Brief Communication

# $SK_{Ca}$ Channels Mediate the Medium But Not the Slow Calcium-Activated Afterhyperpolarization in Cortical Neurons

**Claudio Villalobos,[1] Vikram G. Shakkottai,[2] K. George Chandy,[2] Sharon K. Michelhaugh,[1] and Rodrigo Andrade[1]**

[1]Department of Psychiatry and Behavioral Neuroscience, Wayne State University School of Medicine, Detroit, Michigan 48201, and [2]Department of Physiology and Biophysics, College of Medicine, University of California, Irvine, Irvine, California 92697-4034

**Many neurons, including pyramidal cells of the cortex, express a slow afterhyperpolarization (sAHP) that regulates their firing. Although initial findings suggested that the current underlying the sAHP could be carried through $SK_{Ca}$ channels, recent work has uncovered anomalies that are not congruent with this idea. Here, we used overexpression and dominant-negative strategies to assess the involvement of $SK_{Ca}$ channels in mediating the current underlying the sAHP in pyramidal cells of the cerebral cortex.**

**Pyramidal cells of layer V exhibit robust AHP currents composed of two kinetically and pharmacologically distinguishable currents known as the medium AHP current ($I_{mAHP}$) and the slow AHP current ($I_{sAHP}$). $I_{mAHP}$ is blocked by the $SK_{Ca}$ channel blockers apamin and bicuculline, whereas $I_{sAHP}$ is resistant to these agents but is inhibited by activation of muscarinic receptors. To test for a role for $SK_{Ca}$ channels, we overexpressed $K_{Ca}2.1$ (SK1) and $K_{Ca}2.2$ (SK2), the predominant $SK_{Ca}$ subunits expressed in the cortex, in pyramidal cells of cultured brain slices. Overexpression of $K_{Ca}2.1$ and $K_{Ca}2.2$ resulted in a fourfold to fivefold increase in the amplitude of $I_{mAHP}$ but had no detectable effect on $I_{sAHP}$. As an additional test, we examined $I_{sAHP}$ in a transgenic mouse expressing a truncated $SK_{Ca}$ subunit (SK3-1B) capable of acting as a dominant negative for the entire family of $SK_{Ca}$–$IK_{Ca}$ channels. Expression of SK3-1B profoundly inhibited $I_{mAHP}$ but again had no discernable effect on $I_{sAHP}$. These results are inconsistent with the proposal that $SK_{Ca}$ channels mediate $I_{sAHP}$ in pyramidal cells and indicate that a different potassium channel mediates this current.**

*Key words:* sAHP; mAHP; SK channels; apamin; pyramidal cells; cerebral cortex

## Introduction

Pyramidal neurons of the cortex and hippocampus display a calcium-activated slow afterhyperpolarization (sAHP) that plays a key role in regulating cell firing (Schwindt et al., 1988a,b; Stocker et al., 1999) and is the target for regulation by multiple neurotransmitters (Nicoll, 1988). Biophysical and electrophysiological studies have suggested that this sAHP is mediated by a calcium-activated potassium current. However, despite extensive studies, the identity of the ion channels underlying the sAHP remains uncertain (Sah and Faber, 2002; Vogalis et al., 2003).

In the mid-1990s, with the discovery of the $SK_{Ca}$ family of potassium channels ($K_{Ca}2.x$) (Kohler et al., 1996; Gutman et al., 2003), the search for the ion channels responsible for the sAHP appeared to have reached fruition (Vergara et al., 1998; Bond et al., 1999). However, subsequent studies comparing the properties of channels made by $SK_{Ca}$ subunits with those underlying the sAHP revealed a number of physiological and pharmacological anomalies (for review, see Sah and Faber, 2002; Vogalis et al., 2003). One possible explanation for these anomalies is that the sAHP is indeed mediated by channels composed of $SK_{Ca}$ subunits, but that in neurons, these subunits are assembled into channels with distinct pharmacological–physiological properties. These novel properties could result from the formation of channels with a specific stoichiometry not easily attained in heterologous expression systems or from the incorporation of auxiliary subunits. An alternative interpretation of these anomalies is that the ion channel responsible for the sAHP is not composed of $SK_{Ca}$ subunits. Distinguishing between these two possibilities is an essential step toward the long-awaited elucidation of the molecular substrate responsible for the current underlying the sAHP ($I_{sAHP}$). In the current study, we used overexpression and dominant-negative strategies in cultured brain slices and transgenic animals to test the idea that $SK_{Ca}$ channels are essential components of the channel responsible for $I_{sAHP}$ in the cerebral cortex.

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Correspondence should be addressed to Rodrigo Andrade, 2309 Scott Hall, Department of Psychiatry and Behavioral Neuroscience, Wayne State University School of Medicine, 540 East Canfield, Detroit, MI 48201. E-mail: randrade@med.wayne.edu.

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## Materials and Methods

*Brain slice experiments.* Whole-cell recordings were obtained from pyramidal cells of layer V of the anterior cingulate and somatosensory cortex in acute rat and mouse brain slices [postnatal day 15 (P15)–P30] and in rat brain slices (P8–P12) in culture for 2–3 d. Slices were prepared as described previously (Haj-Dahmane and Andrade, 1996; Beique and An-

drade, 2003) and placed in a recording chamber (Sakmann and Stuart, 1995) on the stage of a Nikon (Melville, NY) E600 microscope. Slices were superfused with Ringer's solution (in mM: 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1 $NaH_2PO_4$, 26.2 $NaHCO_3$, and 22 glucose, bubbled to saturation with 95% $O_2$–5% $CO_2$) at 30°C. Neurons were visualized using differential interference contrast and fluorescence, and recordings were obtained using 3–5 MΩ electrodes filled with a potassium-based intracellular solution (in mM: 125 $KMeSO_4$, 5 KCl, 5 NaCl, 0.02 EGTA, 11 HEPES, 1 $MgCl_2$, 10 $Na_2$ phosphocreatine, 4 MgATP, and 0.3 NaGTP, pH 7.3). Signals were amplified using Axoclamp2B (Axon Instruments, Foster City, CA), Axopatch1B, or EPC-10 amplifiers, digitized, and stored (Haj-Dahmane and Andrade, 1996). AHP currents were elicited using 100-msec-long steps to +20 mV in TTX (1 μM) to allow calcium into the cell and trigger the AHPs (Stocker et al., 1999). The medium AHP current ($I_{mAHP}$) was measured 30–50 msec after the end of the step, a time empirically determined to correspond to the peak of the apamin-sensitive $I_{mAHP}$. $I_{sAHP}$ was measured 300 msec after the end of the depolarizing step, when $I_{sAHP}$ is near its peak and $I_{mAHP}$ has decayed by ~90% (Abel et al., 2004). In some experiments, we compared the amplitude of $I_{mAHP}$ and $I_{sAHP}$ in neighboring transfected [identified by green fluorescent protein (GFP) expression] and untransfected pyramidal neurons in cultured slices. The untransfected cells served as controls in terms of specific location, slice history, and animal of origin. We also compared these currents with those recorded from cells transfected with GFP alone in different slices. Rat $K_{Ca}2.1$ ($rK_{Ca}2.1$; SK1) and $rK_{Ca}2.2$ (SK2) were a gift from Dr. M. Stocker (University College London, London, UK).

*Cell line experiments.* Whole-cell recordings were obtained in cell lines engineered to stably express SK1–SK3, $IK_{Ca}$, and $BK_{Ca}$ potassium channel subunits. Human embryonic kidney 293 (HEK 293) cells expressing human $K_{Ca}2.1$ (hSK1), $rK_{Ca}2.2$ (rSK2), and human $K_{Ca}3.1$ ($hIK_{Ca}1$) were a gift from Dr. Khaled Houamed (University of Chicago, Chicago, IL), and HEK 293 cells stably expressing $hK_{Ca}3.1$ ($hBK_{Ca}$) were a gift from Dr. Andrew Tinker (University College London, London, UK). Chinese hamster ovary 7 cells expressing human $K_{Ca}2.3$ (hSK3) were obtained from Aurora Biosciences (San Diego, CA). Cell lines were grown in DMEM, supplemented with 10% dialyzed fetal calf serum and 2 mM each of glutamine, penicillin, and streptomycin. Each cell line was cultured in the presence of a selection agent (10 μg/ml puromycin for HEK 293T cells stably expressing $K_{Ca}2.1$, $rK_{Ca}2.2$, and $hK_{Ca}3.1$; 0.5 μg/ml blasticidin for cells stably expressing $hK_{Ca}2.3$; and 800 μg/ml G418 for HEK 293 cells stably expressing $hBK_{Ca}$) to preserve channel expression. All cells were kept in a 37°C humidified incubator with 5% $CO_2$ and were split 1:10 twice per week. Mammalian expression vectors containing SK3-1B or GFP were transfected using the Fugene-6 transfection reagent (Roche Products, Indianapolis, IN), as described previously (Tomita et al., 2003).

Whole-cell recordings for $SK_{Ca}$, $IK_{Ca}$, and $BK_{Ca}$ currents were performed using an internal solution containing 1 μM free $Ca^{2+}$ (in mM: 145 K-aspartate, 8.5 $CaCl_2$, 10 EGTA, 10 HEPES, and 2 $MgCl_2$, pH 7.4), as described previously (Kolski-Andreaco et al., 2004). The external recording solution contained the following (in mM): 155 $Na^+$ aspartate, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, pH 7.2, 280–300 mOsm. Cells were held at −80 mV, and SK1-3, $BK_{Ca}$, and $IK_{Ca}$ currents were recorded using 200 msec voltage ramps from −120 to 40 mV (120 mV for $BK_{Ca}$). Slope conductance was analyzed for SK and $IK_{Ca}$ at −80 mV and for $BK_{Ca}$ at 60 mV. Cells in which series resistance was >10 MΩ were excluded. The mean slope conductance of each of the currents in cells transfected with SK3-1B was normalized to the mean conductance in GFP-transfected controls.

## Results

Pyramidal cells of the prefrontal and anterior somatosensory cortices of rats and mice display robust AHP currents (Fig. 1*A*) similar to those seen in pyramidal neurons of the hippocampus and other regions of the cortex (Schwindt et al., 1988a; Stocker et al., 1999). As illustrated in Figure 1*A*, this AHP current comprises two kinetically distinguishable components, an early component known as the $I_{mAHP}$ and a late, slower component known as the $I_{sAHP}$. These two currents can also be differentiated pharmacologically. The $I_{mAHP}$ is inhibited by apamin (Fig. 1$A_1$) (300 nM; $n = 22$ cells in rat slices and 25 cells in mouse slices) and by bicuculline (Fig. 1$A_2$) (30 μM; $n = 8$ cells in rat slices and 5 cells in mouse slices). In contrast, $I_{sAHP}$ is resistant to these agents but is readily inhibited by activation of cholinergic muscarinic neurotransmitter receptors ($n = 12$ cells) (Araneda and Andrade, 1991). These results obtained in the prefrontal–anterior somatosensory cortex are in agreement with previous findings on pyramidal cells in the cortex as well as in the hippocampus (Schwindt et al., 1988a; Stocker et al., 1999).

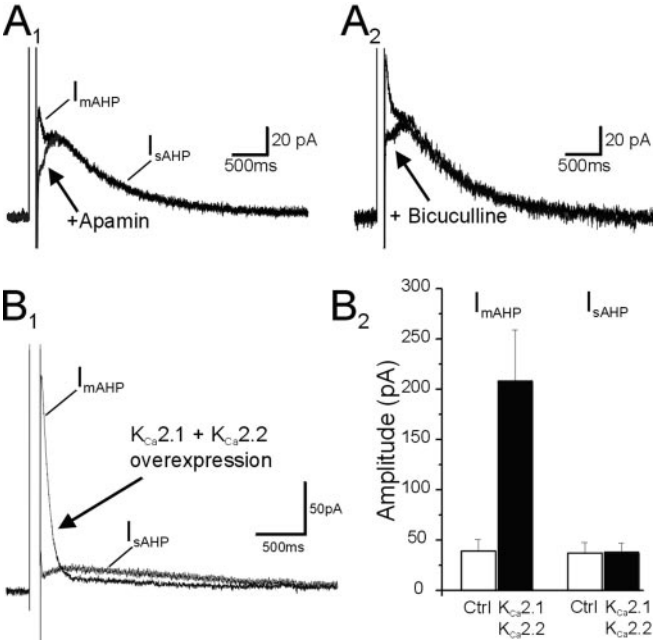


**Figure 1.** Effect of $SK_{Ca}$ channel blockers and $K_{Ca}2.1$ plus $K_{Ca}2.2$ overexpression on $I_{mAHP}$ and $I_{sAHP}$. $A_1$, Apamin (300 nM) inhibits $I_{mAHP}$ but has no effect on $I_{sAHP}$ in a pyramidal cell of layer V in an acute slice. $A_2$, Bicuculline (30 μM) similarly inhibits $I_{mAHP}$ but is without effect on $I_{sAHP}$ in a different pyramidal cell in an acute brain slice. $B_1$, Overexpression of $K_{Ca}2.1$ plus $K_{Ca}2.2$ in cultured brain slices selectively amplifies $I_{mAHP}$. The control trace depicts $I_{AHP}$ in a control untransfected cell. $B_2$, Plot comparing the amplitude of $I_{mAHP}$ and $I_{sAHP}$ in cells transfected with $K_{Ca}2.1$ plus $K_{Ca}2.2$ and control (Ctrl) untransfected cells. Error bars indicate SEM.

Because apamin and bicuculline inhibit all three members of the $K_{Ca}2.x$–$SK_{Ca}$ channel family (Kohler et al., 1996; Ishii et al., 1997; Khawaled et al., 1999; Strobaek et al., 2000; Grunnet et al., 2001), the results outlined above support the idea that $I_{mAHP}$ is carried by channels composed of $SK_{Ca}$ subunits (Stocker et al., 1999). However, it is possible that the lack of sensitivity of $I_{sAHP}$ to these blockers could have resulted from the formation in neurons of yet uncharacterized multimers of $SK_{Ca}$ subunits, possibly in conjunction with auxiliary components. Therefore, in the current study, we used molecular techniques to directly target $SK_{Ca}$ channels in central neurons and test their role in the generation of the sAHP.

Because SK1 and SK2 are the $SK_{Ca}$ isoforms most abundantly expressed in the anterior prefrontal–somatosensory cortices (Stocker and Pedarzani, 2000), it would be expected that these isoforms would also be major components of any $SK_{Ca}$ channels in this region. Therefore, as an initial test to determine whether $SK_{Ca}$ channels contribute to $I_{sAHP}$, we overexpressed $rK_{Ca}2.1$ and $rK_{Ca}2.2$ in pyramidal cells of the anterior cortex and examined the effect of this manipulation on the amplitude of $I_{mAHP}$ and

$I_{sAHP}$. These experiments were conducted on cultured brain slices transfected using particle-mediated gene transfer (gene gun) techniques. Transfected cells were identified using GFP as a marker (Beique and Andrade, 2003). As illustrated in Figure 1*B*, overexpression of $rK_{Ca}2.1$ and $rK_{Ca}2.2$ in pyramidal cells of layer V resulted in a large increase in the amplitude of $I_{mAHP}$. Overall $rK_{Ca}2.1$–$rK_{Ca}2.2$ overexpression resulted in an approximately fourfold to fivefold increase in the amplitude of $I_{mAHP}$ (39 ± 11 pA, $n = 7$ cells in control untransfected cells; 48 ± 12 pA, $n = 9$ cells in control GFP-transfected cells; 208 ± 51 pA, $n = 13$ cells in $rK_{Ca}2.1$–$rK_{Ca}2.2$-transfected cells; $p < 0.05$ against both control groups). Application of apamin (300 nM) greatly inhibited the $I_{mAHP}$ in $rK_{Ca}2.1$–$rK_{Ca}2.2$-transfected cells (81 ± 8% inhibition; $n = 5$ cells; $p < 0.05$), confirming that it reflected an increase in $SK_{Ca}$ channel function in these cells. Strikingly, in contrast to these results, overexpression of $rK_{Ca}2.1$ and $rK_{Ca}2.2$ did not result in any detectable change in the $I_{sAHP}$ (control untransfected, 37 ± 10 pA; $rK_{Ca}2.1$ plus $rK_{Ca}2.2$ overexpression, 38 ± 9 pA; $p = 0.95$) (Fig. 1$B_2$). These results called into question the idea that $K_{Ca}2.1$ or $K_{Ca}2.2$ are constituents of the channel responsible for $I_{sAHP}$ in pyramidal cells.

To further test for the involvement of $SK_{Ca}$ channels in mediating $I_{sAHP}$, we took advantage of the recent discovery of a $K_{Ca}2.3$ transcript (SK3-1B) that suppresses endogenous apamin-sensitive (presumed $SK_{Ca}$) channels in a dominant-negative manner when they are heterologously expressed in mammalian cells (Tomita et al., 2003). To determine the activity spectrum of SK3-1B, we assessed its ability to suppress calcium-activated potassium currents carried by $K_{Ca}2.1$, $K_{Ca}2.2$, and $K_{Ca}2.3$ as well as $K_{Ca}3.1$ ($IK_{Ca}1$) and $K_{Ca}1.1$ ($BK_{Ca}$) in cell lines engineered to stably express the corresponding genes. As illustrated in Figure 2, expression of SK3-1B effectively inhibited currents carried through $K_{Ca}2.1$–$K_{Ca}2.3$ as well as $K_{Ca}3.1$ channels ( $p < 0.01$ for each of the currents) but had little effect on currents carried through $K_{Ca}1.1$. Previous work has shown that SK3-1B expression is without effect on currents carried through voltage-dependent potassium channels, and its dominant-negative activity is not attributable to dysregulated calmodulin homeostasis (Tomita et al., 2003; Kolski-Andreaco et al., 2004; Shakkottai et al., 2004). Thus, SK3-1B appears to be a potent tool to assess the involvement of $SK_{Ca}$–$IK_{Ca}$ subunits in the formation of the channels responsible for $I_{sAHP}$.

To test the effect of SK3-1B on $I_{mAHP}$ and $I_{sAHP}$, we used a transgenic mouse engineered to express the SK3-1B isoform fused to GFP under the control of the Thy-1 promoter (Shakkottai et al., 2004). Figure 3$A_1$ shows that the SK3-1B–GFP transgene is abundantly expressed in layer V of the anterior somatosensory cortex. Layer V of the somatosensory cortex is composed predominantly of pyramidal cells, and consistent with this composition, many GFP-positive cells could be identified as pyramidal by a prominent proximal apical dendrite delineated by the SK3-1B–GFP fluorescence (Fig. 3$A_2$, arrows). Within these neurons, the SK3-1B–GFP fusion protein appeared to be localized to the intracellular region in the soma–proximal dendrites. This distribution was in dramatic contrast to that seen in age-matched animals expressing yellow fluorescent protein (YFP), a spectral variant of GFP, under the control of the Thy-1 promoter in a different transgenic animal (Fig. 3*B*) (Feng et al., 2000). Because previous work in model systems has shown that the SK3-1B isoform acts as a dominant negative by intracellular trapping $SK_{Ca}$ subunits (Tomita et al., 2003), these observations are concordant with the expectation that SK3-1B may function similarly as an $SK_{Ca}$ dominant negative in the cortex.

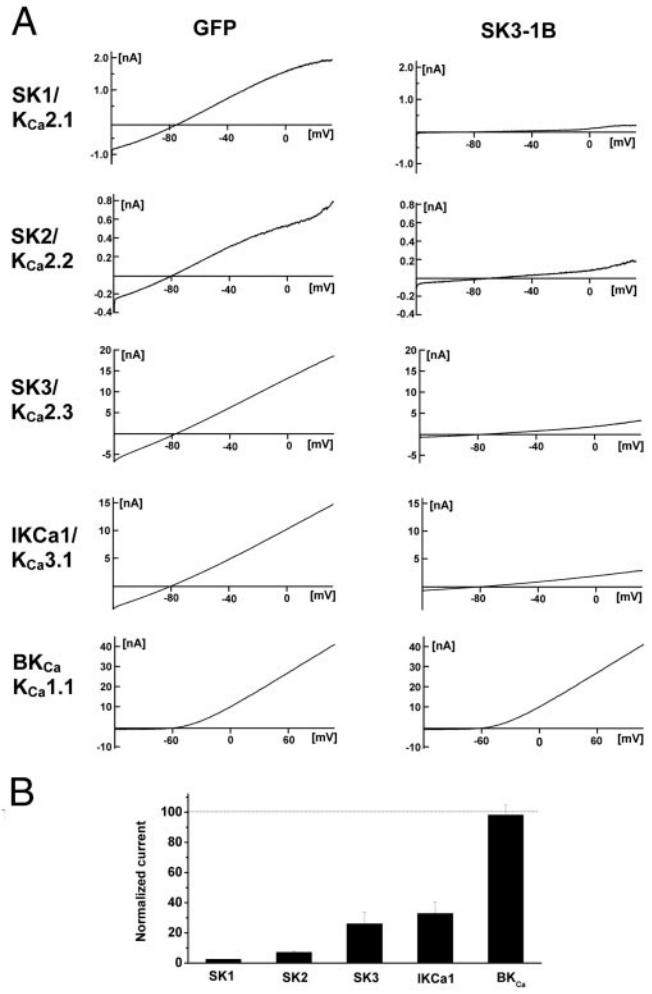


**Figure 2.** SK3-1B suppresses $K_{Ca}2.1$–$K_{Ca}2.3$ (SK1–SK3) and $K_{Ca}3.1$ ($IK_{Ca}1$) but not $K_{Ca}1.1$ ($BK_{Ca}$). *A*, Current traces from cell lines stably expressing indicated channels after GFP vector (left) or SK3-1B (right) transfection. Cells were held at −80 mV, and 200 msec voltage ramps from −120 to 40 mV (120 mV for $BK_{Ca}$) were used for the recordings. The internal pipette solution contained 1 μM free $Ca^{2+}$ to activate the respective $K_{Ca}$ currents. *B*, Summary of current suppression by SK3-1B in cell lines. Slope conductances were measured at −80 mV for the SK and $IK_{Ca}$ currents and at 60 mV for the BK current traces. Slope conductances in the SK3-1B-transfected cells were normalized to the GFP-transfected controls. Error bars indicate SEM.

Figure 4 illustrates that expression of SK3-1B in pyramidal cells of layer V in this transgenic animal resulted in the suppression of $I_{mAHP}$ ( $p = 0.01$). We confirmed that this suppression reflected an inhibition of the function of $SK_{Ca}$ channels by examining the effect of apamin. As shown in Figure 4*B*, the ability of apamin to inhibit $I_{mAHP}$ was almost completely occluded in pyramidal cells derived from mice expressing the SK3-1B transgene (SK3-1B–GFP, $n = 35$ cells; wild type, $n = 25$ cells). Furthermore, because pyramidal cells expressing YFP under the control of the same promoter (Thy-1, in a different transgenic mouse) still displayed robust apamin-sensitive $I_{mAHP}$s (115 ± 19 pA; $n = 5$ cells; data not shown), the almost complete suppression of the apamin-sensitive component of $I_{mAHP}$ in the SK3-1B–GFP transgenic mice was unlikely to have resulted from the activity of the Thy-1 promoter or the expression of GFP. Thus, in these transgenic mice, SK3-1B expression effectively inhibits the function of calcium-activated potassium channels formed by $SK_{Ca}$ subunits in pyramidal cells of layer V. Remarkably, despite this inhibition, we could not detect any significant differences in the amplitude

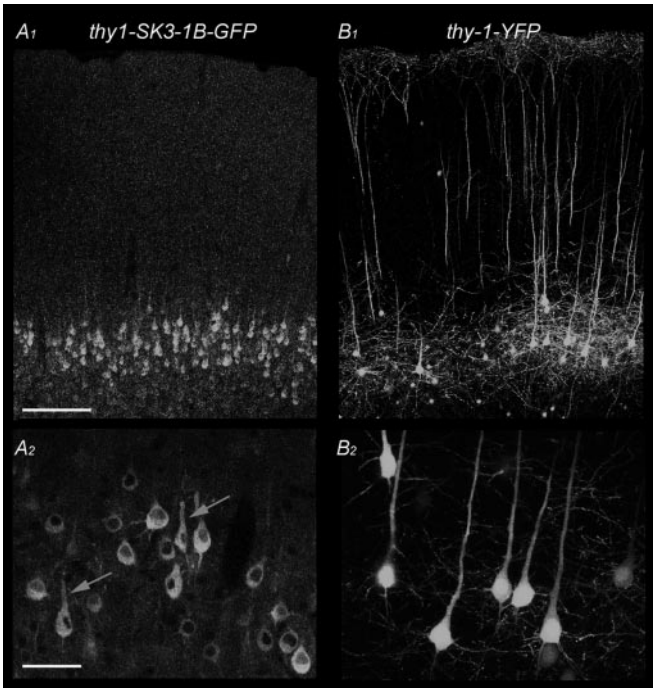


**Figure 3.** Distribution of SK3-1B–GFP in the anterior somatosensory cortex in a transgenic mouse expressing this fusion protein under the control of the Thy-1 promoter. *A*$_1$, *A*$_2$, The SK3-1B–GFP fusion protein is expressed in layer V, but its distribution is restricted to the soma and proximal dendrite (arrows). *B*$_1$, *B*$_2$, YFP expressed under the control of the Thy-1 promoter in a different transgenic mouse is also expressed in layer V, but the YFP completely fills the dendritic arbor of pyramidal cells. Scale bars: *A*$_1$, *B*$_1$, 200 $\mu$m; *A*$_2$, *B*$_2$, 50 $\mu$m.

of $I_{sAHP}$ in these same cells. As expected, carbachol (30 $\mu$M) inhibited $I_{sAHP}$ both in the SK3-1B transgenic animals as well as in their wild-type litter-matched controls ($n$ = 10 and 4 cells, respectively).

## Discussion

We used overexpression of $K_{Ca}2.x$ ($SK_{Ca}$) subunits and expression of an $SK_{Ca}$–$IK_{Ca}$ dominant negative to test for the involvement of these potassium channel subunits in the formation of the ion channels responsible for $I_{sAHP}$. The results from these complementary experiments lead us to conclude that $SK_{Ca}$ channel subunits do not contribute to the formation of the potassium channels underlying $I_{sAHP}$ in pyramidal cells of the cortex.

Pyramidal cells of layer V of the anterior cerebral cortex express calcium-activated AHPs that are mediated by a medium-duration current known as $I_{mAHP}$ and a slower current known as $I_{sAHP}$ (Schwindt et al., 1988a; Stocker et al., 1999). The properties of the first of these currents, including its sensitivity to apamin and bicuculline, have identified it as being carried by channels composed of $SK_{Ca}$ isoforms (Schwindt et al., 1988a,b; Stocker et al., 1999). In contrast, $I_{sAHP}$ is insensitive to apamin and bicuculline but is readily inhibited by the activation of neurotransmitter receptors coupled to heterotrimeric G-proteins of the $G\alpha_s$ and $G\alpha_{q/11}$ families (Nicoll, 1988; Strobaek et al., 2000; Sah and Faber, 2002). The initial report that SK1 could form apamin-insensitive calcium-activated potassium channels leads to the idea that $I_{sAHP}$ could result from the expression of $SK_{Ca}$, and in particular $K_{Ca}2.1$ genes, in pyramidal cells (Kohler et al., 1996; Vergara et al., 1998). However, subsequent work has shown that $K_{Ca}2.1$ exhibits reduced but nevertheless significant apamin sensitivity in mammalian cells (Strobaek et al., 2000; Grunnet et al., 2001), raising doubts as to this interpretation. Furthermore, subsequent work has unveiled differences in the time course of intracellular calcium transients and of the $I_{sAHP}$ as well as a disturbing incongruence between the ready modulation of $I_{sAHP}$ by a variety of neurotransmitters and the relative refractoriness to modulation by currents carried through $SK_{Ca}$ channels (Sah and Faber, 2002; Vogalis et al., 2003; Abel et al., 2004). Together, these observations have questioned the role of $SK_{Ca}$ channels in mediating the $I_{sAHP}$.

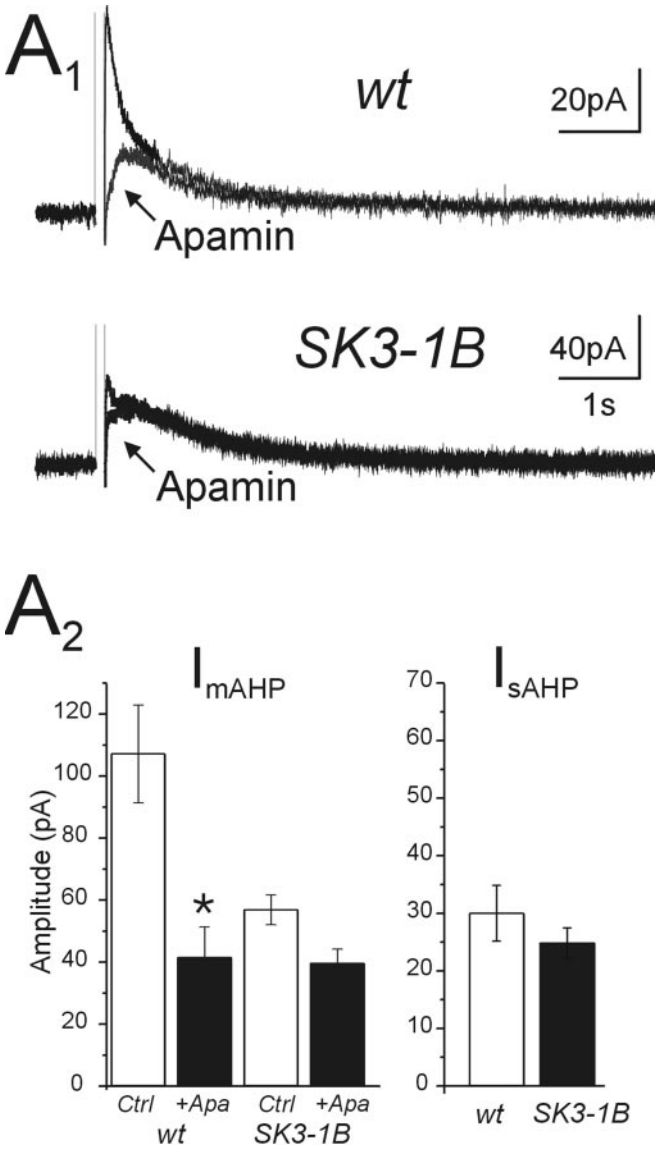


**Figure 4.** SK3-1B–GFP suppresses $I_{mAHP}$ but is without effect on $I_{sAHP}$. *A*$_1$, Comparison of $I_{AHP}$ in pyramidal cells of layer V derived from SK3-1B–GFP-expressing and wild-type mice. *A*$_2$, Plot summarizing the effects of SK3-1B–GFP expression on $I_{mAHP}$ and $I_{sAHP}$. SK3-1B–GFP expression suppressed the apamin-sensitive $I_{mAHP}$ but had no significant effect on $I_{sAHP}$. +Apa, Apamin; Ctrl, control; wt, wild type. Error bars indicate SEM. $*p < 0.005$.

One possible interpretation of these results is that $SK_{Ca}$ channels exhibit unique properties in neurons because of cell-specific modification of the $SK_{Ca}$ isoforms, a unique channel stoichiometry, or the incorporation of auxiliary subunits. Alternatively, it is possible that the ion channels responsible for $I_{sAHP}$ may belong to a molecular family other than $K_{Ca}2.x$–$SK_{Ca}$. Because it seems unlikely that purely pharmacological approaches can satisfactorily distinguish between these possibilities, we used molecular biological approaches to directly target $SK_{Ca}$ channels in pyrami-

dal cells of the cortex and thus distinguish between these two possibilities.

In an initial series of experiments, we overexpressed $rK_{Ca}2.1$ and $rK_{Ca}2.2$, the $SK_{Ca}$ isoforms that predominate in the anterior cerebral cortex (Stocker and Pedarzani, 2000). This overexpression resulted in a large increase in the amplitude of an apamin-sensitive $I_{mAHP}$, confirming the functional overexpression of these genes. However, overexpressing $rK_{Ca}2.1$ and $rK_{Ca}2.2$ produced no detectable effect of $I_{sAHP}$ in the same cells. These results suggested that $K_{Ca}2.x$–$SK_{Ca}$ channels do not mediate $I_{sAHP}$, but they could not completely rule out alternative possibilities. For example, it remained possible that incorporation of an auxiliary subunit could have acted as a rate-limiting step for the formation of the $I_{sAHP}$ channels, thus explaining the failure of the $K_{Ca}2.1$–$K_{Ca}2.2$ overexpression to increase $I_{sAHP}$.

To address this limitation, in a second series of experiments, we took advantage of the recent discovery of a truncated variant of SK3 (SK3-1B) that can trap endogenously expressed $SK_{Ca}$ subunits in intracellular compartments and prevent their trafficking to the plasma membrane (Tomita et al., 2003). Expression of SK3-1B in heterologous cell lines stably expressing $K_{Ca}2.1$–$K_{Ca}2.3$ and $K_{Ca}3.1$ effectively suppressed these currents, although it had no effect on currents mediated by $K_{Ca}1.1$ channels. These results indicate that the SK3-1B isoform can act as an effective dominant-negative inhibitor of $SK_{Ca}$–$IK_{Ca}$ channels.

To examine the effect of SK3-1B on $I_{sAHP}$, we used a transgenic mouse expressing SK3-1B under the control of the Thy-1 promoter (Shakkottai et al., 2004). In these animals, SK3-1B fused to GFP is expressed in pyramidal cells of layer V, where it accumulates in somatic intracellular compartments. This pattern of expression suggests that in pyramidal cells, as seen for other cell types (Tomita et al., 2003), the transgene may be able to trap native $SK_{Ca}$ and $IK_{Ca}$ subunits intracellularly, thus making them unavailable for incorporation into ion channels trafficking to the plasma membrane. Consistent with this idea, pyramidal cells of layer V derived from SK3-1B transgenic animals expressed a greatly reduced apamin-sensitive $I_{mAHP}$. Despite this effective suppression of $SK_{Ca}$ channel function, in these same animals, $I_{sAHP}$ remained essentially unchanged. These results are again inconsistent with the idea that $SK_{Ca}$ channels contribute to the formation of the ion channel mediating $I_{sAHP}$ in pyramidal cells.

Many central and peripheral neurons express sAHPs that play a key role in regulating spike-firing pattern and are the target for a multitude of neurotransmitters. A search for the molecular substrate for these sAHPs has resulted in the identification of several distinct potassium channel subunits capable of assembling calcium-activated potassium currents (Vergara et al., 1998; Sah and Faber, 2002). Among these, $SK_{Ca}$ channels initially emerged as the leading candidates for mediating these sAHPs, although several pharmacological and physiological anomalies have more recently begun to question this idea (Sah and Faber, 2002; Vogalis et al., 2003). In the current study, we find that direct molecular targeting of the $SK_{Ca}$ subunit in pyramidal cells of the cerebral cortex allows us to enhance or inhibit $I_{mAHP}$ but that these same manipulations have no detectable effect on $I_{sAHP}$. These results agree with the idea that $SK_{Ca}$-containing channels mediate the medium AHP in pyramidal cells but do not support the idea that $SK_{Ca}$ isoforms contribute to the formation of the ion channels responsible for the sAHP.

$K_{Ca}$ channels are encoded by eight genes (*$K_{Ca}1.1$*, *$K_{Ca}2.1$*–*$K_{Ca}2.3$*, *$K_{Ca}3.1$*, *$K_{Ca}4.1$*, *$K_{Ca}4.2$*, and *$K_{Ca}5.1$*) in the mammalian genome (Gutman et al., 2003), and our studies exclude *$K_{Ca}2.1$*–*$K_{Ca}2.3$* and *$K_{Ca}3.1$* as being responsible for the sAHP. Of the remaining genes, *$K_{Ca}1.1$* (*$BK_{Ca}$*) and *$K_{Ca}4.1$* (*Slack*) are abundantly expressed in the brain, and these isoforms can coassemble to generate an $IK_{Ca}$ current (Joiner et al., 1998). The $K_{Ca}1.1$–$K_{Ca}4.1$ heterotetramer is a plausible candidate for the $I_{sAHP}$. An alternative possibility is that a different potassium channel with yet unrecognized calcium sensitivity mediates the current underlying the $I_{sAHP}$ in cortical neurons. In addition, it is possible that calcium may also indirectly activate a potassium current through a signal transduction pathway to elicit the sAHP current (Schwindt et al., 1992; Sah and Faber, 2002; Abel et al., 2004). By excluding $K_{Ca}2.1$–$K_{Ca}2.3$ and $K_{Ca}3.1$, the present study narrows the search for the channel responsible for $I_{sAHP}$.

## References


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