Domain Analysis of the Calcium-activated Potassium Channel SK1 from Rat Brain

FUNCTIONAL EXPRESSION AND TOXIN SENSITIVITY*

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Two small conductance, calcium-activated potassium channels (SK channels), SK2 and SK3, have been shown to contribute to the afterhyperpolarization (AHP) and to shape the firing behavior in neurons for example in the hippocampal formation, the dorsal vagal nucleus, the subthalamic nucleus, and the cerebellum. In heterologous expression systems, SK2 and SK3 currents are blocked by the bee venom toxin apamin, just as well as the corresponding neuronal AHP currents. However, the functional role and pharmacological profile of SK1 channels from rat brain (rSK1) is still largely unknown, as so far rSK1 homomeric channels could not be functionally expressed. We have performed a domain analysis to elucidate the pharmacological profile and the molecular determinants of rSK1 channel expression by using channel chimeras in combination with immunocytochemistry, immuno blot analysis, and electrophysiology. Our results reveal that the rSK1 subunit is synthesized in cells but does not form functional homomeric channels. Exchanging the carboxyl terminus of rSK1 for that of hSK1 or rSK2 is sufficient to rescue the functional expression of rSK1 channels. Additionally, transplantation of both amino and carboxyl termini of rSK1 onto hSK1 subunits, normally forming functional homomeric channel, hinders their functional expression, while hSK1 channels containing only the rSK1 carboxyl terminus are functional. These results suggest that the lack of functional expression of rSK1 channels is probably due to problems in their assembly and tetramerization but not in their calmodulin-dependent gating. Finally, we show that chimeric channels containing the core domain (S1–S6) of rSK1, unlike hSK1, are apamin-insensitive.

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Two small conductance, calcium-activated potassium channels (SK channels), SK2 and SK3, have been shown to contribute to the afterhyperpolarization (AHP) and to shape the firing behavior in neurons for example in the hippocampal formation, the dorsal vagal nucleus, the subthalamic nucleus, and the cerebellum. In heterologous expression systems, SK2 and SK3 currents are blocked by the bee venom toxin apamin, just as well as the corresponding neuronal AHP currents. However, the functional role and pharmacological profile of SK1 channels from rat brain (rSK1) is still largely unknown, as so far rSK1 homomeric channels could not be functionally expressed. We have performed a domain analysis to elucidate the pharmacological profile and the molecular determinants of rSK1 channel expression by using channel chimeras in combination with immunocytochemistry, immunoblot analysis, and electrophysiology. Our results reveal that the rSK1 subunit is synthesized in cells but does not form functional homomeric channels. Exchanging the carboxyl terminus of rSK1 for that of hSK1 or rSK2 is sufficient to rescue the functional expression of rSK1 channels. Additionally, transplantation of both amino and carboxyl termini of rSK1 onto hSK1 subunits, normally forming functional homomeric channel, hinders their functional expression, while hSK1 channels containing only the rSK1 carboxyl terminus are functional. These results suggest that the lack of functional expression of rSK1 channels is probably due to problems in their assembly and tetramerization but not in their calmodulin-dependent gating. Finally, we show that chimeric channels containing the core domain (S1–S6) of rSK1, unlike hSK1, are apamin-insensitive.

Neurons encode information in the form of spike frequency. Spike frequency adaptation (SFA)\(^1\) denotes the progressive decrease in firing frequency in response to prolonged depolarizations (1). SFA is mainly due to the activation of ion channels generating the afterhyperpolarizations (AHPs) that follow action potentials. The more pronounced the AHPs, the less the neuron fires and the stronger is SFA (1).

SFA and the underlying AHP show two temporally distinct phases in cortical neurons (2). The medium AHP (mAHP), lasting 100–300 ms, can be observed after single or bursts of action potentials (3, 4). Different K\(^{+}\) currents underlie the mAHP, among which a prominent role is played by the Ca\(^{2+}\)-activated K\(^{+}\) (KCa) current (4). mAHP is activated by Ca\(^{2+}\) entering the neurons during action potentials and hyperpolarizes the membrane, thereby generating the early phase of SFA and regulating the tonic firing frequency of neurons (4). This current is selectively blocked by the toxins apamin, scyllatoxin, and tamapin (4, 5). The slow AHP activates and deactivates around 10-fold slower compared with the mAHP. sAHP is also mediated by a K\(_{Ca}\) current termed sAHP, which is not blocked by apamin or any other known K\(^{+}\) channel blockers in brain slices (2). The suppression of sAHP by a number of neurotransmitters leads to a drastic reduction of late SFA and an enhancement of neuronal excitability (2).

Three small conductance K\(_{Ca}\) channel subunits (rSK1, rSK2, and rSK3) are expressed in the rat central nervous system and have been proposed to generate the two distinct currents I\(_{sAHP}\) and I\(_{mAHP}\), which underlie the mAHP and sAHP, respectively (6). rSK2 and rSK3 subunits form homomeric channels that open upon binding of intracellular calcium to calmodulin (CaM), which is constitutively bound to the COOH-terminal region of each channel subunit (7). Bee and scorpion toxins have been used as reliable tools to correlate K\(_{Ca}\) channels to native currents, revealing for example that the highly apamin-sensitive SK2 channels mediate I\(_{mAHP}\) in hippocampal neurons (4, 8), whereas the less sensitive SK3 channels mediate I\(_{mAHP}\) in dorsal vagal, superior cervical ganglion, and midbrain dopaminergic neurons (9–11).

Currently little is known about the function and no pharmacological data are available for the rat SK1 channel. We have used a domain analysis approach to investigate the functional expression and toxin sensitivity of the SK1 channel cloned from rat brain (rSK1).

MATERIALS AND METHODS

DNA Constructs—The coding regions of hSK1, rSK1, and rSK2 were cloned into pcDNA3 (Invitrogen). In the chimera rSK1_hSK2_CNK2 aa 1–88 of the NH\(_2\) terminus and aa 373–536 of the COOH terminus of rSK1 were replaced by aa 1–121 of the NH\(_2\) terminus and aa 406–580 small conductance K\(_{Ca}\) channel; CaM, calmodulin; HEK293 cells, human embryonic kidney 293 cells; F, farads.

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of the COOH terminus of SK2. In the chimera hSK1_{NSK1-CSK2} aa 1–92 of the NH2 terminus and aa 373–543 of the COOH terminus of hSK1 were replaced by aa 1–88 of the NH2 terminus and aa 373–536 of the COOH terminus of rSK1. In the chimera rSK1_{NSK1}, rSK1_{CSK2}, hSK1_{NSK1}, hSK1_{CSK2}, rSK1_{NSK1}, and rSK1_{CSK2} only the corresponding NH2 or COOH terminus was replaced. All modified sequences were verified by sequencing with a BigDye Terminator Cycle Sequencing Kit and an ABI3100 Avant Genetic-Analyzer (Applied Biosystems).

**Antibody Production and Purification**—The rSK1 (GenBankTM accession number AF000973) NH2 terminus (aa 77–69) was expressed as a glutathion S-transferase fusion protein used to raise the anti-NSK1 antibody (Biogenes). The peptide anti-CSK2 antibody (12) was affinity-purified on a Sulfolink column (Pierce) coupled to the immunizing antibody (Biogenes). The peptide anti-CSK2 antibody (12) was affinity-purified on a Sulfolink column (Pierce) coupled to the immunizing peptide. Generation and characterization of the anti-NSK2 antibody have been described previously (12).

**Transfection of HEK293 Cells**—HEK293 cells were grown in a humidified atmosphere (5% CO2, 95% air) at 37 °C in Dulbecco’s modified Eagle's medium/F-12 supplemented with 1-glutamine, penicillin/streptomycin, and 10% fetal calf serum. Cells used for Western analysis were transfected using the CaPO4 method and harvested 24–48 h after transfection. Cells used for electrophysiology and immunocytochemistry were transfected using LipofectAMINE (Invitrogen). For electrophysiological recordings, a plasmid of rSK1, hSK1, or chimera (1.5 μg) was co-transfected with 0.5 μg of pEGFP (enhanced green fluorescent protein)-C2.

**Immunoblot Analysis**—Cells were lysed in a buffer containing 1 mM EDTA, 1 mM dithiothreitol, 10 mM Tris-HCl at pH 8.0 supplemented with 0.1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM pepstatin A, 1 μM/ml aprotinin, and 5% (v/v) lithium dodecyl sulfate, sonicated for 30 s, and solubilized at 4 °C for 15 min. Samples (0.5–5 μg of protein, estimated by the DC Protein assay, Bio-Rad) were denatured and separated by SDS-PAGE on a 10% polyacrylamide gel, and immunoblot analysis was performed as described previously (12).

**Immunocytochemistry**—HEK293 cells expressing the indicated channel or chimera were processed for immunocytochemistry as described in Ref. 12. Primary antibody dilutions were 1:500: anti-NSK1 and anti-CSK2; or 1:1000: anti-NSK2. The secondary Cy3-conjugated goat anti-rabbit antibody was used at 1:800 (Jackson ImmunoResearch). Immunostaining was visualized with a fluorescence microscope (Axiophot, Zeiss), and pictures were taken with a CCD camera (Micropublisher, QImaging).

**Electrophysiology**—Whole-cell patch clamp recordings of HEK293 cells transiently transfected or stably expressing rSK2 channels (5) were performed (EPC-9, HEKA Elektronik) while perfusing with extracellular solution “high K+” (in mM): 125 KCl, 5 Hepes, 10 EGTA, 10 D-glucose, and 100 nM Ca2+. Electrophysiological recordings, a plasmid of rSK1, hSK1, or chimeras (1.5 μg) was co-transfected with 0.5 μg of pEGFP (enhanced green fluorescent protein)-C2.

**Rearrangement of the COOH terminus and aa 377–69) was expressed as a chimeric rSK1 subunit. rSK1 protein was detected by SDS-PAGE and Western analysis (Fig. 3), no current above background could be measured in rSK1 transfected cells. The anti-NSK1 antibody detected rSK1 protein distributed throughout the cell, with a higher concentration in the endoplasmic reticulum and Golgi apparatus (Fig. 2A). Immunostaining was visualized with a fluorescence microscope (Axiophot, Zeiss), and pictures were taken with a CCD camera (Micropublisher, QImaging).

**RESULTS AND DISCUSSION**

Within the SK channel family, the primary sequences of the rat and human SK2 subunits present 97.6% identity (6, 13, 14). Similarly, the amino acid sequences of the SK3 subunit from the two species display 94.4% identity (13, 15). For both SK2 and SK3, the highly homologous human and rat subunits display very similar functional and pharmacological profiles, with SK2 channels presenting a very high sensitivity to apamin (IC50: 27–400 pm, Refs. 6, 14, and 16–19), and SK3 channels being less sensitive (IC50: 0.6–19 nm, 19–21). By contrast, the rat SK1 subunit presents only 84% sequence identity with the human SK1 (Fig. 1). The hSK1 subunit forms homomeric channels with a sensitivity to apamin that varies depending on the heterologous expression system of choice (IC50: 3.3–12 nm in mammalian cell lines (17, 18); >100 nm (6, 16) or ~700 pm/200 nm (19) in oocytes). No information is instead available on the functional and pharmacological properties of rSK1 channels. To characterize rSK1 channels, we performed a set of immunocytochemical experiments on HEK293 cells transiently transfected with the rSK1 subunit. rSK1 protein was detected with a specific antibody generated against a portion of the rSK1 NH-term region (anti-NSK1). Fig. 2B shows a typical result of an immunofluorescence obtained on rSK1 transfected cells. The anti-NSK1 antibody detected rSK1 protein distributed throughout the cell, with a higher concentration in the endoplasmic reticulum and Golgi apparatus (Fig. 2A). The anti-NSK1 antibody did not reveal any signal when applied to non-transfected cells (Fig. 2C) or to cells expressing rSK2 channels (Fig. 2D), thereby demonstrating its subunit specificity. To assess the functional properties of rSK1 channels, we performed electrophysiological recordings in the whole-cell configuration. The channels were activated by 1 μM free Ca2+ present in the pipette solution, conditions that reliably activated hSK1, rSK2, rSK3, and IK (5). Despite the presence of rSK1 protein as detected by immunofluorescence and in the immunoblot (Fig. 3), no current above background could be measured in rSK1 transfected cells (Fig. 2, D and Q; current density: 13.2 ± 6.3 pA/pF, n = 6). By contrast, cells transfected with rSK2 not only showed clear immunofluorescence signals, as detected with SK2 specific antibodies against its NH2-terminal (anti-NSK2; Fig. 2P) and COOH-terminal region (anti-CSK2; Fig. 2G), but also displayed K+ currents activated by Ca2+ (Fig. 2H, see also Ref. 5).

**Fig. 1.** Alignment of the primary sequences of rSK1 and hSK1 (GenBankTM accession numbers AF000973 and NM_002248). Boxed in gray are the putative transmembrane domains, S1–S6, and the pore region (P-region). Boxed in black are the amino acids that differ in the two sequences. Dashes indicate gaps.
and anti-CSK2 (Fig. 2K) antibodies. Furthermore, electrophysiological recordings demonstrated that the rSK1NSK2-CSK2 chimera assembled and formed functional K<sub>Ca</sub> channels, as shown by the K’ currents recorded in the presence of Ca<sup>2+</sup> (Fig. 2, L and Q; current density: 176.5 ± 25.6 pA/pF; n = 11). To narrow down the region responsible for the lack of rSK1 functional expression, we generated a second chimeric subunit where only the COOH-terminal region of rSK1, necessary for the binding of CaM, was exchanged with that of rSK2 (rSK1CSK2) (Fig. 2M). When expressed in HEK293 cells, the rSK1CSK2 protein was synthesized and could be detected by immunofluorescence using the anti-NSK1 antibody (Fig. 2N) and the anti-CSK2 antibodies (Fig. 2O). Like rSK1NSK2-CSK2, rSK1CSK2 assembled into functional K’ channels as demonstrated by the K’ currents elicited in the presence of Ca<sup>2+</sup> (Fig. 2, P and Q; current density: 100.7 ± 21.6 pA/pF; n = 19). Similarly, functional expression could be obtained when the COOH-terminal region of rSK1 was replaced by that of the human homologue, hSK1 (Fig. 2Q; rSK1CSK2 current density: 98.5 ± 15.9 pA/pF; n = 17). Conversely, the replacement of the NH<sub>2</sub>-terminal region of rSK1 by that of rSK2 (rSK2CSK1) yielded a single band corresponding to a molecular weight of 51 kDa. The same antibody did not detect any bands when tested on lysate from HEK293 cells or cells transfected with rSK2, thereby confirming its specificity. rSK1CSK2, rSK2CSK1, hSK1NSK2, and hSK1NSK2-CSK2 were detected by the anti-NSK1 antibody and, for rSK1CSK2, also by the anti-CSK2 antibody as a single band. The anti-CSK2 antibody did not cross-react with rSK1 and was used to detect the rSK1NSK2-CSK2 and rSK2NSK1 chimeras. Similar results were obtained in Western analysis experiments of three independent transfections.

Fig. 2. Immunofluorescence and functional expression of rSK1 and chimeric channels. A, E, I, and M, nomenclature and schematic drawings of the chimeric channels generated. rSK1 regions correspond to thin lines and rSK2 regions to thick lines. The exact boundary of each segment is given under “Materials and Methods.” B, specific rSK1 staining by anti-NSK1 antibody. C, no signal was observed with anti-NSK1 on non-transfected HEK293 cells (c1) or cells transfected with rSK2 (c2). F and G, specific staining of cells expressing SK2 obtained with the anti-NSK2 (F) and the anti-CSK2 (G) antibodies. J and K, expression of the chimera rSK1CSK2CSK2 detected by using the anti-NSK1 (J) and the anti-CSK2 (K) antibodies. N and Q, expression of the chimera rSK1CSK2 detected by the anti-NSK1 (N) and the anti-CSK2 (O) antibodies. Scale bar: 10 μm in B and F–O; 6.8 μm in C1 and C2. All immunofluorescence experiments were repeated in duplicate on two different transfections, except for rSK1, which was tested on three different transfections. D, H, L, and P, SK currents were elicited in the whole-cell configuration by voltage pulses from −100 to +40 mV in 20 mV steps, lasting 30 ms, in the presence of 1 μM Ca<sup>2+</sup> in the patch pipette and of high K<sup>+</sup>. HEK293 cells expressing rSK1 did not display any currents (D), while cells expressing rSK2 (H), rSK1CSK2CSK2 (L), or rSK1CSK2 (P) showed K<sub>Ca</sub> currents. Q, bar diagram summarizing the current amplitude for non-transfected cells, rSK1, hSK1, and all chimeric channels tested at −80 mV.

In the attempt to elucidate whether the COOH terminus of rSK1 is per se capable of hindering the formation of functional channels when attached to normally expressing subunits, we exchanged the COOH-terminal region of hSK1 with that of rSK1 (Fig. 2Q). Surprisingly, hSK1CSK1 subunits formed functional channels in HEK293 cells (Fig. 2Q; current density: 293.3 ± 64.5 pA/pF; n = 11). Similarly, replacing the NH<sub>2</sub> terminus of hSK1 by that of rSK1 led to the formation of functional channels (Fig. 2Q; current density: 372.4 ± 101.3 pA/pF; n = 3). By contrast, functional expression was hindered when both rSK1 NH<sub>2</sub> and COOH termini substituted the corresponding regions in hSK1 (Fig. 2Q; current density: 17.2 ± 4.6 pA/pF; n = 11). The CaM-binding region (ABC or Ct1 region (7, 22)) plays a critical role in gating SK channels in response to elevations of intracellular Ca<sup>2+</sup> (7, 23, 24). The CaM-binding region of rSK1 is very similar to the one of hSK1 (Fig. 1), presenting only five different amino acids. The gating of hSK1<sub>NSK1</sub> channels argues against an impairment of CaM binding and gating solely due to the rSK1 COOH terminus. This view is supported by three further lines of evidence. First, the two residues that are critical for the constitutive binding of CaM to the COOH terminus of rSK2 and its surface expression (Arg<sup>434</sup> and Lys<sup>437</sup>; Refs. 24 and 25) are conserved in rSK1 (Arg<sup>434</sup> and Lys<sup>437</sup>; Fig. 1). Second, the amino acids forming α-helices important for constitutive CaM binding, as shown by NMR spectroscopy and x-ray crystallography (26, 27), are conserved in rSK1. Third, the region comprising the proximal part of the COOH-terminal domain (up to aa 370 in rSK1), S6, and the pore domain around the selectivity filter, which have been analyzed in rSK2 by substituted cysteine accessibility mutagenesis to identify the SK channel gate (28), is identical in all rSK subunits and thus cannot be responsible for the lack of functional expression of rSK1.

The expression of both rSK1 and of the different chimeric proteins used for the domain analysis was further confirmed by Western analysis. Fig. 3 shows that HEK293 cells transfected with the rSK1 subunit and probed with the NH<sub>2</sub>-terminal antibody anti-NSK1 yielded a band corresponding to a molecular weight of 51 kDa (Fig. 3), which is in good agreement with
the predicted molecular weight of the rSK1 subunit. The anti-
NSK1 antibody was specific as it did not detect any protein in
non-transfected HEK293 cells or in cells transfected with rSK2
(Fig. 3). Similarly, the anti-NSK2 antibody and the anti-CSK2
antibody were specific, as they recognized only a single band
corresponding to the rSK2 channels in transfected but no pro-
tein in non-transfected HEK293 cells (12) or in cells express-
ging rSK1 (Fig. 3). Finally, by using the appropriate antibody on
lysates from transfected HEK293 cells, we showed the expression
of all chimeric proteins at molecular weights close to their
predicted values (Fig. 3), confirming that both rSK1 and the
chimeric subunits used for our domain analysis were expressed
in full length, although only some of them displayed functional
expression.

The analysis of rSK1 chimeras shows first of all that the
substitution of the rSK1 COOH terminus by the rSK2 or the
hSK1 COOH termini, but not the substitution of the corre-
sponding NH2 termini, is per se sufficient to obtain its func-
tional expression. Additionally, transplantation of both NH2
and COOH termini of rSK1 onto hSK1 subunits, normally
capable of forming functional homomeric channel, hinders
their functional expression, in agreement with previous evi-
dence that both the NH2 and the COOH termini of SK channels
are important for the SK channel assembly and tetrameriza-
tion (22, 29). Despite the apparent ability of the rSK1 COOH
terminus to bind CaM and allow channel gating as revealed by
the functional expression of hSK1-rSK1, the role of the COOH
terminus in assembly might explain the lack of functional
expression of rSK1 homomeric channels. First, rSK1 on its own
might not be appropriately assembled and transported to the
plasma membrane, as the COOH-terminal region of SK chan-
nels is important for their multimerization and surface expres-
sion (22). Two domains important for assembly and membrane
targeting have been characterized: the Ct1 domain, corre-
sponding to the CaM-binding region of the channels (also
known as ABC region (7)), and the Ct2 domain, corresponding
to the distal part of the COOH-terminal tail containing a
leucine zipper motif (13, 22). The primary sequence of the rSK1
COOH terminus distal to the CaM-binding region differ-
ificantly from the one of hSK1 and rSK2. This sequence diver-
gence might affect the proper assembly and/or transport to
the membrane of rSK1 channels, although the leucine zipper
motif in the Ct2 domain is conserved in rSK1 (Fig. 1), and no
obvious endoplasmic reticulum or Golgi retention motifs have
been identified in this region. Alternatively, the membrane
targeting of rSK1 might require an associated protein, acting
as a chaperone, a function previously shown for example for
Kv-β subunits (30). The absence of this accessory protein in
heterologous expression systems could then explain the lack of
rSK1 expression. Another possibility is that under native con-
ditions rSK1 might co-assemble with other SK subunits and
reach the membrane as part of heteromeric channels with
peculiar functional and pharmacological features. Indeed, the
existence of SK heteromeric channels is still a controversial
issue (Refs. 16, 29, 31, and 32, but also see Ref. 8), but an
increasing number of voltage-gated K+ channel subunits (Kv5,
Kv6, Kv8, Kv9 families) have been shown to function as mod-
ulatory subunits by forming heteromeric channels with mem-
bers of the Kv2 family while being unable to generate func-
tional homomeric channels (33, 34).

The structural basis of the different apamin and D-tubocu-
arine sensitivity of the three SK subunits (hSK1, rSK2, and
rSK3) has been ascribed to differences in a few amino acids
located in the pore region (16, 35). The pharmacological prop-
erties of the rSK1 channel could not be investigated so far
because this subunit does not form functional channels (Figs. 2,

![Image](317x336 to 564x737)

**FIG. 4.** Chimeric rSK1 currents are not blocked by apamin or D-tubocurarine. A–I, HEK293 cells expressing SK channels and chimeras were measured in the whole-cell configuration in the presence of 1 μM intracellular Ca2+. Voltage ramps from −100 to +40 mV (duration: 400 ms) were applied. At least three different sets of transfected cells were tested for each construct. The ramp protocol is illustrated schematically (A, inset). A, cells expressing rSK1 did not show any current. B–E, the rSK1/NSK2-CSK2 (B, C) and the rSK1/CSK2 chimera (D, E) yielded currents that were not suppressed by 100 nM apamin (B, D) or by 50 μM D-tubocurarine (C, E, dTC). F, similarly, the rSK1/CSK2 chimera was not sensitive to 100 nM apamin. G, under the same recording conditions, hSK1 channels generated a current that was largely blocked by 10 nM apamin (IC50 = 3 ± 1 nM; n = 8). H and I, the hSK1/CSK2 chimera, containing the core region of hSK1, generated currents sensi-
tive to apamin (H: 10 nM; IC50 = 4.3 ± 1.1 nM; n = 7) and to D-
tubocurarine (I: 50 μM; n = 3). The measurements were performed in high symmetrical K+ . In the same cells in low K+ only very small residual currents could be observed. Similar results were obtained in three to eight cells for each chimera. J, time course of current inhibition induced by apamin (100 nM) on the hSK1-filled circles and the rSK1/CSK2-mediated current (open circles) measured at −100 mV. Voltage ramps were repeated every 10 s.
tubocurarine at 50 μM (Fig. 4C; n = 5). Similarly, no block was observed upon application of 100 nM apamin or 50 μM d-tubocurarine to cells expressing rSK1 CSK2 chimeric channels (Fig. 4, D, E, and F; n = 4 for each blocker). Finally, also rSK1CSK1 chimeric channels did not display any sensitivity to apamin (100 nM; Fig. 4F; n = 6) or d-tubocurarine (50 μM; not shown; n = 9). Conversely, apamin (5–100 nM; Fig. 4G, n = 8) and d-tubocurarine (50 μM; n = 3, data not shown) blocked currents generated by hSK1 channels and by chimeric subunits containing the core domain of hSK1, such as hSK1 rSK1, with similar affinities (Fig. 4, H, n = 7, and I, n = 3). The functional expression of the rSK1CSK1 chimera has revealed its very intriguing lack of sensitivity to apamin and curare. This finding is rather surprising as the determinants for apamin binding have been localized in the pore region (16, 35), where the apamin-insensitive rSK1 and the moderately apamin-sensitive hSK1 present identical primary sequences (Fig. 1). One possible explanation for this difference in toxin sensitivity is that amino acids located outside the pore region might contribute to apamin binding. Since our data show that the sensitivity to apamin co-segregates with the core domain of the SK subunits, the critical determinants for apamin binding are most likely located between S1 and S6, where the rSK1 and hSK1 sequences differ by 16 amino acids (Fig. 1). The lack of apamin sensitivity of the rSK1 subunit has important implications for the possible role of rSK1 in determining the pharmacological profile of AHP currents in central neurons, for example by taking part in the formation of heteromeric SK channels (Refs. 16, 29, 31, 32, but also see Ref. 8). In particular, the pharmacological features of the rSK1 channel emerging from this study might revive the hypothesis that it underlies the apamin-insensitive slow AHP current (sAHP) in the central nervous system. However, we believe this is an unlikely scenario based on several lines of evidence, including mismatches between SK and sAHP channels in their kinetics, pharmacology (hSK1 is apamin-sensitive, while sAHP is insensitive in human cortical neurons (36)), and expression patterns in the brain (37, 38). A final conclusion on the contribution of rSK1 to neuronal AHP currents will possibly be reached only by genetic suppression of SK1 expression in animal models.

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