region (**a**, higher magnification in **b**) and in the periventricular area (**c**, higher magnification in **d**), with scattered signals throughout all layers of the cortex (**e**). **f** Negative control (sense-SGK1 probe)

To test whether the kinase was also present in glial cells we performed double antibody stains for the presence of SGK1 and glial-specific markers in combination with the Nissl stain. In sections of dehydrated hippocampus astrocytes did not show any immunoreactivity to the SGK1-antibody (Fig. 3B) whereas many oligodendrocytes stained positive (Fig. 3C). A few microglial cells were SGK1-positive, the majority, however, were not (Fig. 3D).

Transcriptional regulation of SGK1

As shown in Fig. 4A, hSGK1 transcription in SKNSH human neuroblastoma cells increased significantly (1064± 227%, *n*=4) following enhancement of extracellular osmolarity by addition of 50 mmol/l NaCl for 2 h. As illustrated in Fig. 4B, SGK1 mRNA was significantly enhanced (by 166±20%, *n*=4) in the temporal lobe including hippocampus from dehydrated rats compared to transcripts from control rat brains. There was no significant alteration of β-actin mRNA expression.

Activation of the shaker K^+-channel Kv1.3 by co-expression of hSGK1 in *Xenopus laevis* oocytes

Xenopus oocytes expressing Kv1.3, but not oocytes injected with water, responded to cell membrane depolar-

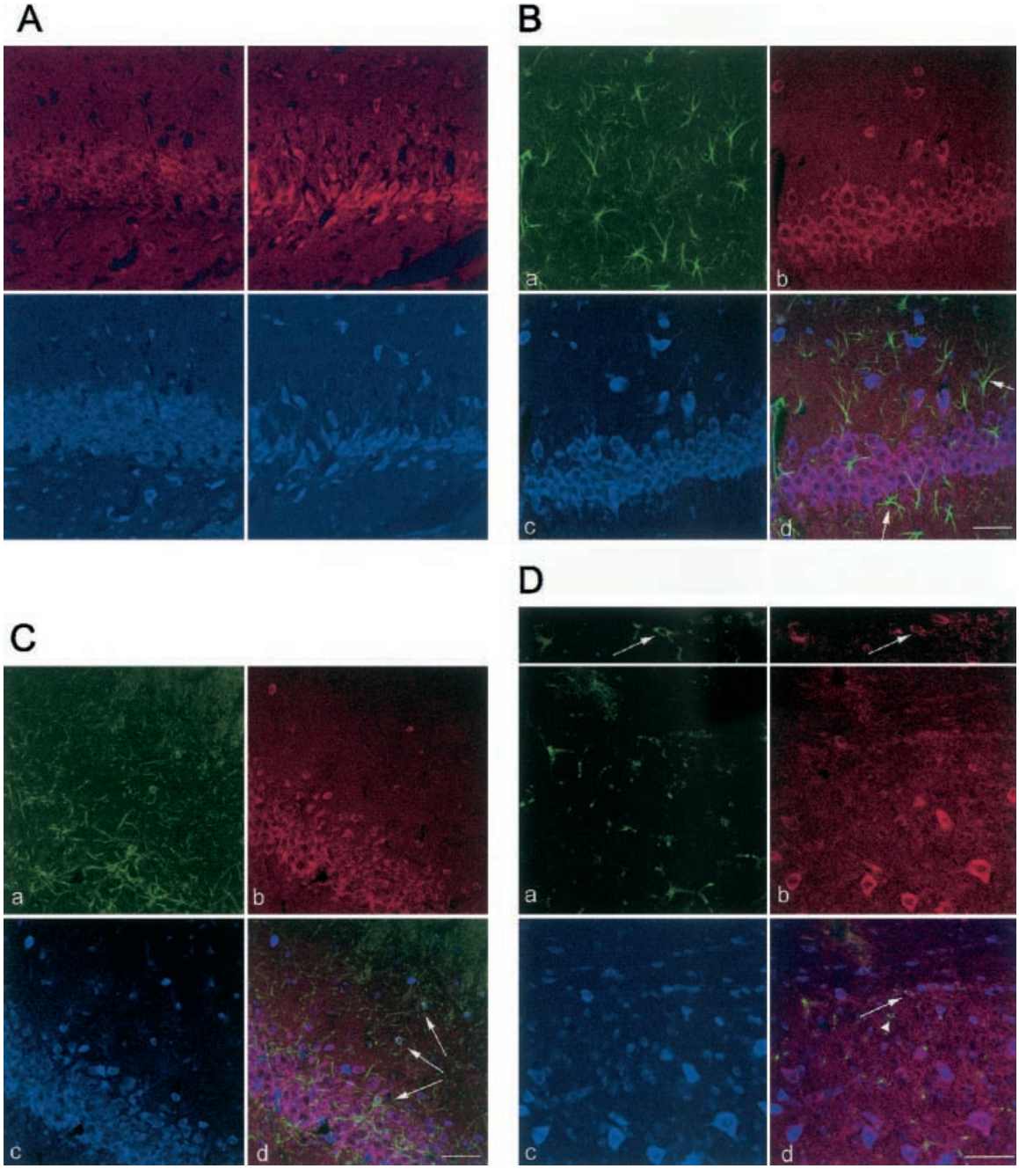

Fig. 3A–D Immunoreactivity of SGK1 in cryosections from the hippocampal region CA3 in brains from normal (**Ab,d**) and dehydrated rats (**Ab,d,B–D**). SGK1-immunoreactivity (*red*), fluorescent Nissl stain (*blue*), the respective cell-type-specific antibodies (*green*) and all three labels combined (*mixed colors*) are represented. Neurons are most prominently labelled by the Nissl stain and most of them are positive for SGK1 antibody stain as demonstrated in all *panels*. *Scale bars* 50 µm. **A** Increased SGK1 immunoreactivity in brain tissue derived from dehydrated rats (*b*, *d*) compared to control rats (*a*, *c*). **B** Double staining for glial fibrillary acidic protein (GFAP, specific for astrocytes) revealed no co-localization with SGK1. In *d*, the *arrows* indicate two astrocytes in which the cell bodies are detected by the Nissl stain but are clearly immuno-negative for SGK1 (*b* and *c*). **C** Double staining with an oligodendrocyte-specific marker (MAB1580) revealed immuno-positive cells which also showed SGK1-immunoreactivity (*arrows*). In *d*, some cells in the neuronal cell layer stained only with the blue Nissl stain, suggesting that not all neurons are positive for the SGK1 antibody. **D** Double staining with a microglial-specific antibody (anti-integrin) indicated a few cells positive for both integrin and SGK1. The cell indicated by the *arrow* in *d* is shown in higher magnification in the *small figures above*. *Below* the integrin-positive cell is a SGK1-negative microglial cell (*arrow head* in *d*). Some capillaries are also stained by the antibody

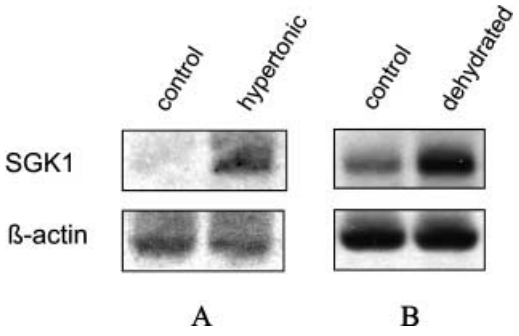

Fig. 4A, B Up-regulation of hSGK1 by hypertonicity in cells of a human neuroblastoma cell line (SKNSH) and in the brain of dehydrated rats. **A** Northern analysis of SKNSH cells (15 µg total RNA) showing increased hSGK1 transcription following stimulation with 50 mM NaCl for 2 h. **B** Total RNA (20 µg) of temporal lobe including hippocampal area of control and dehydrated rat brains was blotted followed by SGK1 detection. For comparison β-actin transcripts from the same cell or tissue samples are shown

ization with a rapidly inactivating outwards current as described previously (Fig. 5). The current was inhibited completely by the specific Kv1.3 inhibitor margatoxin (1 nM). In oocytes co-expressing Kv1.3 and hSGK1, peak currents were significantly larger than in oocytes expressing Kv1.3 alone (Fig. 5).

Discussion

The present study demonstrated that SGK1 is expressed in glial cells and neurons and that its expression can be up-regulated by osmotic shrinkage. SGK1 transcription in neural cells is thus apparently similarly sensitive to cell volume as it is in HepG2 liver cells [37], MDCK kidney cells [37], pancreatic CFPAC cells [19] macrophages [39], endothelial cells and fibroblasts [24]. Similarly, SGK1 in the mouse [3] and the shark [38] is up-

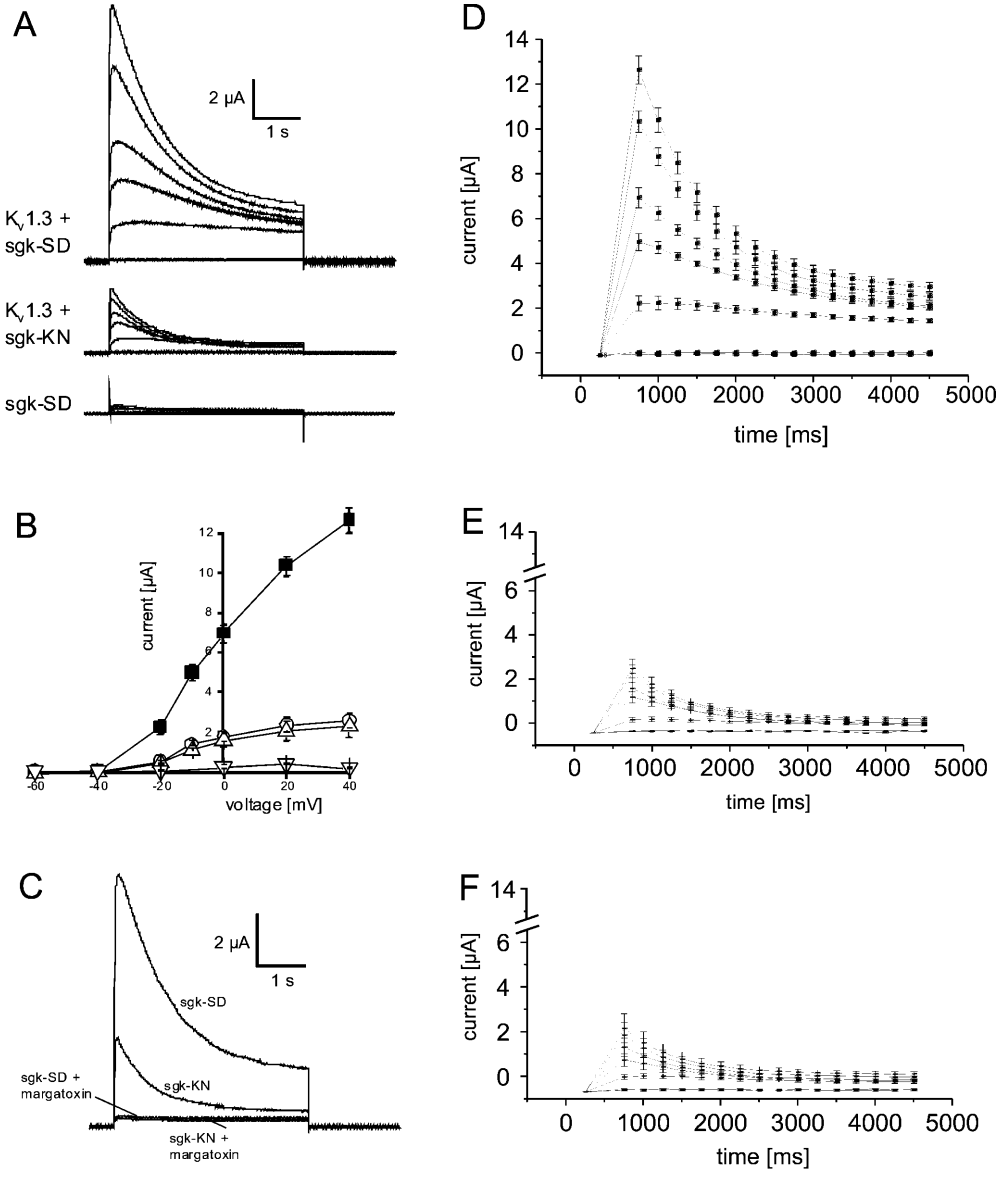

Fig. 5A–F SGK1-induced up-regulation of voltage-gated K^+ (Kv1.3) channels in *Xenopus laevis* oocytes **A** Original current traces recorded by the two-electrode voltage-clamp technique from oocytes co-injected with active kinase SGK1 S422D (SGK1-*SD*+*Kv1.3*), with inactive kinase SGK1 K127N (SGK1-*KN*+*Kv1.3*) and SGK1-SD+H_2O (SGK1-*SD*). Currents were evoked by depolarizing square pulses from –80 mV holding potential to voltages between –60 to +40 mV. **B** Current/voltage relation of the evoked peak currents as recorded in (**A**) from oocytes co-injected with SGK1-SD plus Kv1.3 (*squares*), SGK1-KN plus Kv1.3 (*circles*), H_2O plus Kv1.3 (*triangles*) or SGK1-SD plus H_2O (*inverted triangles*). Means±SEM; *n*=8. **C** Original traces from a (SGK1-SD+Kv1.3)- and a (SGK1-KN+Kv1.3)-co-injected oocyte, recorded in the presence and absence of margatoxin (1 nM). Currents were induced by depolarizing voltage steps from –80 mV to 0 mV. **D–F** Arithmetic means of the currents induced in Kv1.3-expressing oocytes co-injected with sgk-SD (**D**), SGK1-KN (**E**) or water (**F**)

regulated by osmotic cell shrinkage. The up-regulation is mediated by p38 kinase [3, 40]. SGK1 transcription is up-regulated also by aldosterone [6, 26], which, however, decreases following water deprivation [11] and thus cannot account for the increase of SGK1 transcription observed in the present study. As an increase of intracellular Ca^{2+} activity has also been shown to increase SGK1 transcription in pancreatic CFPAC [19] and human endothelial HuVEC [24] cells, activation of the neurons is expected to similarly enhance SGK1 transcription.

The present observations point further to a possible functional role of the kinase in influencing the regulation of neuroexcitability. As shown by the co-expression studies, the kinase markedly up-regulates the activity of Kv1.3, which is distributed widely in brain tissue [7, 9, 10, 20, 21, 22, 28, 33] and contributes to the repolarization following action potentials [28, 32].

The up-regulation of SGK1 in the dehydrated brain on the one hand, and the stimulating effect of SGK1 on Kv1.3 activity on the other, could indicate that SGK1 participates in alterations of neuroexcitability during dehydration. Neuronal excitation is modified by extracellular osmolarity, whereby an increase of plasma osmolarity decreases and a decrease of plasma osmolarity increases the susceptibility to epileptic seizures [1, 31]. A number of different mechanisms may contribute to altered excitability in dehydration. Neuronal excitability depends critically on the function of the glial cells [14], which control extracellular K^+ and neurotransmitter concentrations [15]. Both functions are compromized during glial cell swelling due to depolarization [16] and deranged transport of neurotransmitters across the cell membrane [17, 18]. Cell volume may influence neuronal excitability more directly by influencing intracellular [Cl^-] and/or modifying cell volume regulatory ion transport, such as ion channels and $Na^+K^+2Cl^-$ cotransport [2, 25]. Furthermore, alterations of extracellular osmolarity may modify excitability through alteration of the extracellular space [29, 31]. The present study does not provide conclusive evidence for an SGK1-mediated increase in K^+ channel activity during dehydration in vivo. Even though osmotic cell shrinkage has in some cells been shown to hyperpolarize the cell membrane [30], K^+ channels are activated by cell swelling rather than by cell shrinkage [23]. Whether or not enhanced K^+ channel activity in neurons participates in the decrease of neuroexcitability during dehydration thus remains uncertain. We cannot rule out that the effect of SGK1 on K^+ channels may be overridden by other mechanisms regulating K^+ channels during cell shrinkage. Moreover, as the SGK1 kinase modifies the activity of other ion channels [24, 35, 36] and transporters [24], its influence on neuroexcitability cannot be predicted with certainty. Nevertheless, SGK1 is a kinase that is highly sensitive to the hydration status of the animal and has the capacity to regulate ion channels pertinent for regulation of neuroexcitability.

In conclusion, hSGK1 is expressed in both neurons and glial cells and its expression is stimulated by cell shrinkage. The kinase is able to stimulate the activity of the neuronal voltage-gated Kv1.3 K^+-channel and may thus participate in the regulation of neuronal excitability.

Acknowledgements The authors are indebted to Dr. J. Schwab for supply of the SKNSH cells and Dr. S. Grissmer for providing the mouse Kv13 clone, they acknowledge the technical assistance of E. Faber, U. Hammacher and B. Noll and the meticulous preparation of the manuscript by Tanja Loch and Lejla Subasic. This study was supported by the Deutsche Forschungsgemeinschaft, Grant Nr. La 315/4-4 and La 315/5-1, and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (Centre for Interdisciplinary Clinical Research) 01 KS 9602. C.D. is supported by the Foundation pour la Recherche Médicale, France C.A.W. is a Feodor-Lynen Fellow of the Alexander-von-Humboldt Foundation, Germany.

References

1. Andrew RD (1991) Seizure and acute osmotic change: clinical and neurophysiological aspects. J Neurol Sci 101:7–18
2. Ballanyi K, Grafe P (1988) Cell volume regulation in the nervous system. Renal Physiol Biochem 11:142–157
3. Bell LM, Leong ML, Kim B, Wang E, Park J, Hemmings BA, Firestone GL (2000) Hyperosmotic stress stimulates promoter activity and regulates cellular utilization of the serum- and glucocorticoid-inducible protein kinase (Sgk) by a p38 MAPK-dependent pathway. J Biol Chem 275:25262–25272
4. Böhmer C, Wagner CA, Beck S, Moschen I, Melzig J, Werner A, Lin J-T, Lang F, Wehner F (2000) The shrinkage-activated Na^+ conductance of rat hepatocytes and its possible correlation to rENaC. Cell Physiol Biochem 10:187–194
5. Burg M, Kwon BED, Kültz D (1996) Osmotic regulation of gene expression. FASEB J 10:1598–1606
6. Chen SY, Bhargava A, Mastroberardino L, Meijer OC, Wang J, Buse P, Firestone GL, Verrey F, Pearce D (1999) Epithelial sodium channel regulated by aldosterone-induced protein sgk. Proc Natl Acad Sci USA 96:2514–2519
7. Coleman SK, Newcombe J, Pryke J, Dolly JO (1999) Subunit composition of Kv1 channels in human CNS. J Neurochem 73:849–858
8. De la Rosa DA, Zhang P, Naray-Fejes-Toth A, Fejes-Toth G, Canessa CM (1999) The serum and glucocorticoid kinase sgk increases the abundance of epithelial sodium channels in the plasma membrane of *Xenopus* oocytes. J Biol Chem 274: 37834–37839
9. Fadool DA (1998) Tyrosine phosphorylation downregulates a potassium current in rat olfactory bulb neurons and a cloned Kv1.3 channel. Ann NY Acad Sci 855:529–532
10. Fadool DA, Levitan IB (1998) Modulation of olfactory bulb neuron potassium current by tyrosine phosphorylation. J Neurosci 18:6126–137
11. Huang W, Lee SL, Arnason SS, Sjøquist M (1996) Dehydration natriuresis in male rats is mediated by oxytocin. Am J Physiol 270:R427–R433
12. Huber SM, Gamper N, Lang F (2001) Chloride conductance and volume-regulatory non-selective cation conductance in human red blood cell ghosts. Pflügers Arch 441:551–558
13. Imaizumi K, Tsuda M, Wanaka A, Tohyama M, Takagai T (1994) Differential expression of sgk mRNA, a member of ser/thr protein kinase gene family, in rat brain after injury. Mol Brain Res 26:189–196
14. Kempski O, Rosen S von, Weigt H, Staub F, Peters J, Baethmann A (1991) Glial ion transport and volume control. Ann NY Acad Sci 633:306–317
15. Kettenmann H, Faissner A, Trotter J (1996) Neuronglia interactions in homeostasis and degeneration. In: Greger R, Windhorst U (eds) Comprehensive human physiology. Springer, Berlin Heidelberg New York, pp 533–543
16. Kimelberg HK, O'Connor ER (1988) Swelling of astrocytes causes membrane potential depolarization. Glia 1:219–224

17. Kimelberg HK, Goderie SK, Higman S, Pang S, Waniewski RA (1990) Swelling-induced release of glutamate, aspartate, and taurine from astrocyte cultures. J Neurosci 10:1583–1591
18. Kimelberg HK, Rutledge E, Goderie S, Charniga C (1995) Astrocytic swelling due to hypotonic or high K^+ medium causes inhibition of glutamate and aspartate uptake and increases their release. J Cereb Blood Flow Metab 15:409–416
19. Klingel K, Wärntges S, Bock J, Wagner CA, Sauter M, Waldegger S, Kandolf R, Lang F (2000) Expression of the cell volume regulated kinase h-sgk in pancreatic tissue. Am J Physiol 279:G998–G1002
20. Klumpp DJ, Song EJ, Ito S, Sheng MH, Jan LY, Pinto LH (1995) The Shaker-like potassium channels of the mouse rod bipolar cell and their contributions to the membrane current. J Neurosci 15:5004–5013
21. Klumpp DJ, Song EJ, Pinto LH (1995) Identification and localization of K^+ channels in the mouse retina. Vis Neurosci 12:1177–1190
22. Koch RO, Wanner SG, Koschak A, Hanner M, Schwarzer C, Kaczorowski GJ, Slaughter RS, Garcia ML, Knaus HG (1997) Complex subunit assembly of neuronal voltage-gated K^+ channels. Basis for high-affinity toxin interactions and pharmacology. J Biol Chem 272:27577–27581
23. Lang F, Busch GL, Ritter M, Voelkl H, Waldegger S, Gulbins E, Haeussinger D (1998) Functional significance of cell volume regulatory mechanisms. Physiol Rev 78:247–306
24. Lang F, Klingel K, Wagner CA, Stegen C, Wärntges S, Friedrich B, Lanzendörfer M, Melzig J, Moschen I, Steuer S, Waldegger S, Sauter M, Paulmichl M, Gerke V, Risler T, Gamba G, Capasso G, Kandolf R, Hebert SC, Massry SG, Bröer S (2000) Deranged transcriptional regulation of cell volume sensitive kinase hSGK in diabetic nephropathy. Proc Natl Acad Sci USA 94:8157–8162
25. Misgeld U, Deisz RA, Dodt HU, Lux HD (1986) The role of chloride transport in postsynaptic inhibition of hippocampal neurons. Science 232:1413–1415
26. Naray-Fejes-Toth A, Canessa C, Cleaveland ES, Aldrich G, Fejes-Toth G (1999) Sgk is an aldosterone-induced kinase in the renal collecting duct. Effects on epithelial Na^+ channels. J Biol Chem 274:16973–16978
27. Obermüller N, Gretz N, Kriz W, Reilly RF, Witzgall R (1998) The swelling-activated chloride channel ClC-2, the chloride channel ClC-3, and ClC-5, a chloride channel mutated in kidney stone disease, are expressed in distinct subpopulations of renal epithelial cells. J Clin Invest 101:635–642
28. Ohno-Shosaku T, Kim I, Sawada S, Yamamoto C (1996) Presence of the voltage-gated potassium channels sensitive to charybdotoxin in inhibitory presynaptic terminals of cultured rat hippocampal neurons. Neurosci Lett 207:195–198
29. Osehobo EP, Andrew RD (1993) Osmotic effects upon the theta rhythm, a natural brain oscillation in the hippocampal slice. Exp Neurol 124:192–199
30. Ritter M, Steidl M, Lang F (1991) Inhibition of ion conductances by osmotic shrinkage of Madin-Darby canine kidney cells. Am J Physiol 261:C602–C607
31. Traynelis SF, Dingledine R (1989) Role of extracellular space in hyperosmotic suppression of potassium-induced electrographic seizures. J Neurophysiol 61:927–938
32. Turrigiano GG, Marder E, Abbott LF (1996) Cellular short-term memory from a slow potassium conductance. J Neurophysiol 75:963–966
33. Veh RW, Lichtinghagen R, Sewing S, Wunder F, Grumbach IM, Pongs O (1995) Immunohistochemical localization of five members of the Kv1 channel subunits: contrasting subcellular locations and neuron-specific co-localizations in rat brain. Eur J Neurosci 7:2189–2205
34. Wagner CA, Friedrich B, Setiawan I, Lang F, Bröer S (2000) The use of *Xenopus laevis* oocytes for the functional characterization of heterologously expressed membrane proteins. Cell Physiol Biochem 10:1–12
35. Wagner CA, Bröer A, Albers A, Gamper N, Lang F, Bröer S (2000) The heterodimeric amino acid transporter 4F2hc/LAT1 is associated in *Xenopus* oocytes with a non-selective cation channel that is regulated by the serine/threonine kinase sgk1. J Physiol (Lond) 526:35–46
36. Wagner CA, Ott M, Klingel K, Beck S, Melzig J, Friedrich B, Wild K-N, Bröer S, Moschen I, Albers A, Waldegger S, Tümmler B, Egan ME, Geibel JP, Kandolf R, Lang F (2001) Effects of the serine/threonine kinase SGK1 on the epithelial Na^+ channel (ENaC) and CFTR. Implications for cystic fibrosis. Cell Physiol Biochem 11:209–218
37. Waldegger S, Barth P, Raber G, Lang F (1997) Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. Proc Natl Acad Sci USA 94:4440–4445
38. Waldegger S, Barth P, Forrest JN Jr, Greger R, Lang F (1998) Cloning of sgk serine-threonine protein kinase from shark rectal gland – a gene induced by hypertonicity and secretagogues. Pflügers Arch 436:575–580
39. Waldegger S, Klingel K, Barth P, Sauter M, Lanzendörfer M, Kandolf R, Lang F (1999) *h-sgk* Serine-threonine protein kinase gene as early transcriptional target of TGF-β in human intestine. Gastroenterology 116:1081–1088
40. Waldegger S, Gabrysch S, Barth P, Fillon S, Lang F (2000) h-sgk Serine-threonine protein kinase as transcriptional target of p38/MAP kinase pathway in HepG2 human hepatoma cells. Cell Physiol Biochem10:203–208
41. Webster MK, Goya L, Firestone GL (1993) Immediate-early transcriptional regulation and rapid mRNA turnover of a putative serine/threonine protein kinase. J Biol Chem 268:11482–11485
42. Webster MK, Goya L, Ge Y, Maiyar AC, Firestone GL (1993) Characterization of sgk, a novel member of the serine/threonine protein kinase family which is transcriptionally induced by glucocorticoids and serum. Mol Cell Biol 13:2031–2040