Somatic localization of a specific large-conductance calcium-activated potassium channel subtype controls compartmentalized ethanol sensitivity in the nucleus accumbens

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Introduction
Anatomical, behavioral, and electrophysiological studies have clearly identified the nucleus accumbens (NAcc) as a key region for the addictive properties of drugs of abuse, including alcohol. Although our knowledge of neuronal channels and receptors altered by alcohol has grown significantly, the mechanisms underlying alcohol-mediated modulation of synaptic integration remain poorly understood. Because synaptic integration takes place primarily in the dendritic arborization (for review, see Reyes, 2001), it is particularly important to determine whether the actions of drugs of abuse, such as alcohol on ion channels, differ between the dendritic and somatic compartments. This information has been difficult to obtain, because most electrophysiological studies that examine the effects of alcohol do not differentiate between signals elicited from the soma and the dendrites. In this study, we used excised inside-out patches that allow differential study of channels from each compartment.

Recent findings indicate that dendrite physiology varies from that of the soma. Properties of Na⁺ and some K⁺ channels were found to be different between soma and dendrites in a number of brain regions (Hoffman et al., 1997; Mickus et al., 1999; Martina et al., 2000; Williams and Stuart, 2000). Moreover, Haberstock-Debic et al. (2003) recently reported that chronic morphine exposure induces the internalization of dendritic but not somatic μ-opioid receptors in NAcc medium spiny neurons. We focused on BK channels because they are well known for shaping action potentials (Golding et al., 1999; Shao et al., 1999; Bennett et al., 2000; Pedarzani et al., 2000; Pedarzani et al., 2000; Faber and Sah, 2002; Edgerton and Reinhart, 2003), which is a characteristic likely to be a key element coding the addictive properties of alcohol. In addition, they are very sensitive to intracellular calcium, making them well positioned to respond to modifications of the properties of the Ca²⁺-permeable NMDA receptor, an ionotropic receptor that is regarded as pivotal in synaptic plasticity in general (Malenka and Nicoll, 1993) and in drug addiction in particular (Trujillo and Akil, 1995). Moreover, BK channels are robustly potentiated by relevant concentrations of ethanol (Dopico et al., 1996; Chu et al., 1998; Knott et al., 2002; Crowley et al., 2003), although one study...
reported an inhibitory effect on neurons from dorsal root ganglia (Gruß et al., 2001). Finally, recent evidence obtained using Caenorhabditis elegans suggests that the BK channel is the sole mediator of intoxication in this worm model system (Davies et al., 2003).

BK channels exist as a complex of subunits, including the pore-forming α subunit and four regulatory β subunits, which are the products of four distinct genes (β1–β4). BK channels are characterized by their large conductance (120–300 pS) and by their sensitivity to both voltage and intracellular Ca2+, properties that are modulated by β subunits (Kaczkorowski et al., 1996; Gribkoff et al., 1997; Vergara et al., 1998; Coetzez et al., 1999; Jagota, 2001). The BK α subunit is ubiquitously expressed in the brain (Chang et al., 1997a). In contrast, among the β subunits, only the β4 subunit has been reported in the brain (Behrens et al., 2000; Brenner et al., 2000). Use of the inside-out patch-clamp approach enabled us to examine BK channel properties in distinct neuronal compartments. Our data are consistent with the interpretation that compartment-dependent ethanol (EtOH)-induced potentiation of NAcc BK channel activity depends on the selective expression of the BK channel auxiliary subunit β4 in the cell body.

Materials and Methods

Animals, slice preparation, and experimental solutions. We used young male Sprague Dawley rats (80–150 gm) to prepare NAcc slices as described previously (Martin and Siggins, 2002). Briefly, after decapitation, the brains were rapidly transferred into a cold (4°C), oxygenated, low-calcium HEPES-buffered salt solution containing the following (in mM): 234 sucrose, 2.5 KCl, 2 NaH2PO4, 11 glucose, 4 MgSO4, 2 CaCl2, 1.5 HEPES. Slices were incubated for up to 6 hr at room temperature (20–22°C) in a gas (95% O2 and 5% CO2) NaHCO3-buffered saline solution (in mM): 116.4 NaCl, 1.8 CaCl2, 0.4 MgSO4, 5.36 KCl, 0.89 NaH2PO4, 5.5 glucose, 24 NaHCO3, 100 glutathione, 1 nitro-arginine, 1 kynurenic acid, pH 7.35, adjusted with NaOH (300–305 mOsml). After 1 hr of incubation, we dissected out the region of the nucleus accumbens with the aid of a dissecting microscope. We incubated the tissue for 25 min in an oxygenated (100% O2 with constant stirring) HEPES-buffered solution in the inner chamber of a Cell-Stirr flask (Wheaton, Millville, NJ) containing papain (1 mg/ml) and the following (in mM): 136 NaCl, 0.44 KH2PO4, 2.2 KCl, 0.35 NaH2PO4, 5.5 glucose, 10 HEPES, 100 glutathione, 1 nitro-arginine, 1 kynurenic acid, and 1 pyruvic acid, pH 7.35, 300–305 mOsml. After mechanical trituration of the tissue using fire-polished Pasteur pipettes with successively smaller tip diameters, we plated the supernatant into a 35 mm Petri dish placed on the stage of the inverted microscope. The cells were allowed to attach to the dish for 10 min before replacing the Na+-isethionate solution with normal external solution containing the following (in mM): 145 K-glucolate, 1 MgCl2·2 H2O, 1.2 CaCl2, 15 HEPES, 4 EGTA, 4 HEDTA, and 4-AP (10 mM) to block possible contaminating fast transient IA currents. The capillaries were first filled through the tip and then backfilled with the recording solution. We recorded in voltage-clamp mode with an Axopatch 200B amplifier and a D.A.C. (digital analog converter) TL-1 interface from Axon Instruments (Union City, CA). BK channel currents were recorded at a sampling rate of 5 kHz and low-pass filtered at 1 kHz with an eight-pole Bessel filter. Potentials and currents were digitized, curve-fit, measured, and plotted using the pClamp6 suite of acquisition and analysis software (Heka Elektronik, Lambrecht/Pfalz, Germany). The recording pipette contained the following (in mM): 145 K-glucolate, 1 MgCl2·2 H2O, 15 HEPES, 1.5 HEDTA, 2 Na-ATP.

Open probability analysis and calcium sensitivity. The product of the total number of functional channels present in the membrane patch (N) and the probability that a particular channel is open under steady-state conditions (Po) was used as an index of channel activity. NPo values were calculated from all-points amplitude histograms. In multiple channel patches of unknown N, knowing NPo and the number of openings (X), under the period of observation (T), allowed calculation of all openings (X) from the relationship: T = NPo/X, (NPo data as a function of voltage was fitted with a Boltzmann function of the following type: Pp = [(1 + exp − KV − V0.5)] −1, where K is the logarithmic potential sensitivity and V0.5 is the potential at which Pp is half maximal. When the (NPo)1/2-voltage relationship is fitted by the Boltzmann curve, a plot of LNPNp as a function of voltage is linear at low values of Pp. In this plot, the reciprocal of the slope is the potential at which Pp is half maximal. NPo values were calculated from the following: 1/slope = RT/zF, where R, T, and F have their usual meanings defined in the Nernst equation. EtOH application. Data were sampled for a period of 2 min. As a control, we recorded BK channel activity for 30 sec twice to ensure a stable baseline activity. Then, EtOH was applied and BK channel activity was monitored for 30 sec in successive blocks of 30 sec for 2–3 min. Maximal EtOH effects during this period were used in the analysis, regardless of the time of exposure. Data were expressed as mean ± SE (with the number of cells or patches in parentheses).

Dwell time analysis. In single-channel patches, durations of open times were measured with half-amplitude threshold analysis. A maximum-likelihood minimization routine was used to fit curves to the distribution of open times. Determination of the minimum number of terms for adequate fit was established using a standard F statistic table (significance level, p < 0.01). The slope of the unitary current amplitude current–voltage relationship yielded the unitary conductance (γ). Values for I were obtained from the Gaussian fit of all-points amplitude histograms by measuring the distance between the modes corresponding to the closed state and the first opening level. For all experiments, reported voltages correspond to the potential at the intracellular side the membrane.

Reverse transcription–PCR. RNAs were extracted from four nucleus accumbens punches or from a fragment of rat liver with the RNeasy Protect Mini extraction kit (Qiagen, Valencia, CA) according to the instructions of the manufacturer. After extraction, the RNAs were treated with 20 U of DNase I for 15 min at 37°C to minimize the risk of genomic DNA contamination. The RNA was then purified again on an RNeasy mini-column. The Titan One Tube reverse transcription (RT)-PCR kit (Roche, Basel, Switzerland) was used according to the instructions of the manufacturer to detect the presence of KCNN1A1, KCNNB1, and KCNNB4 RNAs. The following pairs of primers were used: KCNN1A1, GGAACTGTAGTGGAGGCGTACACC (5′ primer) and CCGAGTCGGCTGATACGAAAG-
TCG (3’ primer); KCNMB1, ATCAAGGACCGAAAGACCTG (5’ primer) and CTACTTCTGAGCTGCCAAGAC (3’ primer); KCNMB4, CGACAGTGACGGAAC-AACTC (5’ primer) and CATGGTCTTGCC-CTTGACTGC (3’ primer). Expected amplification product sizes are as follows: 531 bp for KCNMA1, 456 bp for KCNMB1, and 357 bp for KCNMB4. RNAs (200 ng) were used in each reaction. After adding the enzyme mix, the RNAs and primers were denatured at 94°C for 3 min. The reverse-transcription reactions were then performed at 30°C for 30 min, followed by 35 cycles of PCR amplification (30 sec at 94°C, 30 sec at 55°C, 1 min at 68°C; after 10 cycles, 5 sec/cycle were added to the elongation step). To verify that the signals detected were not caused by genomic DNA contamination, RT-PCRs were also performed for each pair of primers after the reverse transcriptase was heat inactivated.

Immunohistochemistry. Male Spraque Dawley adult rats, (22–40 d of age) were obtained from Taconic Farms (Germantown, NY) and killed in accordance with the National Institutes of Health guidelines on the care and use of animal study protocol approved by the University of Massachusetts Animal Care and Use Committee. Brain fragments containing NAcc were rapidly removed from the skull, fixed overnight in 4% paraformaldehyde in PBS, and immersed in 20% sucrose/PBS for at least 24 hr. After plunging into sucrose, tissue was briefly washed in PBS, embedded in 6% gelatin–egg yolk mixture in cardboard molds, and exposed to concentrated formaldehyde vapors for at least 3 d (4°C). After hardening, 40 µm sections were cut on a freezing microtome, and sections were placed in net-well carriers (one section per carrier). The floating sections were permeabilized/blotted in the buffer containing 10% NGS, 0.1% BSA, 0.4% Triton X-100 in PBS/0.02% sodium azide, pH 7.4, for 1 hr at room temperature. Sections were incubated (overnight at 4°C) with specific monoclonal primary antibodies (1:300) against BK channel subunits (α, β1 (Affinity BioReagents, Golden, CO) and β4 (gifts from Millenium Pharmaceuticals, Cambridge, MA)), rinsed, and incubated for 1 hr at room temperature with Alexa 594-tagged, anti-rabbit secondary antibody (1:400; Molecular Probes, Eugene, OR). Next, sections were incubated with neuronal-specific nuclear protein (Chemicon, Temecula CA; overnight at 4°C), a mature neuron marker, rinsed, and incubated for 1 hr at room temperature with Alexa 488-tagged anti-mouse secondary antibody (1:400; Molecular Probes). To probe the presence of BK channel subunits in astrocytes, sections were co-stained with monoclonal antibody against GFAP (1:200; Chemicon) and Alexa 488-tagged, anti-mouse secondary antibody. Sections were rinsed in PBS, mounted on SuperFrost Plus microslides (VWR Scientific, Batavia, IL) in Prolong Gold Antifade Reagent (Invitrogen, San Diego, CA) and use of animal study protocol approved by the University of Massachusetts Animal Care and Use Committee. Brain fragments containing NAcc region. We report that somatic BK channel open probability was strongly potentiated by EtOH in a dose-dependent manner, whereas dendritic BK channels showed much less sensitivity to EtOH. A combination of electrophysiological, molecular biological, and immunohistochemical approaches established the presence of the BK β4 in the cell body and absence in the dendrite, consistent with a role of the β4 subunit in compartment-specific ethanol sensitivity.

NAcc neurons express functional BK channels

Although the presence of the BK channel α subunit has been reported in the striatal region (Behrens et al., 2000), the BK channel has not been identified in NAcc neurons. To assess BK channel α subunit expression, we used an antibody specifically directed against its pore-forming region. The specificity of this antibody was confirmed by evidence that it stained only HEK293 cells expressing the BK channel α subunit (Fig. 1A, B) but not untransfected cells (Fig. 1C,D). Using a specific neuronal marker (neuronal-specific nuclear protein) to label neurons specifically, we found that ~90% of NAcc neurons express BK channels. This percentage was corroborated by whole-cell voltage-clamp data. After block of IA and the delayed rectifier (IDR) currents with 10 mM 4-AP and 20 mM quinidine, respectively, 8 of 12 NAcc neurons had a TEA-sensitive outward current, whereas four died out without recovery (data not shown). Figure 1E shows that the α subunit was not confined to the soma region but was visible on processes; the single arrowhead points to a primary dendrite and the double arrowhead points to what may be labeled secondary dendrites (Fig. 1F).

The presence of functional BK channels in the membrane of medium spiny neurons was established by assessing their basic electrophysiological properties in inside-out patches. First, we examined voltage sensitivity by recording currents at various membrane potentials ranging from −100 to +100 mV while exposing the cytosolic face of the channels to 5 mM Ca2+ Figure 2A shows the activity of a single BK channel recorded between −60 and +20 mV. At −60 mV, the channel opens sporadically (P0 = 0.034). As the potential across the membrane is depolarized, the time spent in the open state increases steadily to reach an almost permanent open state at +20 mV (P0 = 0.95). We exposed the intracellular face of the same channel to Ca2+ ranging from 1 to 10 µM while holding the membrane potential at −40 mV (Fig. 2B). As expected, BK channel activity was extremely low
(Pₒ = 0.001) at 1 µM Ca²⁺ but increased to Pₒ = 0.24 at 5 µM before reaching an almost permanent open state at 10 µM Ca²⁺ (Pₒ = 0.89) (Fig. 2 B). We plotted BK current amplitude of the patch shown in Figure 2 A as a function of membrane potential (Fig. 2 C). As expected ([Kᵢ] = [Kₒ]), BK current reversed at 0 mV, and the current–voltage relationship was well fitted with a linear regression (r = 0.99), giving a slope conductance of 234 pS, in line with values reported for BK channels in brain and smooth muscle (for review, see McManus, 1991).

**EtOH potentiates somatic but not dendritic BK channels**

We examined the sensitivity of somatic BK channels to 50 mM EtOH in inside-out patches in the presence of 5 µM Ca²⁺ (Fig. 3). In control conditions (Vₛ = −30 mV), BK channels opened only briefly (NPₒ = 0.031). Within 1 min after exposure to 50 mM EtOH, the activity of BK channels increased dramatically to reach an NPₒ of 0.59 (Fig. 3 A), returning to a level close to control after washout of the drug (NPₒ = 0.095). When averaged over eight patches, 50 mM EtOH increased somatic BK channel activity more than eightfold (8.68 ± 2.23) (Fig. 3 C). Acute EtOH-mediated enhancement of BK channel activity was concentration dependent between 1 and 50 mM EtOH (Fig. 3 C). Thus, 1, 10, and 25 mM EtOH increased BK channel activity in the soma 1.45 ± 0.05-fold, 2.5 ± 0.78-fold, and 4.05 ± 1.57-fold, respectively. However, the effects of EtOH were bimodal, and at the highest concentration tested (100 mM), EtOH increased BK channel open probability only 2.39 ± 0.53-fold, possibly indicating the presence of a lower-affinity inhibitory alcohol-binding site. Interestingly, we found significant variability in the effect of EtOH on somatic BK channels from patch to patch, at all EtOH concentrations tested. For example, potentiation by 50 mM EtOH ranged from 1.15-fold to 18.03-fold. Inhibition by the drug was never observed. Because we recorded only one patch per neuron, we cannot determine whether this variability exists within or be-
Acute EtOH increases somatic but fails to alter dendritic BK channel activity. A, Traces of somatic BK channels before, during (50 mM EtOH), and 2 min after (Wash) acute EtOH exposure ($V_h = -30$ mV, $5 \mu$M Ca$^{2+}$) in inside-out patches. EtOH strongly potentiated the activity of somatic BK channels. B, Shows the lack of effects of 50 mM EtOH on dendritic BK channels recorded at $V_h = -30$ mV in the presence of $5 \mu$M Ca$^{2+}$ (wash not shown). The membrane patch was excised 25 $\mu$m from the soma. Downward deflections indicate inward currents; C and O represent the closed and open states of the channels, respectively. C, Plot of averaged effects of various EtOH concentrations on somatic and dendritic BK channels. The dashed line indicates control level (onefold), and the numbers within bars represent the number of patches tested.

**Figure 3.** Acute EtOH increases somatic but fails to alter dendritic BK channel activity. A, Traces of somatic BK channels before, during (50 mM EtOH), and 2 min after (Wash) acute EtOH exposure ($V_h = -30$ mV, $5 \mu$M Ca$^{2+}$) in inside-out patches. EtOH strongly potentiated the activity of somatic BK channels. B, Shows the lack of effects of 50 mM EtOH on dendritic BK channels recorded at $V_h = -30$ mV in the presence of $5 \mu$M Ca$^{2+}$ (wash not shown). The membrane patch was excised 25 $\mu$m from the soma. Downward deflections indicate inward currents; C and O represent the closed and open states of the channels, respectively. C, Plot of averaged effects of various EtOH concentrations on somatic and dendritic BK channels. The dashed line indicates control level (onefold), and the numbers within bars represent the number of patches tested.

across patches; EtOH failed to alter BK channel activity in any of the patches tested, suggesting a greater homogeneity of BK channel phenotype in this compartment. Given the striking difference in EtOH sensitivity between somatic and dendritic BK channels in NAcc medium spiny neurons, we explored the biophysical properties of the channel populations in the two compartments. Our results supported the presence of a more heterogeneous BK channel population in the soma, compared with that in the dendrite.

**Conductance**

The mean conductance of somatic BK channels was $251 \pm 7$ (n = 6), $227 \pm 11$ (n = 13), and $216 \pm 6.9$ (n = 9) pS with 1, 5, and 10 $\mu$M Ca$^{2+}$, respectively. In dendrites, BK conductance at the same Ca$^{2+}$ concentrations was $239 \pm 11$ (n = 6), $217 \pm 9.4$ (n = 9), and $213 \pm 5.6$ (n = 7) pS. There were no statistically significant differences between soma and dendrite (Fig. 4A).

**Calcium dependence**

Figure 4B shows the normalized open probability of individual patches as a function of membrane potential in the presence of 10 $\mu$M Ca$^{2+}$. The potentials at which BK channels were open half of the time ($V_{0.5}$) showed a wider range in the soma (from −38 to +17 mV) than in the dendrites (−35 to −20 mV), resulting from the presence of a population of channels from somatic membrane exhibiting a $V_{0.5}$ more depolarized than those observed in the dendrites. Figure 4C shows the normalized $N_{P_o}$ of BK channels from soma (filled symbols) and dendrites (open symbols) as a function of the membrane potential. The $N_{P_o}$–voltage relationship could be well fitted using the Boltzmann equation. The graph shows that BK channel open probability increases as the membrane is depolarized for all [Ca$^{2+}$] tested. The lesser Ca$^{2+}$ sensitivity of somatic BK channels is demonstrated by a shift to the right, showing that at a given [Ca$^{2+}$], the membrane potential needs to be more depolarized to induce a similar open probability. The different Ca$^{2+}$ sensitivities of somatic and dendritic BK channels are further illustrated in Figure 4D, where the $V_{0.5}$ values were $6.25 \pm 2.47$ and +5.59 $\pm$ 3.68 mV for dendrite and soma, respectively (p = 0.035). A similar difference was found in the presence of 10 $\mu$M Ca$^{2+}$, with $V_{0.5}$ values of $-28.14 \pm 2.2$ and $-19.1 \pm 3.7$ mV (p = 0.038). $V_{0.5}$ values at 25 $\mu$M Ca$^{2+}$ (−69.3 $\pm$ 2.3 and −63.5 $\pm$ 3.5 mV) were not statistically different (p = 0.4).

**Soma and dendrites differ in their BK channel kinetics and gating**

Although whole-cell patch clamp is usually used to study kinetic properties (activation, deactivation, and inactivation) of ion channels, we could not use this approach, because it would not have enabled us to independently examine BK properties in each compartment. To circumvent this problem, we compiled a cumulative current trace built from the summation of repetitively evoked single-channel sweeps. The resulting current trace resembles the classical macroscopic current recorded in whole-cell configuration. The membrane of an inside-out patch was stepped from a holding potential of 0 to +40 or +50 mV 100 times. A typical example showing 9 of 100 consecutive traces from a somatic patch is shown in Figure 5A. In both soma and dendrites, we found two populations of BK currents, one for which the activation was best fitted with a single exponential ($\tau = 23.9 \pm 3.6$ msec in the soma and 27.01 msec in the dendrites) and the other
best fitted with a double exponential ($\tau_{\text{fast}}$ and $\tau_{\text{slow}}$) were $7.6 \pm 2.2$ and $27.9 \pm 4.64$ msec in the soma and $6.6 \pm 1.8$ and $33.8 \pm 5.77$ msec in the dendrites, respectively. A typical example of a somatic BK current with a single $\tau$ of 28.34 msec is shown in Figure 5B, whereas BK currents that were best fitted with a double exponential are shown in Figure 5, C and D. The ratio of channel subtypes observed was strikingly different in the two compartments: in somata, BK channels in seven of nine patches contained channels showing fast activation (Fig. 5E). We interpret this data to mean that although each compartment can contain similar BK subtypes, they are not equivalently distributed.

Soma and dendrites differ in their BK open times

The mean open time of BK channels from the soma and dendrites was measured while holding the potential at a value that produced an $NP_\text{o}$ of 0.5. In soma, and to a lesser extent in dendrites, the BK channel mean open time showed calcium dependency. Thus, in the soma, mean open time decreased from $6.55 \pm 1.01$ (n = 10) to $2.44 \pm 0.39$ (n = 6) msec between 1 and 10 $\mu$M Ca$^{2+}$, respectively (Fig. 5F, Table 1). Interestingly, in the dendrites, the relationship between [Ca$^{2+}$], and mean open time was reversed, and the mean open time increased from $4.47 \pm 0.42$ to $6.59 \pm 1.01$ msec for similar free-Ca$^{2+}$ concentrations (Fig. 5F, Table 1).

The expression of $\beta$1 and $\beta$4 BK channel subunits in the NAcc is compartment specific

A potential explanation for the compartment-specific ethanol sensitivity of BK that is compatible with much of the biophysical and pharmacological characterization of the channel in each compartment involves a differential distribution of the BK $\beta$4 subunit, with a greater representation in the cell body than in the dendrite. These data include the following: (1) $V_{\text{o,5}}$ values for $\beta$4-containing channels would be expected to shift $V_{\text{o,5}}$ to more depolarized levels, as seen for a subpopulation in the soma; (2) activation rates were slower in the cell body, as would be expected for $\beta$4-containing channels (Behrens et al., 2000; Brenner et al., 2000; Lippiat et al., 2003); (3) open times were markedly different in the two compartments, consistent with reports that mean open time is influenced by $\beta$ subunits (Nimigean and Magleby, 1999). Finally, 100 nM ChTX strongly inhibited dendritic BK channels in five of six patches (open probability decreased by $88 \pm 7\%$), whereas in somata, ChTX inhibited BK activity by $82 \pm 11\%$ in only 4 of 10 cases (data not shown), supporting the proposition that the toxin-resistant $\beta$4-containing channels (Meera et al., 2000) were more common in the cell body.

RT-PCR confirmed the expression of three different BK subunits in the NAcc: KCNMA1 ($\alpha$), KCNMB1 ($\beta$1), and KCNMB4 ($\beta$4). We did not examine the presence of transcripts coding for $\beta$2 and $\beta$3 subunits, because there are no sequences currently available for these subunits for the rat. In addition, we never observed inactivating BK currents that would be expected in BK channels containing $\beta$2 and $\beta$3 subunits.

As expected, we obtained a robust signal with primers for the pore-forming subunit KCNMA1. We also detected a robust signal with primers amplifying the KCNMB4 RNA, indicative of strong $\beta$4 subunit expression in the NAcc (Fig. 6A). In addition, we observed a weaker signal with primers for KCNMB1 (Fig. 6A). These results support the notion that BK channels present in the NAcc contain $\alpha$, $\beta$1, and $\beta$4 subunits.

We could not rule out the possibility that the message coding for these BK channel subunits originated not from NAcc medium spiny neurons but rather from small blood vessels or astrocytes. $\beta$1 subunits are reportedly very abundant in smooth muscle (Behrens et al., 2000; Weiger et al., 2000). However, a $\beta$1-specific antibody clearly shows $\beta$1 subunits on the membrane of both soma and dendrites of NAcc neurons (Fig. 6B). In contrast, the $\beta$4 subunit is not observed in the dendrites of these neurons (Fig. 6C). Finally, a specific astrocyte marker (GFAP) revealed that these cells were rare in the NAcc, where they are confined to the lower part of the wall of the ventricles, adjacent to the dorsal region of the NAcc core (Fig. 6D). Therefore, these cells cannot account for the intense labeling found throughout the NAcc. The number of $\beta$1- and $\beta$4-labeled neurons as a proportion of the total number of neurons in several samples (Fig. 6E) indicated a predominance of $\beta$4 subunit (53 $\pm$ 3% of neurons), compared with 27 $\pm$ 2% labeled for $\beta$1, suggesting that a fraction of NAcc neurons coexpress $\beta$1 and $\beta$4 subunits.
Acute EtOH potentiates αβ4 but not αβ1 BK channels in HEK cells

Our data suggest that the differential effects of EtOH on somatic versus dendritic BK channels reflect the selective expression of the β4 subunit in the soma. To test the validity of this interpretation, we transfected human BK channel message encoding the α subunit with either β1 or β4 subunit into HEK293 cells and tested the acute effects of 25 and 50 mM EtOH. We found that neither concentration of EtOH affected αβ1 BK channel open probability (Fig. 7). In contrast, EtOH increased the open prob-

ability of αβ4 BK channels in a dose-dependent manner (Fig. 7). Thus, 25 mM EtOH increased BK channel open probability almost twofold (1.85 ± 0.25; n = 4), whereas the effect of 50 mM EtOH was almost fourfold (3.7 ± 0.81; n = 5). These data suggest that the sensitivity of somatic BK channels is likely associated with the expression of β4, whereas its insensitivity in the den-
drites might be explained by the predominant expression of the β1 subunit and the relative absence of the β4 subunit.

EtOH modulates NAcc action potentials

We examined the physiological consequences of EtOH by determining the effects of the drug on NAcc action potentials recorded in current clamp. We found that 50 mM EtOH strongly accelerated the repolarization phase of action potentials (APs) (Fig. 7B) (n = 3), which is in agreement with its excitatory effect on BK channel open probability.
selective sensitivity of BK channels to EtOH; and (4) potentiation plex may be specifically found in one or the other group. et al., 2000; Brenner et al., 2000; Weiger et al., 2000). Interest-
showed that BK expression of BK channels in NAcc medium spiny neurons. In the Our electrophysiological and anatomical results reveal the ex-
Expression of BK channels in the NAcc
Discussion
The data presented in this study demonstrate: (1) the expression of functional BK channels on the membrane of soma and den-
drites of nucleus accumbens medium spiny neurons; (2) the concentration-dependent potentiation by EtOH of somatic, but not dendritic, BK channel open probability; (3) cell body-specific expression of the BK channel β4 subunit, possibly underlying the selective sensitivity of BK channels to EtOH; and (4) potentiation of β4-containing, but not β1-containing, BK channels expressed in HEK293 cells.
Expression of BK channels in the NAcc
Our electrophysiological and anatomical results reveal the ex-
pression of BK channels in NAcc medium spiny neurons. In the adult rat brain, Knaus et al. (1996) and Chang et al. (1997a) showed that BK α subunit mRNA is found in the neocortices and olfactory cortices, hippocampus, cerebellum, striatum, thalamus, and amygdala, corroborating a number of electrophysiological studies (Bielefeldt et al., 1992; Dopico et al., 1996; Kang et al., 1996; Chavis et al., 1998; Hicks and Marrion, 1998; Martin et al., 2001; Faber and Sah, 2002; Edgerton and Reinhart, 2003). Among the four auxiliary subunits (β1–β4) identified so far, β4 has been found to be the predominant form in the brain (Behrens et al., 2000; Brenner et al., 2000; Weiger et al., 2000). Interest-
ingly, approximately half of all NAcc neurons were labeled with the β4 antibody, correlating with the percentage labeled for the α subunit, suggesting that these two subunits are coexpressed. Because there are also two major populations of cells in the NAcc, the enkephalin-containing and substance-P-containing neurons (for review, see Meredith, 1999), it is possible that the α/β4 complex may be specifically found in one or the other group. The presence of the BK channel β1 subunit in the NAcc was surprising, because it had been believed to be present exclusively in smooth muscles of peripheral organs (Behrens et al., 2000; Weiger et al., 2000). It is unlikely that our findings are artifactual for several reasons: (1) positive identification was made for both mRNA, using RT-PCR, and for protein, using antibody; (2) a highly selective antibody commonly used against β1 subunit clearly showed that the staining originated from NAcc neurons; (3) the same immunostaining also revealed strong β1 subunit staining in magnocellular neurons of the supraoptic nucleus (data not shown), whereas none of the surrounding brain regions were stained, clearly demonstrating the specificity of the antibody used in our study; and (4) only ~25% of all medium spiny neu-
EtOH effects on BK channels and its compartmentalization
With the exception of one study, which reported an inhibition (Walters et al., 2000), the potentiation of BK channel activity by acute alcohol has been documented in a number of preparations such as the brain (Dopico et al., 1996), growth hormone 3 pitu-
yitary clonal cells (Jakab et al., 1997), and reconstituted planar lipid bilayers (Chu et al., 1998; Crowle et al., 2003). Our results in the NAcc demonstrate a potentiation of the open probability of somatic BK channels by relevant concentrations of EtOH, whereas dendritic channels were unaffected by similar concentra-
tions. Compartment-specific effects of EtOH have been docu-
menced previously for hormone-releasing magnocellular neu-
rons, in which BK channels in the cell bodies of SON are not affected by ethanol at relevant concentrations, whereas they are potentiated in terminals of the neurohypophysis (Dopico et al., 1999). This is the first report of a similar dichotomy between the somatic and dendritic compartments.

We suggest that the differences we observed in subunit com-
position between soma and dendrites in the NAcc could explain the differential effects of EtOH on BK channels. A correlation between subunit composition and ethanol sensitivity has been reported for other ion channels (for review of calcium channels, see Crews et al., 1996) and ionotropic receptors (e.g., GABA and NMDA). The NMDA receptor NR2B subunit is believed to favor EtOH-mediated inhibition of NMDA currents (Chu et al., 1995; Kalluri and Ticku, 1999; Peoples and Stewart, 2000), and splice variants of the NR1 subunit are similarly differentially sensitive to the drug (Koltchine et al., 1993). The enhancement of GABA-
mediated current by EtOH is thought to be mediated in part by its α1 subunit (Mihic et al., 1997). In the NAcc, the differential subcellular expression of β1 and β4 is confirmed by the immu-
nohistochemistry and by their biophysical properties. Thus, BK channel slower activation rate and lower calcium dependency in the soma is in agreement with studies showing similar properties for αβ4 BK channels in expression systems (Brenner et al., 2000; Lippiat et al., 2003). Our pharmacological results lead to a similar conclu-
sion. Data from our laboratory showing that BK channels expressed in terminals of hypothalamic magnocellular neurons are sensitive to EtOH, unlike their somatic counterparts (Dopico et al., 1999), re-
semble the dichotomy found in the NAcc. Indeed, we recently found that the BK channel β4 subunit is almost exclusively expressed in the terminals, whereas β1 is found only in the soma of these neurons (in preparation), mirroring closely the present results of the effects of EtOH on BK channels from the soma and dendrites of NAcc me-
dium spiny neurons. Finally, the fact that almost all dendritic BK channels, unlike somatic ones, are sensitive to CbTx further strengthens the idea that BK channels in the former compartment are composed of β1 subunits rather than β4-containing channels.

Although subunit composition is a likely candidate to explain the compartment-specific effects of EtOH on BK channels, a number of additional factors may contribute. These include dif-
fferences in membrane lipid composition of soma and dendrites. The plasma membrane is a highly heterogeneous environment in which lipid domains exist that can serve important roles in cell function (Welti and Glaser, 1994; Bevers et al., 1999; London,
2002). Thus, modification of the lipid environment can alter BK channel activity (Moczydlowska et al., 1985; Clarke et al., 2002) as well as the sensitivity of this channel to EtOH (Crowley et al., 2003). However, although it is known that lipid composition varies from region to region in the brain (Chavko et al., 1993), variability between dendrites and soma within the same neuron has not been reported to our knowledge. Other possibilities also must be considered, such as post-translational modulation of the BK channel by phosphorylation, which is known to affect the activity and alcohol pharmacology of the BK channel.

**Physiological consequences**

The physiological role of the BK channel has been extensively studied in a number of preparations. BK channels are activated by membrane depolarization and by an increase in intracellular calcium, as occurs during calcium influx through the voltage-gated calcium channels to which they are coupled (Marrion and Tavalin, 1998). BK channels play a role in controlling neurotransmitter release and also in modulating action potential shape and frequency at the somatic level (Shao et al., 1999). In the NAcc, we found that 50 mM EtOH accelerated the repolarization of action potentials. Although we have not conclusively demonstrated that this effect is solely caused by actions on BK channels, the increase of BK channel open probability by EtOH strongly suggests that EtOH does alter the shape of medium spiny neuron APs. This likely modulates the pattern of action potentials sent to the terminals, influencing the release of GABA and the resulting integration within and beyond the NAcc.

Interestingly, Dopico et al. (1999) reported a similar finding in the hypothalamic-neurohypophysial (NH) system, where BK channels in NH terminals that predominantly contain the α2δ channel (our unpublished observation) participate in shaping action potentials, whereas BK channels in the associated cell bodies within the hypothalamus, where the αβ1 form of the channel predominates, do not participate in shaping the spike.

The lack of sensitivity of NAcc dendritic BK channels to acute EtOH might suggest that alcohol effects on NAcc neurons mediated through BK channels are circumscribed to the soma and have little impact on the integration of dendritic electrical activity. This last point is critical, because the vast majority of glutamate, GABA, and serotonin projections to the NAcc medium spiny neurons establish synaptic contact primarily on dendrites of these cells (for review, see Meredith, 1999). However, in addition to the role that BK channels in the NAcc cell body may play by their direct response to EtOH, dendritic BK channels may also play a role in the response to EtOH, despite their lack of direct response. EtOH is likely to lower intracellular calcium levels by inhibiting NMDA receptors and voltage-gated calcium channels (Nie et al., 1994; Widmer et al., 1998), two heteromultimeric complexes colocalized with BK channels (Marrion and Tavalin, 1998; Isaacson and Murphy, 2001). These changes in dendritic calcium levels may be transduced by the associated BK channels, ultimately influencing input and output patterns of NAcc neurons. Thus, the presence of BK channels in NAcc neurons, as well as their differential sensitivity to ethanol, may play an important role in the response to ethanol in this important component of the reward pathway.

**References**


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