Molecular characterization of volume-sensitive SKCa channels in human liver cell lines

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Molecular characterization of volume-sensitive SKCa channels in human liver cell lines. Am J Physiol Gastrointest Liver Physiol 282: G116–G122, 2002.—In human liver, Ca2+-dependent changes in membrane K+ permeability play a central role in coordinating functional interactions between membrane transport, metabolism, and cell volume. On the basis of the observation that K+ conductance is partially sensitive to the bee venom toxin apamin, we aimed to assess whether small-conductance Ca2+-sensitive K+ (SKCa) channels are expressed endogenously and contribute to volume-sensitive K+ efflux and cell volume regulation. We isolated a full-length 2,140-bp cDNA (hSK2) highly homologous to rat brain rSK2 cDNA, including the putative apamin-sensitive pore domain, from a human liver cDNA library. Identical cDNAs were isolated from primary human hepatocytes, human HuH-7 hepatoma cells, and human Mz-ChA-1 cholangiocarcinoma cells. Transduction of Chinese hamster ovary cells with a recombinant adenovirus encoding the hSK2-green fluorescent protein fusion construct resulted in expression of functional apamin-sensitive K+ channels. In Mz-ChA-1 cells, hypotonic (15% less sodium glutamate) exposure increased K+ current density (1.9 ± 0.2 to 37.5 ± 7.1 pA/pF; P < 0.001). Apamin (10–100 nM) inhibited K+ current activation and cell volume recovery from swelling. Apamin-sensitive SKCa channels are functionally expressed in liver and biliary epithelia and likely contribute to volume-sensitive changes in membrane K+ permeability. Accordingly, the hSK2 protein is a potential target for pharmacological modulation of liver transport and metabolism through effects on membrane K+ permeability.

hepatocyte; cholangiocyte; cell volume; apamin

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stress in liver and biliary cell lines (22, 23). Moreover, there appear to be ~1,700 apamin binding sites per liver cell, although more than one target protein may be involved (7, 13). On the basis of these observations, the purpose of these studies was to assess whether apamin-sensitive $SK_{Ca}$ channels are expressed in liver epithelia and to evaluate whether they contribute to cell volume regulation and modulation of volume-sensitive liver functions.

**EXPERIMENTAL PROCEDURES**

Reagents. Apamin, charybdotoxin, and barium chloride were obtained from Sigma (St. Louis, MO). Calmidazolium and 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA)-AM were purchased from Calbiochem (San Diego, CA). Other chemicals were obtained from Sigma.

Cell models. Hepatocytes and cholangiocytes represent the primary epithelial cell types in liver. For in vitro studies, human HuH-7 hepatoma cells and Mz-ChA-1 cholangiocarcinoma cells were utilized as models for these different cell types since they have been shown to retain many functions and differentiated features of primary cells (17). Mz-ChA-1 cells also express an apamin-sensitive $K^+$ conductance that can be activated during metabolic stress (22). Chinese hamster ovary (CHO) cells were used for cDNA expression. All cells were grown in MEM containing 5% FCS at 37°C in 5% CO₂.

Isolation and characterization of $SK_{Ca}$ cDNAs. cDNAs were isolated from a human liver cDNA library (SuperScript, Gibco-BRL, Gaithersburg, MD) using PCR and from human liver, primary human hepatocyte, human Mz-ChA-1 cholangiocarcinoma cell, and human HuH-7 hepatoma cell mRNA using RT-PCR. Sense and antisense primers were synthesized using brain 5K sequence obtained from GeneBank. A high-fidelity $Pfu$ DNA polymerase (Stratagene) was used for all reactions. Using $Taq$ polymerase (GIBCO-BRL), we performed 5’- and 3’-rapid amplification of cDNA ends (RACE) RT-PCR. cDNAs were directly inserted into the pcR II-TOPO plasmid vector (Invitrogen) for sequencing and further manipulation.

Northern analysis. Poly(A) RNA (3 µg/lane) was denatured, fractionated by electrophoresis through a 1% agarose-formaldehyde gel, and transferred to a Magna Nylon membrane (MSI, Westboro, MA). Prehybridization was performed at 42°C for 2 h in 5 × SSC (750 mM NaCl and 75 mM sodium citrate), 50% formamide, 1X Denhardt’s solution, 1% SDS, 50 mM Tris, pH 7.5, 5 mM EDTA, 100 µg/ml salmon sperm DNA, and 50 µg/ml tRNA. cDNA probes for hSK2 (complete sequence) were labeled by random priming with [$\alpha$-32P]dCTP (GIBCO-BRL). The blot was subsequently hybridized overnight at 42°C with [$\alpha$-32P]-labeled hSK2 probe, washed twice at 42°C for 10 min with 2 × SSC and 0.1% SDS, twice at 42°C for 30 min with 0.1 × SSC and 0.1% SDS, and once at 60°C for 30 min with 0.1 × SSC and 0.1% SDS. The blot was then exposed to X-ray film (Biomax MS, Amersham Pharmacia Biotech).

Development of adenovirus expression vector. The full-length hSK2 cDNA without the stop codon was inserted in frame with green fluorescent protein (GFP) into the shuttle plasmid pACCMV under the control of the cytomegalovirus (CMV) major immediate early promoter. The hSK2-GFP fusion construct (GFP added to the extreme COOH terminus) was cotransfected with BstB1-digested Ad5d327Bst-β-gal, and the corresponding protein complex into HEK-293 cells using calcium chloride precipitation. Transfected cells were incubated for 7 days. Viruses were harvested from the cells by freezing and thawing, and recombinant adenovirus encoding the hSK2-GFP fusion protein was plaque purified twice with screening for fluorescence (19). Transduction of CHO cells with adenovirus (10 µl of 5 × 10³ plaque forming units/ml) led to >50% cellular fluorescence by day 2. For controls, cells were transduced with the same recombinant adenovirus expressing GFP alone. Protein expression was regulated by exposure to hydroxyurea to inhibit adenoviral replication.

Western analysis. After being washed in PBS, cells were solubilized in 5 × PAGE buffer (5% SDS, 25% sucrose, 50 mM Tris, 5 mM EDTA, 5% β-mercaptoethanol, and protease inhibitor cocktail). Equimolar samples (20 µg/lane) were assayed for GFP content by Western blotting using GFP monoclonal antibody (Clontech) (14).

Measurement of $K^+$ currents. Whole cell currents were measured using patch-clamp recording techniques (9). Studies were performed at room temperature (22–25°C) 24–48 h after plating of cells on 35-mm collagen-covered plates. Immediately before study, the medium was replaced with an NaCl-rich extracellular solution (see below). Cells were viewed through an inverted phase-contrast microscope using Hoffman optics at a magnification of ×600 (Olympus IMT-2). For study of transient transfectants, hSK2-expressing cells were identified through green fluorescence encoded by the hSK2-GFP construct. Patch pipettes were pulled from Cornin 7052 glass and had resistances of 3–6 MΩ. Recordings were made with an Axopatch IC amplifier (Axon Instruments, Foster City, CA), and signals were filtered at a bandwidth of 2 kHz using a four-pole low-pass Butterworth filter. Currents were recorded on a Gould 2400 chart recorder (Cleveland, OH) and were also digitized (5 kHz) for storage on a computer (Compaq Deskpro 386/20e, Houston, TX). Currents were analyzed using pClamp software (version 6.0, Axon Instruments). Pipette voltages are referred to the bath where $V_p$ corresponds to the membrane potential, and upward deflections of the current trace represent outward membrane current. The standard extracellular solution contained (in mM) 140 NaCl, 4 KCl, 1 KH₂PO₄, 2 MgCl₂, 1 CaCl₂, 5 glucose, and 10 HEPES-NaOH (pH 7.3). The standard pipette (intracellular) solution contained (in mM) 130 KCl, 10 NaCl, 2 MgCl₂, and 10 HEPES-KOH (pH 7.3). Free Ca²⁺ was adjusted to ~100 nM or 1 µM as indicated previously (6). With these solutions, the $K^+$ equilibrium potential is ~−82 mV, and outward currents at a test potential of 0 mV are carried by $K^+$ (15). In other studies, the concentration of Cl⁻ was decreased by partial replacement with glutamate to minimize the contribution of volume-sensitive Cl⁻ currents to the observed response. The low-CI⁻ extracellular solution contained (in mM) 144 sodium glutamate, 8 NaCl, 4 KCl, 1 KH₂PO₄, 2 MgCl₂, 2 CaCl₂, 10 D-glucose, and 10 HEPES-NaOH. The low-Cl⁻ pipette (intracellular) solution contained (in mM) 130 potassium glutamate, 20 NaCl, 2 MgCl₂, 10 HEPES-KOH, 1 EGTA, and 0.5 CaCl₂ (calculated free Ca²⁺ ~100 nM). All reagents were obtained from Fisher Scientific (St. Louis, MO) unless indicated otherwise.

Cell volume measurements. Changes in cell volume were measured electronically using a Coulter Multisizer (Accupack software version 1.19, Hialeah, FL) with an aperture of 100 µM as previously described (15, 16). Measurements of ~20,000 cells in suspension at specified time points after exposure to isotonic or hypotonic buffer were compared with basal values (time 0). Hypotonic buffer was prepared by decreasing NaCl concentration 15%–40% as indicated. Changes in values are expressed as relative volume normalized to the basal period in standard isotonic buffer. Percent recovery was calculated as follows: (peak relative volume – relative volume at 15 min)/ (peak relative volume – 1) × 100.
Statistics. Results are presented as means ± SE, with n representing the number of cells for patch clamp studies and the number of culture plates or repetitions for other assays. Student’s paired or unpaired t-test was used to assess statistical significance as indicated, and P < 0.05 was considered to be statistically significant.

RESULTS

Identification of SKCa homologue hSk2 in human liver epithelia. Initially, both a human liver cDNA library (PCR) and total human liver tissue (RT-PCR) were screened for SKCa cDNAs using degenerate cDNA probes compatible with rSK1, rSK2, rSK3, and hSK4 cDNAs as defined by GeneBank sequence analysis. A single cDNA highly homologous with the rat brain rSK2 cDNA (12) was isolated from both sources and is referred to as hSK2. Subsequently, the full hSK2 sequence was deduced by RT-PCR and by 3′- and 5′-RACE analysis using human liver mRNA. The same sequence was identified from mRNA from human Mz-ChA-1 cholangiocarcinoma cells, which have been previously shown to exhibit apamin-sensitive K+ currents (22). The hSK2 cDNA contains 2,140 bp, including a 1,743-bp open reading frame from position 78 to 1821. Additional sequence encoded the 3′ polyA tail. The open reading frame predicts a protein of 580 amino acids with an amino acid sequence ~97% homologous to rat brain rSK2. Most of the sequence differences, all in frame, are in the NH₂ terminus and do not affect the six putative transmembrane spanning domains (Table 1). Importantly, the hSK2 protein contains the putative pore region between transmembrane domains 5 and 6 identical to the apamin-sensitive channel rSK2, including the apamin-binding motifs (Asp³⁴¹ and Asn⁵⁶⁸, Fig. IA) (11).

Expression of hSK2 transcripts in liver epithelial cells. The expression of hSK2 in different human liver cells was then ascertained. First, using RT-PCR, cDNAs identical to the hSK2 identified in total human liver and in Mz-ChA-1 cells were identified in primary isolated human hepatocytes and human HuH-7 hepatoma cells. Full sequencing showed no differences with hSK2. Second, expression of hSK2 mRNA was detected by Northern analysis of HuH-7 and Mz-ChA-1 cells (Fig. 1B). These findings indicate that the human liver hSK2 cDNA and mRNA are expressed in the two predominant liver epithelial cell types, hepatocytes and cholangiocytes.

Expression of hSK2 in mammalian cells. Previous studies (12) indicate that rSK2 from rat brain encodes a functional channel since heterologous expression results in the appearance of Ca²⁺-activated and apamin-inhibitable K⁺ currents. To confirm that hSK2 identified in liver also encodes functional channels, the hSK2 cDNA, except for the stop codon, was inserted in frame NH₂ terminus to pEGFP-N1 (Clontech) under the control of a CMV promoter in an adenovirus shuttle vector. This design creates an hSK2-GFP fusion protein, with GFP on the extreme COOH terminus. Using this vector, we sequentially purified an hSK2-GFP-expressing adenovirus (see EXPERIMENTAL PROCEDURES). Exposure of CHO cells to hSK2-GFP adenovirus (10 μl of 5 × 10⁶ plaque-forming units/ml) led to a high level of fluorescence in >50% of cells within 48 h. Cellular transduction did not alter cellular viability for up to 72 h. hSK2-GFP protein expression was confirmed by

![Fig. 1. Expression of hSK2 in liver cells. A: the amino acid sequence for the pore domain is overlined and shows no differences with rSK2 identified in neuronal cells. *Sites important for apamin binding. B: Northern analysis was performed using 3 μg of poly(A) mRNA from HuH-7 (liver) and Mz-ChA-1 (biliary) cells and a full-length hSK2 cDNA probe labeled by random priming. A predominant band near 4 kb was detected in both cell types. Size standards (Markers) are shown at right.](image-url)

Amino acid(s) refers to the amino acid position for both hSK2 and rSK2. *In-frame insertion (AAAA) and deletion (GGGG) in hSK2; amino acid positions are for hSK2. †Preceding the first predicted transmembrane domain. ‡After the sixth predicted transmembrane domain.

Table 1. Differences in protein sequence between hSK2 and rSK2

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hSK2. Second, expression of hSK2 mRNA was detected by Northern analysis of HuH-7 and Mz-ChA-1 cells (Fig. 1B). These findings indicate that the human liver hSK2 cDNA and mRNA are expressed in the two predominant liver epithelial cell types, hepatocytes and cholangiocytes.
Western blot analysis of total cellular protein probed with antibodies to GFP. As shown in Fig. 2, an ~77-kDa band was evident in CHO cells transduced with hSK2-GFP, compared with a smaller ~27-kDa band (GFP) in cells transduced with the control adenovirus expressing GFP alone. To assess whether the proteins produced functional channels, CHO cells with detectable GFP fluorescence, indicating expression of the hSK2-GFP construct, were selected for patch clamp analysis as shown in Fig. 3. With the standard solutions used, outward currents at 0 mV correspond to I_{K_v}, and the time course of currents measured from individual cells is shown in Fig. 3, top. Time 0 refers to rupture of the plasma membrane to achieve the whole cell configuration. When the pipette solution dialyzing the cell interior contained ~0.1 μM Ca^{2+}, no currents were detectable. Increasing the Ca^{2+} concentration to 1 μM resulted in transient activation of I_{K_v}, and the response was decreased from 160 ± 41 (n = 5) to 7 ± 9 pA (n = 4, P < 0.01) in the presence of apamin (50 nM). These findings confirm that hSK2 encodes a functional apamin-sensitive K^+ channel as previously described (12). Consequently, additional studies were performed in liver cells using apamin sensitivity as a measure of endogenous SK_{Ca} channel function.

Endogenous SK_{Ca} channels are activated by increases in cell volume and contribute to cell volume recovery from swelling. Previous studies (2, 17, 22) indicate that recovery from liver cell swelling depends on activation of a K^+ conductance pathway with properties similar to that activated by metabolic stress. Using whole-cell patch-clamp recording and the same Cl^-containing solutions, Mz-ChA-1 cell swelling induced by exposure to hypotonic buffer (15%–20% less NaCl) resulted in activation of macroscopic K^+ currents (1,652 ± 233 pA at 0 mV, n = 12). The response was markedly inhibited in the presence of apamin (50 nM; 38 ± 29 pA, n = 5, P < 0.01). Volume-sensitive currents were also effectively inhibited by the nonselective K^+ channel blocker Ba^{2+} (5 mM, P < 0.01) and chelation of intracellular Ca^{2+} concentration (5 mM EGTA in the pipette solution, P < 0.01) as previously described (17).

Because cell swelling also activated a large Cl^- conductance, additional studies were performed using low concentrations of Cl^- in the pipette and bath solutions (EXPERIMENTAL PROCEDURES) to better define the properties of the volume-sensitive K^+ current. The results are shown in Fig. 4. In Fig. 4A, the time course of current activation is depicted. After achieving the whole cell configuration (time 0), exposure to hypotonic buffer (15% less sodium gluconate) was followed after a delay by an increase in current density from 1.9 ± 0.2 to 37.5 ± 7.1 pA/pF (n = 5, 0 mV, P < 0.001). In three of
five cells, currents showed an oscillatory pattern of activation, and in two of five cells the current showed a single peak followed by a more gradual decline toward values above basal levels. In the presence of apamin (50 nM), the response to hypotonic exposure was decreased to 2.8 ± 0.6 pA/pF (50 nM, n = 5, P < 0.01). Current-voltage relationships between -100 and +100 mV were measured by voltage steps in 20-mV increments (Fig. 4B) as well as ramp protocols (Fig. 4C) and showed current reversal near the calculated K⁺ equilibrium potential and showed inactivation at depolarizing potentials. This inactivation was only observed when ramp protocols were obtained near the peak conductance response. Volume-sensitive currents were inhibited by apamin (50 nM).

To assess whether these apamin-sensitive channels contribute to cell volume recovery from swelling, we measured changes in cell volume using a Coulter Multisizer (Fig. 5). In the representative study shown in Fig. 5A, exposure to hypotonic buffer (15% less NaCl) led to a rapid increase in relative cell volume (1.072 ± 0.004 at 2 min, n = 6), which was followed by regulatory volume decrease (RVD) toward basal values (relative volume, 1.008 ± 0.002 at 15 min) similar to previous results (17). Incubation of cells with apamin (100 nM, time 0, n = 6) led to a significant increase in maximal relative volume (1.091 ± 0.004, P < 0.02). Moreover, volume recovery was inhibited ~55% by apamin; percent RVD for control and apamin conditions was 82.7% and 45.1%, respectively (average of 3 or more study days, P < 0.01). Addition of the nonselective K⁺ channel blocker Ba²⁺ (5 mM, n = 6) also increased peak swelling (relative volume, 1.092 ± 0.005, P < 0.02) and completely prevented RVD. Finally, chelation of intracellular Ca²⁺ with BAPTA-AM

![Fig. 4. Volume-activated K⁺ currents in Mz-ChA-1 cells. Whole cell currents were measured using bath and pipette solutions containing low Cl⁻ concentrations (EXPERIMENTAL PROCEDURES). A: in this representative recording, the time course of outward currents at 0 mV (Iₒ) is shown. Immediately after achieving the whole cell configuration (time 0), currents were <50 pA. Exposure to hypotonic buffer (15% decrease in sodium glutamate) to increase cell volume was followed after a delay by appearance of large currents that activated in an oscillatory manner. B: currents measured from the same cell using voltage steps from -100 to +100 mV in 20-mV increments. C: current-voltage relationships were measured using a voltage ramp from -100 to +100 mV. Currents activated by hypotonic exposure reversed near the calculated K⁺ equilibrium potential and showed inactivation at depolarizing potentials. This inactivation was only observed when ramp protocols were obtained near the peak conductance response. Volume-sensitive currents were inhibited by apamin (50 nM).

![Fig. 5. Effect of apamin on Mz-ChA-1 cell volume recovery from swelling. A: a representative study is shown. Exposure of cells to hypotonic buffer (15% decrease in NaCl) caused a rapid increase in cell volume. Under control conditions there was a recovery toward basal values despite the continued presence of hypotonic buffer. However, the rate of recovery was significantly inhibited by the K⁺ channel blockers apamin and barium. B: the concentration dependence of the apamin effects are summarized. Relative regulatory volume decrease (RVD) reflects the RVD observed in the presence of inhibitor compared with control studies performed on the same day. Each point represents the mean ± SE of 3 or more study days. Apamin significantly inhibited cell volume recovery from swelling, but high concentrations of barium had greater inhibitory effects, suggesting the presence of additional apamin-insensitive K⁺ efflux pathways.]
(50 μM, 30 min preincubation, n = 5) also abolished volume recovery, consistent with previous studies (data not shown) (17). The effects of apamin were concentration dependent (Fig. 5B), but even at high concentrations, there was residual volume recovery. Similar results were obtained in separate studies of HuH-7 hepatoma cells, in which percent RVD for control and apamin conditions was 93.7% and 37.6%, respectively (3 study days, P < 0.01).

**DISCUSSION**

Collectively, these findings provide both molecular and biophysical support for the concept that apamin-sensitive SKCa channels encoded by hSK2 are expressed in liver and biliary cells and contribute to volume-sensitive changes in membrane K+ permeability and cell volume homeostasis. The hSK2 cDNA identified in liver cells shares a high degree of identity with the rSK2 cDNA cloned from rat brain, and none of the minor amino acid substitutions involved the putative K+ channel pore, transmembrane, or apamin binding motifs (12). Apamin binding is thought to require expression of an Asp and Asn on each side of the pore domain, modulating electrostatic and hydrophobic interactions between the peptide and the channel (11). Accordingly, it is reasonable to use apamin as a probe for liver SKCa channel function since it is not known to inhibit other members of these (or other) K+ channels. To date, SKCa channels have been best studied in excitable cells of the central nervous system, in which they are fundamentally important in generating slow afterhyperpolarizations. In contrast, SKCa channels in liver epithelia appear to have a different cellular function. A role in cell volume regulation is supported by the observations that 1) heterologous expression of hSK2 cloned from liver cells leads to appearance of Ca2+-activated, apamin-sensitive K+ currents; 2) volume-sensitive K+ currents in Mz-ChA-1 cholangiocarcinoma cells that constitutively express hSK2 mRNA are also Ca2+ activated and apamin sensitive; and 3) in both Mz-ChA-1 and HuH-7 cells, apamin partially prevents cell volume recovery from swelling. Thus SKCa channels represent a potential physiological effector pathway for hormonal and other stimuli that utilize changes in cell volume as a signal affecting liver function.

To our knowledge, the hSK2 cDNA reported here represents the first human SK family member cloned from human liver models. Interestingly, an SK3 homologue has been identified recently (1) in rat liver, but its functional role has not been fully defined. In these studies, hSK2 cDNA was purified from multiple sources and was the only SKCa homologue detected in both hepatocyte and cholangiocyte models. In view of the different functional roles of SKCa channels in neuron vs. liver cells, it is interesting to note that the Ca2+ sensitivity of rSK2 does not appear to involve a direct interaction between Ca2+ and the rSK2 protein. Instead, it depends on a constitutive association of calmodulin with the proximal portion of the intracellular COOH terminus (27). Because activation of volume-sensitive currents in liver cells does not appear to involve calmodulin (unpublished observation), it seems likely that there may be a distinct complement of channel-related proteins or signaling pathways in liver cells that contribute to volume-sensitive channel gating.

Although SKCa channels encoded by hSK2 represent a candidate for a physiologically important volume-sensitive K+ channel in liver cells, several additional points merit further clarification. First, the observation that the nonselective K+ channel blocker Ba2+ causes a greater inhibition of cell volume recovery than apamin suggests that there are likely to be additional apamin-insensitive channels that contribute to volume-sensitive K+ efflux. Similarly, the selectivity of apamin for rSK2 compared with other cloned SKCa channels has recently been questioned (20). Both hSK1 and rSK2 stably expressed in HEK-293 cells were sensitive to apamin (IC50 of 3.3 nM and 83 pM, respectively), findings that differ from the apamin insensitivity observed for hSK1 expressed in Xenopus oocytes (20). Despite the fact that no other SKCa family members could be identified in these studies, the findings reinforce the need for caution in interpretation of inhibitor effects and the need for continued assessment of other channel types (1). Second, little is known regarding the cellular regulation and gating of SKCa channels in liver cells. Whereas calmodulin has been shown to regulate rSK2 in neurons, activation of apamin-sensitive K+ channels in liver cells depends on translocation of the α-isof orm of protein kinase C (PKCα) to the plasma membrane (14). The hSK2 protein contains multiple consensus PKC phosphorylation motifs, but investigation of the potential role of PKCα has been limited by variable expression results in different cell models. The simplest interpretation is that there are likely to be cell type-dependent factors that influence channel regulation, so definition of channel function based on heterologous expression needs to be interpreted with caution. Finally, although these studies emphasize a potential role for SKCa channels in regulation of cell volume, it seems likely that they contribute to other cellular functions as well. Apamin, for example, inhibits K+ efflux induced by adrenergic stimulation of guinea pig hepatocytes (5), and SK family members modulate transepithelial Cl− secretion across colonic epithelia (24). Analogous pathways may exist in bile duct cells, which utilize transepithelial Cl− secretion to modulate the volume and composition of bile.

Collectively, these findings suggest that the liver hSK2 cDNA encodes an apamin-sensitive SKCa channel that contributes to the apamin-binding and apamin-sensitive K+ conductance of liver cells. Moreover, several lines of evidence suggest that these channels are activated by increases in cell volume and contribute to RVD. Accordingly, apamin-sensitive SKCa channels are positioned to play a critical role in the dynamic interactions between metabolism, trans-
port, and cell volume essential for normal liver function.

REFERENCES


