REGULATION OF SGK SERINE/THREONINE PROTEIN KINASE TRANSCRIPTION BY SECRETAGOGUES AND TONICITY IN RECTAL GLAND OF *SQUALUS ACANTHIAS*

Siegfried Waldegger[1], Petra Barth[1], John Forrest[2], Rainer Greger[3], Florian Lang[1]
[1] Physiologisches Institut, Eberhard-Karls-Universität, 72076 Tübingen, Germany
[2] Department of Internal Medicine, Yale University, New Haven CT 06510, USA
[3] Physiologisches Institut, Albert-Ludwigs-Universität, 79104 Freiburg, Germany

Recently, the cell volume regulated sgk (serum- and glucocorticoid dependent) serine/threonine-protein kinase was cloned from the human hepatoma cell line HepG2 (Waldegger, S. et al., *Proc. Natl. Acad. Sci. USA* 94(9): 4440-4445, 1997). The sgk serine-threonine protein kinase was shown to be a cell shrinkage induced gene in many different mammalian cell lines. Since chloride secretion is associated with cell shrinkage and since sgk-mRNA is highly expressed in secretory organs like the pancreas, the question arose, whether stimulation of epithelial chloride secretion is associated with transcriptional activation of sgk kinase in the shark rectal gland.

The shark (*Squalus acanthias*) sgk kinase was identified by a combination of degenerate PCR- and RACE techniques (Waldegger, S. et al., *Eur. J. Physiol.*, submitted). Two alternatively spliced transcripts were identified and termed sgk-1 and sgk-2. Sgk-1 showed an over all sequence identity of 85% with human and rat sgk protein. The open reading frame of sgk-2 cDNA predicted a protein of 595 amino acids, thus surpassing the sgk-1 protein by 162 amino acids. Alignment of sgk-2 with sgk-1 protein proved sequence identity from - referring to sgk-1 - amino acid position 25 to 433. The additional amino acids of sgk-2 formed a new N-terminus, which showed no sequence homology to any of the known protein kinases. The following sequence features, which characterize serine-threonine protein kinases, were found in both sgk-1 and sgk-2 protein: the ATP-binding domain (Aa 106-138 and Aa 267-299 in sgk-1 and sgk-2, respectively) and the serine-threonine protein kinase active-site signature (Aa 220-232 and Aa 381-393 in sgk-1 and sgk-2, respectively). Interestingly, a tyrosine kinase phosphorylation site within the ATP binding domain of the shark sgk proteins is highly conserved between *S. acanthias*, rat and man.

The distribution of sgk RNA in different tissues from *S. acanthias* was analyzed. For this purpose a multiple tissue Northern blot containing RNAs from heart, rectal gland, kidney, brain, intestine, testis, liver and gills was probed with a shark sgk cDNA probe. To gain simultaneously information on quantitative differences between sgk-1 and sgk-2 expression, a cDNA probe was derived from homologous sequences of sgk-1 and sgk-2 (corresponding to nucleotides 701-919 and 1336-1554 of sgk-1 and sgk-2 cDNA, respectively). High expression levels were detected in kidney, intestine, liver, and heart. The expression in rectal gland, brain, and gills was lower, and the lowest level was detected in testis. Almost equal amounts of 2.6 kb sgk-1 and 3.3 kb sgk-2 transcripts were identified in heart and rectal gland. In kidney and brain sgk-2 transcripts were more dominant. In intestine, testis, liver, and gills only sgk-1 transcripts were detectable.

The influence of extracellular tonicity and stimulators of chloride secretion on sgk transcript levels was investigated. Slices of shark rectal gland were incubated for 8 hours in shark Ringer's solution at 12 °C. For hypertonic stimulation, 100 mmol/l NaCl was added to the control solution. Hypotonic stimulation was achieved by removal of 100 mmol/l NaCl from the control solution. Chloride secretion was induced by addition of carbachol (10 μM) or vasoactive intestinal polypeptide (VIP, 10 nM). Whereas removal of NaCl had no effect on sgk transcript levels, hypertonic exposure resulted in a two-fold increase of sgk transcript levels. A five-fold increase of sgk transcript levels was observed after activation of chloride secretion by carbachol and VIP.

The present data demonstrate the existence of the evolutionary highly conserved sgk protein kinase in shark rectal gland and indicate a transcriptional regulation of the sgk gene in response to secretagogues and alterations of extracellular tonicity.

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