ELSEVIER

Comparative Biochemistry and Physiology Part A 130 (2001) 367–376

CBP

www.elsevier.com/locate/cbpa

Review

Serum- and glucocorticoid-dependent kinase, cell volume, and the regulation of epithelial transport ☆

S. Fillon[a], S. Wärntges[a], J. Matskevitch[a], I. Moschen[a], I. Setiawan[a], N. Gamper[a], Y.X. Feng[a], C. Stegen[a], B. Friedrich[a], S. Waldegger[a], S. Bröer[a], C.A. Wagner[a], S.M. Huber[a], K. Klingel[b], A. Vereninov[c], F. Lang[a,*]

[a] *Department of Physiology, University of Tübingen, D-72076 Tübingen, Germany*
[b] *Department of Molecular Pathology, University of Tübingen, D-72076 Tübingen, Germany*
[c] *Institute of Cytology, Russian Academy of Sciences, St. Petersburg 194064, Russia*

Received 23 November 2000; received in revised form 21 February 2001; accepted 22 February 2001

Abstract

Ample pharmacological evidence points to a role of kinases in the regulation of cell volume. Given the limited selectivity of most inhibitors, however, the specific molecules involved have remained largely elusive. The search for cell volume regulated genes in liver HepG2 cells led to the discovery of the human serum- and glucocorticoid-dependent serine/threonine kinase hsgk1. Transcription and expression of hsgk1 is markedly and rapidly upregulated by osmotic and isotonic cell shrinkage. The effect of osmotic cell shrinkage on hsgk1 is mediated by p38 kinase. Further stimuli of hsgk1 transcription include glucocorticoids, aldosterone, TGF-β1, serum, increase of intracellular Ca^{2+} and phorbolesters, whereas cAMP downregulates hsgk1 transcription. The hsgk1 protein is expressed in several epithelial tissues including human pancreas, intestine, kidney, and shark rectal gland. Co-expression of hsgk1 with the renal epithelial Na^+-channel ENaC or the $Na^+/K^+/2Cl^-$-cotransporter NKCC2 (BSC1) in *Xenopus oocytes*, accelerates insertion of the transport proteins into the cell membrane and thus, stimulates channel or transport activity. Thus, hsgk1 participates in the regulation of transport by steroids and secretagogues increasing intracellular Ca^{2+}-activity. The stimulation of hsgk1 transcription by TGF-β1 may further bear pathophysiological relevance.

Keywords: Intracellular Ca^{2+} activity; sgk; Cell volume regulation; Pancreas; Intestine; Kidney; Epithelial Na^+ channel; $Na^+/K^+/2Cl^-$-cotransporter

☆ This paper was originally presented at a symposium dedicated to the memory of Marcel Florkin, held within the ESCPB 21st International Congress, Liège, Belgium, July 24–28, 2000.

* Corresponding author. Tel.: +49-7071-2972-194; fax: +49-7071-2956-18.
E-mail address: florian.lang@uni-tuebingen.de (F. Lang).

1095-6433/01/$ - see front matter
PII: S1095-6433(01)00422-6

1. Introduction

Transcellular epithelial transport leads to alterations of cell volume, whenever entry and exit of osmotically active substances are not precisely balanced (for review see Lang et al., 1998a). To avoid excessive alterations of cell volume, the respective transport systems must be under control of or paralleled by cell volume regulatory mechanisms.

The major cell volume regulatory mechanisms comprise activation of KCl co-transport, K^+ channels, anion, Cl^- and/or osmolyte channels during cell swelling (for review see Fürst et al., 2000; Junankar and Kirk, 2000; Kinne et al., 2000; Lang et al., 1998b; Lauf and Adragna, 2000; Nilius et al., 2000; Valverde et al., 2000), as well as $Na^+/K^+/2Cl^-$ co-transport, Na^+/H^+ exchanger and Na^+ channels during cell shrinkage (for review see Böhmer et al., 2000; Wehner et al., 2000; Lang et al., 1998b; Szászi et al., 2000). The corresponding release or entry of ions with the obliged water accomplishes regulatory cell volume decrease (RVD) or increase (RVI), respectively. Furthermore, the cellular generation or accumulation of osmolytes contributes to the maintenance of cell volume constancy (for review see Burg, 1995; Pasantes-Morales et al., 2000).

The cell volume regulatory transport systems are under the control of a wide variety of signaling mechanisms including activation of protein kinases (Burg, 2000; Hoffmann, 2000a,b; Kinnunen, 2000a,b; Lepple-Wienhues et al., 2000; Papakonstanti et al., 2000; Schliess and Häussinger, 2000; Tinel et al., 2000; van der Wijk et al., 2000; Weiergräber and Häussinger, 2000). The present brief review will concentrate on properties and role of a serine/threonine kinase, which has been cloned as a cell volume regulated gene (Waldegger et al., 1997) and may apparently participate in the regulation of transport systems. The human cell volume-sensitive kinase is highly homologous to the serum and glucocorticoid-dependent kinase (sgk) previously cloned from rat mammary tumor cells (Webster et al., 1993) and has thus, been labeled hsgk. From shark rectal gland the shark sgk (ssgk) has been cloned and again found to be highly homologous to the mammalian kinases (Waldegger et al., 1998). As the isoforms (sgk2 and sgk3) have been cloned in the meanwhile (Kobayashi et al., 1999), the appropriate labeling is now sgk1 or hsgk1.

2. Localized distribution of hsgk1

As shown by Northern blot analysis, hsgk1 is expressed in all human tissues studied, including pancreas, liver, heart, lung, skeletal muscle, placenta, kidney and brain (Waldegger et al., 1997). Within the tissues, hsgk1 is not expressed in all cell types. In pancreas particularly high transcript levels are found in acinar cells (Klingel et al., 2000). As shown in Fig. 1, marked transcription of hsgk1 is found within enterocytes, whereas crypt cells are virtually mRNA free (Waldegger et al., 1999). In the kidney, transcript levels are under appropriate conditions (see below) maybe high in distal nephron and thick ascending limb epithelial cells (Lang et al., 2000).

Apart from epithelial cells, high transcript levels are found in macrophages (Waldegger et al., 1999) and some renal mesangial cells (Lang et al., 2000), which are similarly phagocytosing cells.

3. Regulation of hsgk1

Originally, the sgk1 has been cloned from rat mammary tumor cells as a glucocorticoid inducible kinase (Webster et al., 1993). The human isoform was cloned as a cell volume sensitive gene (Waldegger et al., 1997). Transcription of hsgk1 is not only upregulated by hyperosmotic cell shrinkage, but as well by isotonic cell shrinkage elicited by simultaneous inhibition of the Na^+/H^+ exchanger and the $Na^+/K^+/2Cl^-$ co-transporter (Waldegger et al., 1997). The transcriptional upregulation is rapid, and significant increases of transcript levels are observed within 30 min of exposure of the cells to hypertonic environment (Waldegger et al., 1997).

The transcriptional upregulation of (h)sgk1 is mediated by p38 kinase, which is activated upon cell shrinkage and in turn is required for upregulation of hsgk1 (Bell et al., 2000; Waldegger et al., 2000).

Further stimulators of (h)sgk1 transcription include mineralocorticoids (Chen et al., 1999; Naray-Fejes-Toth et al., 1999; Shigaev et al., 2000) and TGF-β1 (Waldegger et al., 1999; Lang et al., 2000). Moreover, hsgk1 transcription is also markedly upregulated by excessive extracellular glucose concentrations (Lang et al., 2000), an effect blunted in the presence of neutralizing

Fig. 1. Transcription of hsgk1 in intestine as shown by in situ hybridization. Highest levels of hsgk1 mRNA are found in apical villus enterocytes. The transition from hsgk1 expressing enterocytes to virtually hsgk1 mRNA-free crypt cells is sharp. Tissue specimens were fixed in 4% paraformaldehyde, 0.1 M sodium phosphate buffer (pH 7.2) for 4 h and embedded in paraffin. Tissue sections (4 μm) were dewaxed and hybridized basically as described (Hohenadl et al., 1991; Kandolf et al., 1987; Klingel et al., 1992). The mixture contained either the ^{35}S-labelled RNA antisense or sense control hSGK1 probe (Waldegger et al. 1999) (500 ng/ml) in hybridization buffer (10 mM Tris–HCl, pH 7.4, 50% (v/v) deionized formamide, 600 mM NaCl, 1 mM EDTA, 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.05% bovine serum albumin, 10% dextrane sulfate, 10 mM dithiothreitol, denatured sonicated salmon sperm DNA at 200 μg/ml, and rabbit liver tRNA at 100 μg/ml. Hybridization with RNA probes proceeded at 42°C for 18 h. Slides were then washed as described (Hohenadl et al., 1991; Kandolf et al., 1987; Klingel et al., 1992) followed by 1 h at 55°C in 2 × standard saline citrate. Non-hybridized single-stranded RNA probes were digested by RNAse A (20 μg/ml) in 10 mM Tris–HCl, pH 8.0, 0.5 M NaCl for 30 min at 37°C. Tissue slide preparations were autoradiographed (Klingel et al., 1992) and stained with hematoxylin/eosin.

antibodies against TGF-β 1, 2 and 3 (Lang et al., 2000).

An increase of cytosolic Ca^{2+} activity by calcium ionophore ionomycin (1 μM) strongly upregulates hsgk1 transcription, as shown in endothelial (Lang et al., 2000) and pancreatic epithelial (Klingel et al., 2000) cells. Moreover, the stimulating effect of excessive glucose concentrations is blunted in the presence of Ca^{2+} channel blocker nifedipine (Lang et al., 2000), again pointing to a role of Ca^{2+} in the transcriptional regulation of hsgk1.

Transcription of hsgk1 is further stimulated by phorbol esters and is inhibited by protein kinase inhibitor staurosporine (Klingel et al., 2000). Thus, protein kinase C may be involved in the transcriptional regulation of the kinase.

Cytosolic Ca^{2+} and possibly protein kinase C may mediate a regulation of sgk1 transcription by secretagogues. Indeed, transcription of the ssgk1 is stimulated in shark rectal gland by vasoactive intestinal polypeptide and carbachol (Waldegger et al., 1998), secretagogues in this model epithelium for Cl^- secretion (Bleich et al., 1998; Forrest et al., 1983; Greger et al., 1986, 1998, 1999a,b; Thiele et al., 1998; Warth et al., 1998a,b). Surprisingly, however, hsgk1 transcription is downregulated by cAMP (Klingel et al., 2000), which acts as a second messenger during stimulation of Cl^- secretion by several secretagogues (Greger et al., 1984).

Although transcription is upregulated by hyperosmotic incubation (Waldegger et al., 1997; Bell et al., 2000), the activity of the expressed sgk1 protein is reduced by exposure of the cells to hypertonic sorbitol (Kobayashi and Cohen, 1999). This observation casts some doubt on the role of sgk1 in regulatory cell volume increase, which would require activation of the kinases during osmotic cell shrinkage. At this point, more information is needed on the interplay between cell volume and sgk1 activity. The skg1 kinase is activated by H_2O_2 and insulin-like growth factor IGF-1 (Kobayashi and Cohen, 1999; Park et al., 1999). The signaling of IGF-1 to sgk1 involves PI3-kinase and subsequent activation of the serine/threonine kinase PDK1 (Kobayashi and Cohen, 1999; Park et al., 1999).

4. Role of hsgk1 in transport regulation

As illustrated in Fig. 2, co-expression of (h)sgk1 with the rat epithelial Na^+ channel ENaC in *Xenopus* oocytes leads to a strong increase of its activity (Chen et al., 1999; De la Rosa et al., 1999; Lang et al., 2000; Naray-Fejes-Toth et al., 1999). The amiloride sensitive current reflecting ENAC activity is in ENAC expressing oocytes significantly higher at co-expression with the active kinase (sgkSD) as compared to oocytes co-expressing the inactive mutant (sgkKN). Elimination of the only hsgk1 consensus site of ENaC by site directed mutagenesis does not affect the stimulating effect of hsgk1 on the channel, suggesting that it is not the channel itself which is phosphorylated by the kinase (Fig. 2b).

Furthermore, hsgk1 stimulates the $Na^+/K^+/2Cl^-$ co-transporter BSC-1 (NKCC2) of the thick ascending limb. The effect on both ENaC and BSC-1 requires a catalytically activated kinase as an inactive mutant of hsgk1, does not stimulate these two transport proteins. Only the α-subunit of ENaC contains a consensus site for

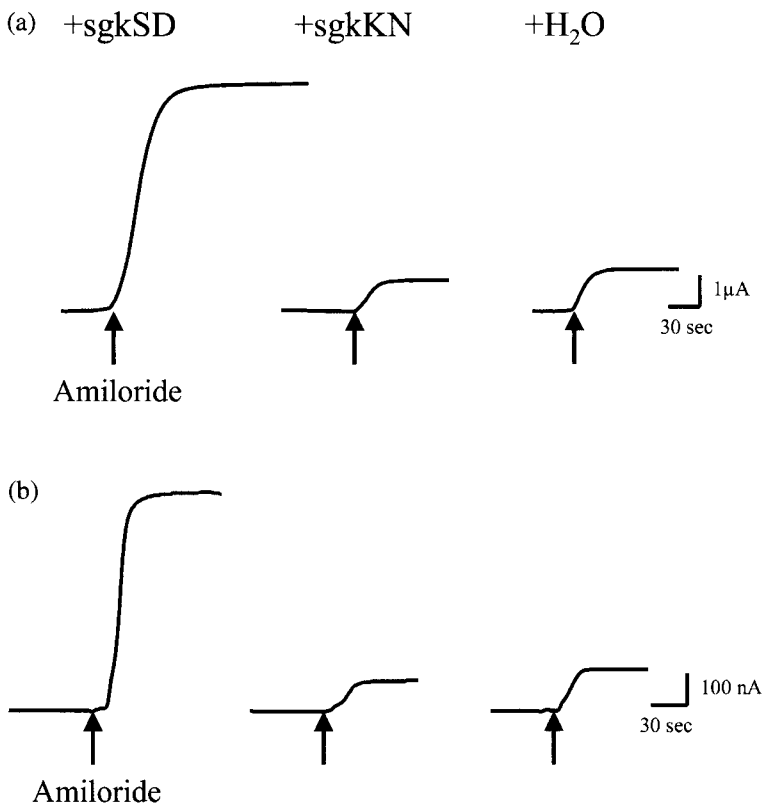

Fig. 2. Stimulation of ENaC by co-expression with hsgk1 in *Xenopus laevis* oocytes. If α-, β-, and γ-subunits of ENaC were injected together with the active kinase sgkKD, the amiloride sensitive current was much larger than in oocytes co-injected with the inactive sgk mutant (sgkKN) or with ENaC alone ($+H_2O$). Oocytes (stages V and VI) were isolated by collagenase treatment as described (Wagner et al., 2000) and allowed to recover overnight. Plasmid DNA of the α-, β-, and γ-subunits of wild type rat epithelial Na^+ channel (ENaC) (Canessa et al., 1993, 1994) or the mutant α^{S622A}-, β-, and γ-rENaC devoid of the hsgk1 consensus site, were linearized with Not I and transcribed in vitro with T7 RNA polymerase in the presence of the cap analog $m^7G(5')ppp(5')G$ at a concentration of 1 mM. The active hSGK1 mutant $hSGK1^{[S422D]}$ (sgkSD), and the inactive hSGK1 mutant $hSGK1^{[K127N]}$ (sgkKN), were transcribed from a PCR product containing the T7 promotor. cRNA encoding the α- and β-subunits of rat epithelial Na^+ channel (ENaC) were linearized with BglII and transcribed in vitro with SP6 polymerase. 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 volumes of 7.5 M ammonium acetate and 2.5 volumes of ethanol to remove unincorporated nucleotides. The integrity of the transcript was checked by denaturing agarose gel electrophoresis. Two-electrode voltage clamp measurements were performed to determine amiloride-sensitive currents (50 μM amiloride) at a holding potential of −80 mV, 1–3 days after the injection. (a) Original traces of amiloride sensitive currents of wildtype α-, β-, and γ−rENaC + hsgk1SD/hsgk1KN/H_2O; and (b) original traces of amiloride sensitive currents of mutant α^{S622A}-, β-, and γ-rENaC + hsgk1SD/hsgk1KN/H_2O.

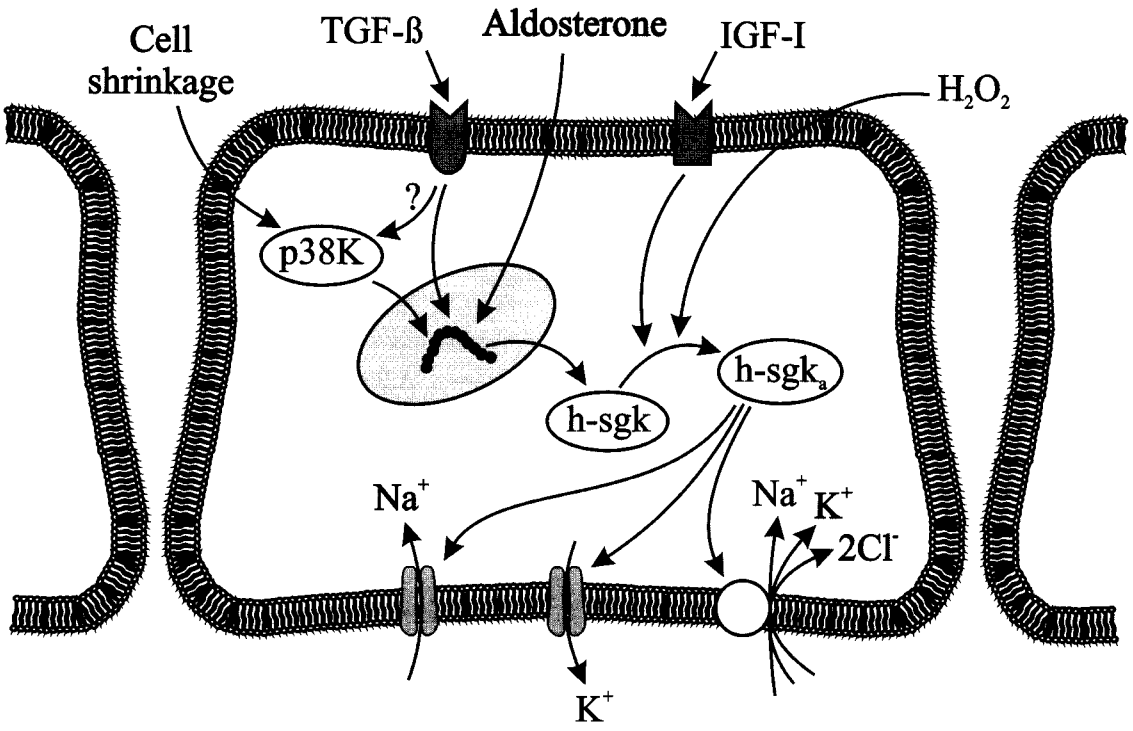

Fig. 3. Tentative model illustrating the role of hsgk1 in the regulation of epithelial transport.

phosphorylation by hSGK (Kobayashi and Cohen, 1999). Mutation of this site in the ENaC protein does, however, not eliminate the stimulatory effect of hSGK1. Thus, it is unlikely that hsgk1 activates ENaC by direct phosphorylation. Instead, hsgk1 apparently stimulates trafficking of its target proteins into the cell membrane (De la Rosa et al., 1999). We and others have tested for an effect of sgk1 on a wide variety of further transport proteins. While preliminary evidence points to activation of Kv1.3 by hsgk1 (Friedrich et al., 2001), other K^+ channels tested including ROMK2 (Chen et al., 1999) proved to be insensitive to co-expression of sgk1. We have not seen upregulation of the osmolyte transporters BGT1, TAUT and SMIT when co-expressed with hsgk1 in *Xenopus* oocytes (J. Matskevitch et al., unpublished observation). This does not conclusively rule out regulation of those carriers by hsgk1 in a different cellular background, but it demonstrates the specificity of the trafficking effect observed in *Xenopus laevis* oocytes. The domains mediating this specificity are yet unknown. A clear picture of the regulatory role of sgk1 must await the identification of its immediate targets.

Given the regulation of sgk1 by aldosterone and secretagogues the stimulation of epithelial ion transport is one of the major physiological functions of skg1. Most likely it operates as an early gene in the stimulation of Na^+ reabsorption by mineralocorticoids. Its role in the regulation of secretion is less clear. The abundant expression of sgk1 in acinar cells of the pancreas and shark rectal gland strongly suggests a role in regulation of Cl^- secretion.

Fig. 3 summarizes the role of h-sgk in transport regulation: The protein is upregulated by cell shrinkage, TGFβ and aldosterone, it is activated by IGF1 and oxidative stress and it modulates transport by insertion of Na^+ channels, $Na^+/K^+/2Cl^-$ co-transport, and possibly K^+ channels into the cell membrane.

5. Putative pathophysiological role of hsgk

The upregulation of hsgk1-transcription by TGF-β1 (Lang et al., 2000; Waldegger et al., 1999) probably bears pathophysiological relevance, as TGF-β1 is considered a crucial pathophysiological component in the generation of fibrosing disease (Border and Noble, 1994) as oc-

curs in diabetic nephropathy (Cohen et al., 1998; Hoffman et al., 1998; Kumar et al., 1999; Lang et al., 2000; Reeves and Andreoli, 2000; Sharma and Ziyadeh, 1995; Sharma et al., 1997; Ziyadeh and Han, 1997; Ziyadeh and Sharma, 1995; Ziyadeh et al., 2000), glomerulonephritis (Bitzer et al., 1998; Border et al., 1990, 1995; Border and Noble, 1993, 1994, 1997; Ketteler et al., 1995; Yamamoto et al., 1996), liver cirrhosis (Annoni et al., 1992; Bayer et al., 1998; De Bleser et al., 1997; Okuno et al., 1999; Roulot et al., 1999; Tiggelman et al., 1995; Tsushima et al., 1999; Zhang et al., 1999), inflammatory bowel disease (Babyatsky et al., 1996; Rugtveit et al., 1997), and lung fibrosis (Agarwal et al., 1996; Coker et al., 1997; Eickelberg et al., 1999; Korfhagen et al., 1994; Maniscalco and Campbell, 1994; Phan and Kunkel, 1992). One of the factors which are thought to contribute to fibrosis is the promotion of cell hypertrophy by TGF-β1 (Fine et al., 1985; Ling et al., 1995; Sharma et al., 1996), which has similarly been observed in diabetes mellitus (Burg and Kador, 1988; McManus et al., 1995; Morocutti et al., 1997). The stimulation of Na^+-reabsorption via activation of ENaC and BSC-1 might increase cell volume and hence, could contribute to TGF-β-induced cell hypertrophy (Lang et al., 1998a,b).

Within the kidney, stimulation of the $Na^+/K^+/2Cl^-$ co-transporter in thick ascending limb (Lang et al., 2000) is expected to decrease the delivery of NaCl to the macula densa and thus, through triggering of tubuloglomerular feedback enhance glomerular filtration rate (Schnermann and Briggs, 1982). Hyperfiltration is in turn, one of the early and deleterious derangements of renal function in diabetic nephropathy (Hostetter et al., 1982). Indeed, reduced sensitivity of tubuloglomerular feedback has been shown to participate in the hyperfiltration of diabetic nephropathy (Blantz et al., 1991; Vallon and Richter, 1998).

The stimulation of the renal Na^+ channel and $Na^+/K^+/2Cl^-$ co-transporter should lead to renal Na^+ retention, which in turn, should favor the development of arterial hypertension.

Acknowledgements

The authors acknowledge the meticulous preparation of the manuscript by Tanja Loch. The study was supported by the Deutsche Forschungsgemeinschaft, Nr. La 315/4-3 and La 315/5-1, 436 RUS 113/488/0 (R), RFBR 98-04-041125, the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (Center for Interdisciplinary Clinical Research) 01 KS 9602, and the Fortüne program of the University of Tübingen (Nr. 302).

References

Agarwal, A.R., Goldstein, R.H., Lucey, E., Ngo, H.Q., Smith, B.D., 1996. Cell-specific expression of the alpha 1(I) collagen promoter CAT transgene in skin and lung: a response to TGF-beta subcutaneous injection and bleomycin endotracheal instillation. J. Cell. Biochem. 63, 135–148.

Annoni, G., Weiner, F.R., Zern, M.A., 1992. Increased transforming growth factor-beta 1 gene expression in human liver disease. J. Hepatol. 14, 259–264.

Babyatsky, M.W., Rossiter, G., Podolsky, D.K., 1996. Expression of transforming growth factors alpha and beta in colonic mucosa in inflammatory bowel disease. Gastroenterology 110, 975–984.

Bayer, E.M., Herr, W., Kanzler, S. et al., 1998. Transforming growth factor-beta 1 in autoimmune hepatitis: correlation of liver tissue expression and serum levels with disease activity. J. Hepatol. 28, 803–811.

Bell, L.M., Leong, M.L.L., Kim, B., Wang, E., Park, J., Hemmings, B.A., Firestone, G.L., 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. (in press).

Bitzer, M., Sterzel, R.B., Bottinger, E.P., 1998. Transforming growth factor-beta in renal disease. Kidney Blood Press. Res. 21, 1–12.

Blantz, R.C., Peterson, O.W., Thomson, S.C., 1991. Tubuloglomerular feedback responses to acute contralateral nephrectomy. Am. J. Physiol. 260, F749–F756.

Bleich, M., Warth, R., Thiele, I., Greger, R., 1998. pH-regulatory mechanisms in in vitro perfused rectal gland tubules of *Squalus acanthias*. Pflügers Arch. 436, 248–254.

Böhmer, C., Wagner, C.A., Beck, S. et al., 2000. The shrinkage-activated Na^+ conductance of rat hepatocytes and its possible correlation to rENaC. Cell Phys. Biochem. 10, 187–194.

Border, W.A., Noble, N.A., 1993. Cytokines in kidney disease: the role of transforming growth factor-beta. Am. J. Kidney. Dis. 22, 105–113.

Border, W.A., Noble, N.A., 1994. Transforming growth factor beta in tissue fibrosis. New. Engl. J. Med. 331, 1286–1292.

Border, W.A., Noble, N.A., 1997. TGF-beta in kidney fibrosis: a target for gene therapy. Kidney Int. 51, 1388–1396.

Border, W.A., Noble, N.A., Ketteler, M., 1995. TGF-beta: a cytokine mediator of glomerulosclerosis and a target for therapeutic intervention. Kidney Int. 49, S59–S61.

Border, W.A., Okuda, S., Languino, L.R., Sporn, M.B., Ruoslahti, E., 1990. Suppression of experimental glomerulonephritis by antiserum against transforming growth factor beta 1. Nature 346, 371–374.

Burg, M.B., 1995. Molecular basis of osmotic regulation. Am. J. Physiol. 268, F983–F996.

Burg, M.B., 2000. Macromolecular crowding as a cell volume sensor. Cell Physiol. Biochem. 10, 251–256.

Burg, M.B., Kador, P.F., 1988. Sorbitol, osmoregulation, and the complications of diabetes. J. Clin. Invest. 81, 635–640.

Canessa, C.M., Horisberger, J.D., Rossier, B.C., 1993. Epithelial sodium channel related to proteins involved in neurodegeneration. Nature 361, 467–470.

Canessa, C.M., Schild, L., Buell, G. et al., 1994. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. Nature 367, 463–467.

Chen, S.Y., Bhargava, A., Mastroberardino, et al., 1999. Epithelial sodium channel regulated by aldosterone-induced protein sgk. Proc. Natl. Acad. Sci. USA 96, 2514–2519.

Cohen, M.P., Sharma, K., Guo, J., Eltayeb, B.O., Ziyadeh, F.N., 1998. The renal TGF-beta system in the db/db mouse model of diabetic nephropathy. Exp. Nephrol. 6, 226–233.

Coker, R.K., Laurent, G.J., Shahzeidi, S. et al., 1997. Transforming growth factors-beta 1, -beta 2, and -beta 3 stimulate fibroblast procollagen production in vitro, but are differentially expressed during bleomycin-induced lung fibrosis. Am. J. Pathol. 150, 981–991.

De Bleser, P.J., Niki, T., Rogiers, V., Geerts, A., 1997. Transforming growth factor-beta gene expression in normal and fibrotic rat liver. J. Hepatol. 26, 886–893.

De la Rosa, D.A., Zhang, P., Naray-Fejes-Toth, A., Fejes-Toth, G., Canessa, C.M., 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.

Eickelberg, O., Kohler, E., Reichenberger, F. et al., 1999. Extracellular matrix deposition by primary human lung fibroblasts in response to TGF-beta1 and TGF-beta3. Am. J. Physiol. 276, L814–L824.

Fine, L.G., Holley, R.W., Nasri, H., Badie-Dezfooly, B., 1985. BSC-1 growth inhibitor transforms a mitogenic stimulus into a hypertrophic stimulus for renal proximal tubular cells: relationship to Na^+/H^+ antiport activity. Proc. Natl. Acad. Sci. USA 82, 6163–6166.

Forrest J.N. Jr., Wang, F., Beyenbach, K.W., 1983. Perfusion of isolated tubules of the shark rectal gland. Electrical characteristics and response to hormones. J. Clin. Invest. 72, 1163–1167.

Friedrich, B., Wagner, C.A., Stegen, C., Beck, S., Moschen, I., Bröer, S., Lang, F., 2001. The aldosterone-regulated serine/threonine kinase SGK1 is a potent stimulator of the voltage gated K^+-channel Kv1.3. Kidney Blood Press. Res. (in press).

Fürst, J., Jakab, M., König, M. et al., 2000. Structure and function of the ion channel ICln. Cell Physiol. Biochem. 10, 329–334.

Greger, R., Bleich, R., Warth, R., Thiele, I., Forrest, J.N., 1999a. The cellular mechanisms of Cl^- secretion induced by C-type natriuretic peptide (CNP). Experiments on isolated in vitro perfused rectal gland tubules of *Squalus acanthias*. Pflügers Arch. 438, 15–22.

Greger, R., Heitzmann, D., Hug, M.J., Hoffmann, E.K., Bleich, M., 1999b. The $Na^+/2Cl^-/K^+$ co-transporter in the rectal gland of *Squalus acanthias* is activated by cell shrinkage. Pflügers Arch. 438, 165–176.

Greger, R., Schlatter, E., Gögelein, H., 1986. Sodium chloride secretion in rectal gland of dogfish *Squalus acanthias*. News Physiol. Sci. 1, 134–136.

Greger, R., Schlatter, E., Wang, F., Forrest J.N. Jr., 1984. Mechanism of NaCl secretion in rectal gland tubules of spiny dogfish (*Squalus acanthias*). III. Effects of stimulation of secretion by cyclic AMP. Pflügers Arch. 402, 376–384.

Greger, R., Thiele, I., Warth, R., Bleich, M., 1998. Does stimulation of NaCl secretion in in vitro perfused rectal gland tubules of *Squalus acanthias* increase membrane capacitance? Pflügers Arch. 436, 538–544.

Hoffman, B.B., Sharma, K., Zhu, Y.Q., Ziyadeh, F.N., 1998. Transcriptional activation of transforming growth factor-beta 1 in mesangial cell culture by high glucose concentration. Kidney Int. 54, 1107–1116.

Hoffmann, E.K., 2000a. Intracellular signalling involved in volume regulatory decrease. Cell Physiol. Biochem. 10, 273–288.

Hoffmann, E.K., 2000b. Volume sensing and signal transduction. Comp. Biochem. Physiol. 126A, S68.

Hohenadl, C., Klingel, K., Mertsching, J., Hofschneider, P.H., Kandolf, R., 1991. Strand-specific detection of enteroviral RNA in myocardial tissue by in situ hybridization. Mol. Cell. Probes 5, 11–20.

Hostetter, T.H., Rennke, H.G., Brenner, B.M., 1982. The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. Am. J. Med. 72, 375–380.

Junankar, P.R., Kirk, K., 2000. Organic osmolyte chan-

nels: a comparative view. Cell Physiol. Biochem. 10, 355–360.

Kandolf, R., Ameis, D., Kirschner, P., Canu, A., Hofschneider, P.H., 1987. PH. In situ detection of enteroviral genomes in myocardial cells by nucleic acid hybridization: an approach to the diagnosis of viral heart disease. Proc. Natl. Acad. Sci. USA 84, 6272–6276.

Ketteler, M., Noble, N.A., Border, W.A., 1995. Transforming growth factor-beta and angiotensin II: the missing link from glomerular hyperfiltration to glomerulosclerosis? Annu. Rev. Physiol. 57, 279–295.

Kinne, R.K.H., Tinel, H., Kipp, H., Kinne-Saffran, E., 2000. Regulation of sorbitol efflux in different renal medullary cells: similarities and diversities. Cell Physiol. Biochem. 10, 371–378.

Kinnunen, P.K.J., 2000a. Lipid bilayers as osmotic response elements. Cell Physiol. Biochem. 10, 243–250.

Kinnunen, P.K.J., 2000b. Lipid bilayers as osmotic response elements. Cell Physiol. Biochem. 10, 243–250.

Klingel, K., Hohenadl, C., Canu, A. et al., 1992. Ongoing enterovirus-induced myocarditis is associated with persistent heart muscle infection: quantitative analysis of virus replication, tissue damage, and inflammation. Proc. Natl. Acad. Sci. USA 89, 314–318.

Klingel, K., Wärntges, S., Bock, J., Wagner, C.A., 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(5), G998–G1002.

Kobayashi, T., Cohen, P., 1999. Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2. Biochem. J. 339, 319–328.

Kobayashi, T., Deak, M., Morrice, N., Cohen, P., 1999. Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. Biochem. J. 344, 189–197.

Korfhagen, T.R., Swantz, R.J., Wert, S.E. et al., 1994. Respiratory epithelial cell expression of human transforming growth factor-alpha induces lung fibrosis in transgenic mice. J. Clin. Invest. 93, 1691–1699.

Kumar, J.M., Brooks, D.P., Olson, B.A., Laping, N.J., 1999. Sgk, a putative serine/threonine kinase, is differentially expressed in the kidney of diabetic mice and humans. J. Am. Soc. Nephrol. 10, 2488–2494.

Lang, F., Busch, G.L., Ritter, M. et al., 1998a. Functional significance of cell volume regulatory mechanisms. Physiol. Rev. 78, 247–306.

Lang, F., Lepple-Wienhues, A., Paulmichl, M., Szabo, I., Siemen, D., Gulbins, E., 1998b. Ion channels, cell volume and apoptotitc cell death. Cell. Physiol. Biochem. 8, 285–292.

Lang, F., Klingel, K., Wagner, C.A. et al., 2000. Deranged transcriptional regulation of cell volume sensitive kinase hSGK in diabetic nephropathy. Proc. Natl. Acad. Sci. USA 94, 8157–8162.

Lauf, P.K., Adragna, N.C., 2000. K–Cl co-transport: properties and molecular mechanism. Cell Physiol. Biochem. 10, 341–354.

Lepple-Wienhues, A., Szabò, I., Wieland, U., Heil, L., Gulbins, E., Lang, F., 2000. Tyrosine kinases open lymphocyte chloride channels. Cell Physiol. Biochem. 10, 307–312.

Ling, H., Vamvakas, S., Busch, G.L. et al., 1995. Suppressing role of transforming growth factor-β1 on cathepsin activity in cultured tubule cells. Am. J. Physiol. 269, F911–F917.

Maniscalco, W.M., Campbell, M.H., 1994. Transforming growth factor-beta induces a chondroitin sulfate/dermatan sulfateproteoglycan in alveolar type II cells. Am. J. Physiol. 266, L672–L680.

McManus, M.L., Churchwell, K.B., Strange, K., 1995. Regulation of cell volume in health and disease. New. Engl. J. Med. 333, 1260–1266.

Morocutti, A., Earle, K.A., Rodemann, H.P., Viberti, G.C., 1997. Premature cell ageing and evolution of diabetic nephropathy. Diabetologia 40, 244–246.

Naray-Fejes-Toth, A., Canessa, C., Cleaveland, E.S., 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.

Nilius, B., Eggermont, J., Droogmans, G., 2000. The endothelial volume-regulated anion channel, VRAC. Cell Physiol Biochem 10, 313–320.

Okuno, M., Sato, T., Kitamoto, T. et al., 1999. Increased 9,13-di-*cis*-retinoic acid in rat hepatic fibrosis: implication for a potential link between retinoid loss and TGF-beta mediated fibrogenesis in vivo. J. Hepatol. 30, 1073–1080.

Papakonstanti, E.A., Vardaki, E.A., Stournaras, C., 2000. Actin cytoskeleton: a signaling sensor in cell volume regulation. Cell Physiol. Biochem. 10, 257–264.

Park, J., Leong, M.L., Buse, P., Maiyar, A.C., Firestone, G.L., Hemmings, B.A., 1999. Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. EMBO J. 18, 3024–3033.

Pasantes-Morales, H., Franco, R., Torres-Marquez, M.E., Hernández-Fonseca, K., Ortega, A., 2000. Amino acid osmolytes in regulatory volume decrease and isovolumetric regulation in brain cells: contribution and mechanisms. Cell Physiol. Biochem. 10, 361–370.

Phan, S.H., Kunkel, S.L., 1992. Lung cytokine production in bleomycin-induced pulmonary fibrosis. Exp. Lung Res. 18, 29–43.

Reeves, W.B., Andreoli, T.E., 2000. Transforming growth factor β contributes to progressive diabetic nephropathy. Proc. Natl. Acad. Sci. USA 97, 7667–7669.

Roulot, D., Sevcsik, A.M., Coste, T., Strosberg, A.D., Marullo, S., 1999. Role of transforming growth factor beta type II receptor in hepatic fibrosis: studies of human chronic hepatitis C and experimental fibrosis in rats. Hepatology 29, 1730–1738.

Rugtveit, J., Nilsen, E.M., Bakka, A., Carlsen, H., Brandtzaeg, P., Scott, H., 1997. Cytokine profiles differ in newly recruited and resident subsets of mucosal macrophages from inflammatory bowel disease. Gastroenterology 112, 1493–1505.

Schliess, F., Häussinger, D., 2000. Cell hydration and insulin signalling. Cell Physiol. Biochem. 10, 403–408.

Schnermann, J., Briggs, J., 1982. Concentration-dependent sodium chloride transport as the signal in feedback control of glomerular filtration rate. Kidney Int. 12, S82–S89.

Sharma, K., Ziyadeh, F.N., 1995. Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-beta as a key mediator. Diabetes 44, 1139–1146.

Sharma, K., Jin, Y., Guo, J., Ziyadeh, F.N., 1996. Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. Diabetes 45, 522–530.

Sharma, K., Ziyadeh, F.N., Alzahabi, B. et al., 1997. Increased renal production of transforming growth factor-beta(1) in patients with type II diabetes. Diabetes 46, 854–859.

Shigaev, A., Asher, C., Latter, H., Garty, H., Reuveny, E., 2000. Regulation of sgk by aldosterone and its effects on the epithelial Na^+ channel. Am. J. Physiol. 278 (4), F613–F619.

Szászi, K., Grinstein, S., Orlowski, J., Kapus, A., 2000. Regulation of the epithelial Na^+/H^+ exchanger isoform by the cytoskeleton. Cell Physiol. Biochem. 10, 265–272.

Thiele, I., Warth, R., Bleich, M., Waldegger, S., Lang, F., Greger, R., 1998. Osmotically -induced conductance and capacitance changes in in vitro perfused rectal gland tubules of *Squalus acanthias*. Kidney Blood Press. Res. 21, 317–324.

Tiggelman, A.M., Boers, W., Linthorst, C., Sala, M., Chamuleau, R.A., 1995. Collagen synthesis by human liver (myo)fibroblasts in culture: evidence for a regulatory role of IL-1 beta, IL-4, TGF beta and IFN gamma. J. Hepatol. 23, 307–317.

Tinel, H., Kinne-Saffran, E., Kinne, R.K.H., 2000. Calcium signalling during RVD of kidney cells. Cell Physiol. Biochem. 10, 297–302.

Tsushima, H., Kawata, S., Tamura, S. et al., 1999. Reduced plasma transforming growth factor-beta1 levels in patients with chronic hepatitis C after interferon-alpha therapy: association with regression of hepatic fibrosis. J. Hepatol. 30, 1–7.

Vallon, V., Richter, K., 1998. Glomerular hyperfiltration in experimental Diabetes Mellitus: potential role of tubular reabsorption. Kidney Blood Press. Res. 21, 105.

Valverde, M.A., Vázquez, E., Muñoz, F.J. et al., 2000. Murine CFTR channel and its role in regulatory volume decrease of small intestine crypts. Cell Physiol. Biochem. 10, 321–328.

van der Wijk, T., Tomassen, S.F.B., de Jonge, H.R., Tilly, B.C., 2000. Signalling mechanisms involved in volume regulation of intestinal epithelial cells. Cell Physiol. Biochem. 10, 289–296.

Wagner, C.A., 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.

Waldegger, S., Barth, P., Forrest J.N. 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.

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.

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. Biochem. 10, 203–208.

Waldegger, S., Klingel, K., Barth, P. et al., 1999. h-sgk Serine–threonine protein kinase gene as early transcriptional target of TGF-β in human intestine. Gastroenterology 116, 1081–1088.

Warth, R., Bleich, M., Thiele, I., Lang, F., Greger, R., 1998a. Regulation of the $Na^+2Cl^-K^+$ co-transporter in in vitro perfused rectal gland tubules of *Squalus acanthias*. Pflügers Arch. 436, 521–528.

Warth, R., Thiele, I., Bleich, M., Greger, R., 1998b. The role of cytosolic Ca^{2+} in the secretion of NaCl in isolated in vitro perfused rectal gland tubules of *Squalus acanthias*. Pflügers Arch. 436, 133–140.

Webster, M.K., Goya, L., Ge, Y., Maiyar, A.C., Firestone, G.L., 1993. Characterization of sgk, a novel

member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. Mol. Cell. Biol. 13, 2031–2040.

Wehner, F., Böhmer, C., Heinzinger, H., van den Boom, F., Tinel, H., 2000. The hypertonicity-induced Na^+ conductance of rat hepatocytes: physiological significance and molecular correlate. Cell Physiol. Biochem. 10, 335–340.

Weiergräber, O., Häussinger, D., 2000. Hepatocellular hydration: signal transduction and functional implications. Cell Physiol. Biochem. 10, 409–416.

Yamamoto, T., Noble, N.A., Cohen, A.H. et al., 1996. Expression of transforming growth factor-beta isoforms in human glomerular diseases. Kidney Int. 49, 461–469.

Zhang, L.P., Takahara, T., Yata, Y. et al., 1999. Increased expression of plasminogen activator and plasminogen activator inhibitor during liver fibrogenesis of rats: role of stellate cells. J. Hepatol. 31, 703–711.

Ziyadeh, F.N., Han, D.C., 1997. Involvement of transforming growth factor-beta and its receptors in the pathogenesis of diabetic nephropathy. Kidney Int. 60, S7–S11.

Ziyadeh, F.N., Hoffman, B.B., Han, D.C. et al., 2000. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal anti-transforming growth factor-β antibody in db/db diabetic mice. Proc. Natl. Acad. Sci. USA 97, 8015–8020.

Ziyadeh, F.N., Sharma, K., 1995. Role of transforming growth factor-beta in diabetic glomerulosclerosis and renal hypertrophy. Kidney Int. 51, S34–S36.