SK Channel Regulation of Dendritic Excitability and Dendrodendritic Inhibition in the Olfactory Bulb

Brady J. Maher and Gary L. Westbrook
Vollum Institute, Oregon Health and Science University, Portland, Oregon

Submitted 28 July 2005; accepted in final form 14 August 2005

INTRODUCTION

The unique morphology of mitral cell dendrites in the olfactory bulb provides an instructive model of dendritic excitability. Mitral cells receive direct afferent input on tufts of distal apical dendrites in the glomerular layer. Apical dendrites act nearly like axons in that they can initiate action potentials that are propagated to the soma (Chen et al. 1997). These actions potentials then backpropagate into the extensive lateral dendrites within the external plexiform layer (EPL). Lateral dendrites taper along their course and are the site of lateral and recurrent inhibition mediated by dendrodendritic synapses with granule cells. Action potentials in lateral dendrites are more labile than in apical dendrites and thus are particularly subject to modulation by both intrinsic and synaptic conductances (Christie and Westbrook 2003; Davison et al. 2004). In other areas of the brain, calcium-activated potassium channels regulate action potential interspike interval and spike frequency adaptation (Sah and Faber 2002; Vergara et al. 1998). Small-conductance calcium-activated potassium (SK) channels, responsible for slow afterhyperpolarizations in CA1 pyramidal cells (Bond et al. 2004), are also expressed in the olfactory bulb (Stocker and Pedarzani 2000).

We examined the role of SK channels in olfactory bulb using whole cell recordings. Mitral cells, but not granule cells, had apamin-sensitive potassium currents consistent with expression of SK channels. Inhibition of SK channels increased action potential firing in mitral cells and thus increased dendrodendritic inhibition. SK currents and NMDA autoreceptor currents had a similar time course, and NMDA receptor-mediated calcium influx was capable of activating SK channels.

METHODS

Preparation of slices

Olfactory bulb slices were prepared from 9- to 20-day-old C57BL/6J. Mice were deeply anesthetized with halothane and then decapitated per protocols approved by the IACUC at Oregon Health and Science University. Bulbs were rapidly removed and immersed in ice-cold oxygenated (95% O2-5% CO2) artificial cerebrospinal fluid (ACSF). This solution was the same as our standard extracellular recording solution (see following text) except that 2 mM CaCl2 and 1 mM MgCl2 were replaced with 0.5 mM CaCl2 and 4 mM MgCl2. Horizontal slices (290 μM) were cut using a vibrating blade vibrotome (VT1000S; Leica, Bannockburn, IL). Slices were incubated in an oxygenated holding chamber for 30 min at 37°C and then stored at room temperature.

Slices were visualized using infrared differential interference contrast microscopy (IR/DIC, Zeiss Axioskop) equipped with a CCD camera (XC-ST30, Sony, Japan) and 40× Zeiss water-immersion objective (0.75 N.A.). Mitral and granule cells could be discriminated easily on the basis of morphology. All experiments were performed at 31–34°C.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Voltage-clamp recordings

Whole cell patch recording from mitral and granule cells were obtained using previously described methods (Stuart et al. 1993). For all experiments, oxygenated (95% O₂-5% CO₂) ACSF contained (in mM) 125 NaCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 3 KCl, and 25 dextrose, pH 7.3. Depending on the experiment, as indicated, we added either 100 µM or 1 mM MgCl₂ and 0.5 mM or 2 mM CaCl₂. For some experiments, synaptic currents were blocked with 100 µM D,L-2-amino-5-phosphonopentanoic acid (AP5), 10 µM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide (NBQX), 5 µM gabazine (Tocris, Ellisville, MO); sodium conductances were blocked with 0.5 µM TTX; and voltage-dependent K⁺ channels and BK channels were blocked with 1 mM tetraethylammonium chloride (TEA). Patch pipettes were fabricated from borosilicate glass (TW150F-6; WPI, Sarasota, FL) with resistances of 1–3 MΩ for mitral cells and 3–7 MΩ for granule cells. Except where indicated, pipettes were filled with (in mM) 135 Kgluconate, 10 KCl, 1 MgCl₂, 10 HEPES, 2 Na-ATP, and 0.4 Na-GTP; pH was adjusted to 7.3 with KOH. To minimize the effect of exogenous buffers on intracellular Ca²⁺, pipettes contained 0.5 mM EGTA. Mitral cell IPSCs were recorded using an internal solution containing (in mM) 125 KCl, 2 MgCl₂, 10 HEPES, 2 Na-ATP, 0.4 Na-GTP, and 0.5 EGTA, adjusted to pH 7.3 with KOH.

Voltage-clamp currents, recorded with a Multiclamp 700A amplifier (Axon instruments, Foster City, CA), were filtered at 2 kHz (4-pole Bessel) and digitized at 10 kHz. Data were acquired using Axograph 4.9 (Axon Instruments). In all recordings membrane voltage \( V_m \) and access resistance \( R_s \) were monitored. Data acquisition was terminated when \( V_m \) was more positive than -48 mV or \( R_s \) was >15 MΩ. The holding potential for current recordings was 55 or 70 mV as indicated for each experiment. For electrical stimulation of the glomerular layer, we used a tungsten bipolar electrode (FHC, Brunswick, ME) placed on the surface of the slice. Computer-generated stimulation pulses triggered a stimulus isolation unit (10–100 V, 0.1 ms; Digitimer, Herfordshire, UK).

Current-clamp recordings

For measurements of cell firing patterns, mitral and granule cells were held at -65 to -72 mV by steady current injection. Action potential trains were evoked with current pulses (100–400 pA, 3 s). Experiments were terminated if action potential threshold or amplitude changed during recordings.

Data analysis and statistics

We used AxoGraph 4.9 on a Macintosh G4 computer for data analysis. For quantification of SK currents, we integrated the currents beginning at the peak of the response (ca. 5 ms following the end of a voltage command pulse, see e.g., Fig. 3C). Results are reported as means ± SE. For all experiments, statistical significance was determined using ANOVA with repeated measures and Student’s \( t \)-test within Microsoft EXCEL (Richmond, WA). Significance was set at \( P \leq 0.05 \).

RESULTS

SK channels in mitral cells

We first were interested in identifying cells in the olfactory bulb that express SK currents. To isolate SK currents,
we blocked transmitter and voltage-gated channels with NBQX, AP5, gabazine, TTX, and TEA. Under these conditions, somatic depolarizing steps of mitral cells (+10 mV, 100 ms) produced long-lasting outward currents (12.3 ± 1.1 pC, n = 27) that were inhibited by the SK channel antagonist 100 nM apamin (2.5 ± 0.4 pC (n = 18); P < 0.0001 Fig. 1). In granule cells, the same protocol did not evoke an outward current (n = 6, Fig. 1C). Likewise, apamin had no effect on granule cells. Thus mitral cells, but not granule cells express SK currents. Calcium-activated potassium channels can couple with several classes of voltage-gated calcium channels (Davies et al. 1996; Marrion and Tavalin 1998; Prakriya and Lingle 1999; Wolfart et al. 2001; Womack et al. 2004). As shown in Fig. 1B, the nonselective calcium channel blocker Cd2+ (200 μM) strongly inhibited slow outward currents in mitral cells [2.98 ± 0.5 pC (n = 9); P < 0.001 Fig. 1D]. Co-application of Cd2+ and apamin did not produce any further inhibition, indicating that Cd2+ completely blocked the apamin-sensitive current under these conditions. Thus voltage-activated calcium channels are a primary source of Ca2+ for SK channel activation.

FIG. 2. Brief depolarizing steps in mitral cells also elicited small-conductance calcium-activated potassium channel (SK) currents. A: amplitude and duration of apamin-sensitive currents increased with the duration of the depolarizing voltage step (+10 mV, 2–100 ms). B: data points represent average charge (pC) of the apamin-sensitive current obtained by subtraction of control traces from traces after apamin. The extracellular solution contained NBQX, AP5, gabazine, TTX, and TEA.

FIG. 3. SK channel currents mask NMDA autoreceptor currents. A: in low extracellular Mg2+ (100 μM), outward currents evoked by depolarizing steps (+10 mV, 100 ms) were biphasic. Apamin blocked the outward current revealing an AP5-sensitive inward current (A1), characteristic of NMDA autoreceptor activation. B: apamin-sensitive outward currents and an underlying NMDA autoreceptor current were also evoked by 2 ms depolarizing voltage steps to +10 mV. The extracellular solution contained NBQX, gabazine, TTX, and TEA.
SK channel activation by short voltage steps

Although 100 ms pulses are typically used to evoke SK currents, the duration and pattern of depolarization will affect the calcium entry and thus the activation of SK channels. In particular, the duration of action potentials in neurons is much briefer, ca. 2 ms. As expected, the apamin-sensitive current decreased as we decreased the duration of the step depolarization (Fig. 2). However, even 2 ms depolarizations activated apamin-sensitive currents [1.86 ± 0.25 pC (n = 6); Fig. 2B]. Thus single backpropagating action potentials may be sufficient to activate SK channels in mitral cell dendrites.

Mitral cell dendrites release glutamate after somatic current injection (Isaacson 1999; Nicoll and Jahr 1982), resulting in activation of dendritic AMPA and NMDA autoreceptors (Friedman and Strowbridge 2000; Schoppa and Westbrook 2002). NMDA autoreceptor currents can last hundreds of milliseconds and are an additional source of dendritic calcium transients (Isaacson 1999). We reasoned that currents evoked by depolarizing step pulses should contain SK as well as NMDA autoreceptor components. To more easily detect NMDA autoreceptor currents, we lowered extracellular Mg$^{2+}$ to 100 μM. Under these conditions, currents evoked by 100 ms voltage steps often had a prominent shoulder that followed the peak response (Fig. 3A, control). Apamin blocked the outward current and unmasked an underlying inward current (Fig. 3A, apamin) that was subsequently blocked by AP5, consistent with an NMDA autoreceptor current (Fig. 3A1, AP5 + apamin). Similar results were obtained with 2 ms voltage steps (Fig. 3B). Interestingly, both the SK and NMDA autoreceptor currents had similar durations. When measured after a 100 ms voltage step, the duration for the SK current was 337 ± 38 and 449.2 ± 97 ms for the NMDA autoreceptor current. Thus under these conditions the NMDA current was obscured by the outward SK current.

We next examined whether NMDA autoreceptor-mediated calcium influx could activate SK channels in mitral cells. Block of the autoreceptor current with AP5 actually increased the net outward current evoked by 100 ms voltage steps (Fig. 4A), Therefore Ca$^{2+}$ influx via NMDA autoreceptors is not necessary for full activation of SK channels. Rather, calcium entry through voltage-activated Ca$^{2+}$ channels appears sufficient. However currents evoked by 2 ms voltage steps were decreased by AP5 [49 ± 9% of control (n = 8); Fig. 4, B and C]. As the duration of voltage step increased AP5 increased the apamin-sensitive current, suggesting that the contribution of autoreceptors to SK activation was overwhelmed by calcium influx through voltage-gated Ca$^{2+}$ channels (Fig. 4C). In physiological concentrations of Mg$^{2+}$ (1 mM), SK channel activation with 2 ms voltage steps was not affected by AP5 [110 ± 10% of control (n = 8); P = 0.65], consistent with the small amplitude of the NMDA autoreceptor current under these conditions.

SK channels and mitral cell firing patterns

The afterhyperpolarizations due to SK channels have prominent effects on cell firing (Sah and Faber 2002; Vergara et al. 1998). We thus examined mitral cell firing patterns after blocking synaptic receptors with NBQX, AP5, and gabazine. Long depolarizing current pulses (3 s) were used to produce trains of action potentials that showed relatively little accommodation. As previously shown in rat olfactory bulb (Friedman and Strowbridge 2000), a reduction in extracellular Ca$^{2+}$ increased the firing frequency in
action potential trains [278 ± 65% of control (n = 3); Fig. 5A]. Reduction of extracellular Ca\(^{2+}\) also reduced the afterhyperpolarization following each action potential in the train (Fig. 5A). As shown in Fig. 6A, apamin had a similar effect as low extracellular Ca\(^{2+}\) on firing frequency in action potential trains [214 ± 27% of control (n = 9)], suggesting that SK channels regulate cell firing in mitral cells. In the absence of AP5, the NMDA autoreceptor response could be seen as a persistent depolarization at the end of the current pulse (Fig. 6A, inset). However, AP5 did not influence the effect of apamin on cell firing, consistent with voltage-clamp recordings that showed little SK activation by NMDA autoreceptors in physiological Mg\(^{2+}\).

Apamin had no effect on action potential frequency in granule cells (107 ± 8% of control; P = 0.41, n = 5 Fig. 6B).

Mitral cells and granule cells form reciprocal dendrodendritic synapses. Thus action potentials in mitral cell dendrites drive recurrent inhibition by activating GABA release from granule cells. To examine the effect of SK current on dendrodendritic inhibition, we voltage clamped mitral cells at -70 mV using KCl-filled pipettes. Electrical stimulation in the glomerular layer evoked barrages of inward currents mediated by GABA\(_{A}\) receptors (Fig. 7A) (Schoppa et al. 1998). Apamin strongly increased dendrodendritic inhibition (Fig. 7A2) regardless of the extracellular Mg\(^{2+}\) concentration: 170 ± 10% of control in 1 mM Mg\(^{2+}\) (n = 6); 193 ± 34% of control in 0.1 mM Mg\(^{2+}\) (n = 10; Fig. 7B). As in previous studies (Schoppa et al. 1998), application of AP5 considerably reduced dendrodendritic inhibition in all neurons tested [44 ± 22% of control in 1 mM Mg\(^{2+}\) (n = 5); 12 ± 5% of control in 0.1 mM Mg\(^{2+}\) (n = 4); Fig. 7B].

**FIG. 5.** Action potential frequency in mitral cells is dependent on extracellular calcium. A: in current-clamp, depolarizing currents (3 s, 150 pA) produced trains of action potentials with prominent afterhyperpolarizations (top). The extracellular solution contained 5 μM gabazine, 10 μM NBQX, 2 mM Ca\(^{2+}\), 1 mM Mg\(^{2+}\). In low extracellular Ca\(^{2+}\) (0.5 mM, middle) the average frequency increased from 9 to 17 Hz for this cell. A1: expanded time scale for traces found in A show that low external Ca\(^{2+}\) blocked the afterhyperpolarizations. The action potential frequency recovered after return to 2 mM Ca\(^{2+}\) (bottom).

**FIG. 6.** Block of SK channels increases action potential frequency in mitral cells. A: a train of action potentials was evoked by a depolarizing current pulse in control conditions (5 μM gabazine, 10 μM NBQX, 2 mM Ca\(^{2+}\), 1 mM Mg\(^{2+}\), top). Apamin (100 nM) increase the average action potential frequency from 12 to 40 Hz in this cell (middle), similar to the effect of low extracellular calcium. At the end of the current pulse, apamin (100 nM) unmasked a residual depolarization (ap) due to NMDA autoreceptors (inset). A1: expanded time scale for traces found in A show that apamin blocked afterhyperpolarizations. B and B1: in granule cells, apamin did not affect the frequency of evoked action potentials. For this cell, the frequency of action potentials in the train was 10 Hz in control and 11 Hz in the presence of apamin (100 nM). Apamin had no effect on hyperpolarization at the end of the current pulse (inset) or following individual action potentials.
DISCUSSION

Neuronal excitability and apamin-sensitive currents

Our results indicate that an apamin-sensitive current is present in mitral cells of the olfactory bulb. By reducing action potential frequency in mitral cells, the SK current can dampen inhibition driven by dendrodendritic synapses with granule cells. Apamin-sensitive currents are present in neurons in many brain regions, in agreement with in situ hybridization and immunohistochemical studies depicting a partially overlapping but distinct distribution pattern of SK subunits (Gehlert and Gackenheimer 1993; Sailer et al. 2004; Stocker and Pedarzani 2000). Calcium entry during action potentials activates SK channels that underlie a component of the afterhyperpolarization, thus positioning these current to control the frequency and regularity of action potentials.

Depending on the neuronal subtype and combination of SK channel subunits, SK channels contribute to distinct patterns of neuronal output including the shaping of synaptic responses as well as rhythmic and burst firing (Bond et al. 2005). For example, in dopaminergic midbrain neurons that are tonically active, block of SK channels increases spontaneous action potentials and also reduces the precision of evoked action potential trains (Wolfart et al. 2001). In the subthalamic nucleus, apamin converts tonic action potential firing into burst activity (Hallworth et al. 2003). SK channels contribute to rhythmic bursting activity early in the development of Purkinje cells (Cingolani et al. 2002). Consistent with the presence of SK currents, mitral cells showed the expected loss of the afterhyperpolarization and increased evoked firing in the presence of apamin. However, natural stimuli cause bursts of action potentials in mitral cells locked to the respiratory cycle (Cang and Isaacson 2003), thus SK currents may also affect these more complex firing patterns.

SK channel subunits

In the olfactory bulb, SK1 and SK3 are highly expressed in the glomerulus and the EPL with SK2 expressed at lower levels in these layers. None of the subunits are highly expressed in the granule cell layer (Sailer et al. 2004). This pattern is consistent with the presence of robust SK current in mitral cells but not in granule cells. The strong immunostaining pattern in the EPL and glomerular layer could indicate that SK channels are preferentially expressed in mitral cell dendrites, although our data do not directly address the distribution of SK channels in mitral cells. In the neocortex and hippocampus, SK1 and SK2 appear to be expressed in pyramidal cell dendrites, whereas SK3 has been reported to be located in hippocampal presynaptic terminals (Obermair et al. 2003; Sailer et al. 2002). Recently, transfected myc-tagged SK2 subunits have been localized to dendritic spines in cultured hippocampal neurons (Ngo-Anh et al. 2005). The prominent staining of SK3 in the EPL (Sailer et al. 2004) suggests that the expression of SK subunits in mitral cells may differ from hippocampal pyramidal cells. This may not be surprising given the distinctive morphology of mitral cell dendrites that lack spines and have presynaptic glutamate release sites.

SK channels and NMDA autoreceptors

The dendritic release of glutamate from mitral cells activates not only the postsynaptic granule cells but also glutamate receptors on the surrounding dendritic membrane. This can be
observed after block of dendrodendritic inhibition as a long-lasting excitation of mitral cells (Nicoll and Jahr 1982; Nowycky et al. 1981) due to activation of dendritic NMDA autoreceptors (Isaacson 1999). Our results indicate NMDA autoreceptor currents and SK currents have an overlapping time course after membrane depolarization.

This situation suggests an interaction between NMDA autoreceptor currents and SK channel currents in controlling action potential patterning. SK currents could shunt auto-receptor-mediated depolarization of dendrites without altering NMDA receptor-mediated calcium influx, the latter of which may be important for cell signaling or transmitter release. Block of NMDA receptors reduces the frequency and clustering of action potentials evoked in mitral cells (Friedman and Strowbridge 2000), suggesting that NMDA autoreceptors can increase dendritic excitability.

It was initially suggested that SK channels are tightly coupled to L-type calcium channels (Marrion and Tavalin 1998). However, it is now clear that multiple calcium channel subtypes and calcium sources can activate SK channels in certain situations (Stocker 2004). Ca2⁺ influx through NMDA receptors can also activate calcium-activated potassium channels. BK-type Ca2⁺-activated potassium channels can be directly activated by NMDA receptors in granule cells of the olfactory bulb (Isaacson and Murphy 2001). In hippocampal pyramidal cells, long somatic application of NMDA can evoke SK currents (Sah and Faber 2002). This action can be quite localized because NMDA receptor-dependent activation of SK channels in CA1 dendritic spines can reduce evoked excitatory postsynaptic potentials (EPSPs) (Ngo-Anh et al. 2005). Likewise in the amygdala SK channels, activated by Ca2⁺ influx through synaptically activated NMDA receptors, reduced EPSPs and the resulting LTP (Faber et al. 2005). In our experiments using somatic recording, we were only able to detect an NMDA receptor contribution to SK channel activation under some conditions (low Mg2⁺, brief voltage steps). However, this does not exclude local activation of SK channels by NMDA autoreceptors under physiological conditions.

**SK channels and dendritic excitability**

Dendritic excitability is regulated by the cell-specific complement of voltage- and ligand-gated ion channels distributed across dendritic compartments (Hauesser et al. 2000). In mitral cells, action potentials attenuate in lateral dendrites, but not in apical dendrites, as a result of A-type potassium currents as well as GABAergic inhibition (Christie and Westbrook 2003; Lowe 2002; Margrie et al. 2001; Xiong and Chen 2002). Focal application of apamin onto apical dendrites of CA1 pyramidal neurons increases the duration of dendritic plateau potentials (Cai et al. 2004), suggesting that SK channels can regulate local excitability. In this case, A and SK currents have distinctive effects on plateau potentials, perhaps reflecting their different biophysical characteristics. Because mitral cell dendrites reliably transmit backpropagating action potentials that are necessary for glutamate release, SK channels likely have a dual role in regulating dendritic excitability and transmitter release.

**Acknowledgments**

We thank J. Adelman for helpful discussions.

**Grants**

This work was supported by National Institutes of Health Grant NS-26494 to G. L. Westbrook and Fellowship S-T32-DA-07262 to B. J. Maher.

**References**


