Striatal Cholinergic Interneurons Express a Receptor-Insensitive Homomeric TASK-3–Like Background K⁺ Current

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INTRODUCTION

Acetylcholine (ACh) is essential for striatal function and changes in striatal cholinergic drive are implicated in the pathophysiology of movement disorders (Calabresi et al. 2000). A sparse population of large aspiny cholinergic interneurons provides the sole source of striatal ACh and regulation of membrane excitability of interneurons is therefore integral to many aspects of striatal function.

Cholinergic interneurons discharge spontaneously in either a tonic or bursting pattern. The expression of these distinct firing patterns involves complex interactions among various voltage-dependent ion channels—TTX-sensitive sodium channels; hyperpolarization-activated HCN channels; voltage-sensitive, inwardly rectifying, and calcium-activated K⁺ channels (Bennett et al. 2000; Wilson 2005)—the activity of which is determined, in part, by additional leak conductances that influence the prevailing membrane potential (Wilson 2005). It is clear that leak K⁺ currents are major determinants of membrane potential, but the channels that underlie those currents have not yet been determined in most cell populations, including striatal cholinergic interneurons.

Molecular candidates for native leak K⁺ currents were revealed with the identification and cloning of the KCNK family of two-pore-domain K⁺ channels (Goldstein et al. 2001; Lesage 2003; Talley et al. 2003). Members of this KCNK family are expressed throughout the brain, with differential but overlapping distributions (Talley et al. 2001). In striatum, TREK-1 (K₂p2.1, Kcnk2) is evenly distributed in a pattern most consistent with expression in medium spiny neurons (Heurteaux et al. 2004; Talley et al. 2001). On the other hand, TASK-3 is expressed in relatively large cells in a diffuse pattern similar to that of cholinergic interneurons (Karschin et al. 2001; Talley et al. 2001). In this study, we used histochemical and electrophysiological approaches to show that TASK-3 channels are functionally expressed in these cells, where they provide a background K⁺ conductance that contributes to the membrane potential; these predominantly homomeric, native TASK-3–like currents proved resistant to modulation by Gq-coupled receptors, suggesting that some aspect of the signaling mechanism linking receptors to TASK channels is disrupted in striatal cholinergic interneurons.

MATERIALS AND METHODS

All animal use was in accordance with guidelines approved by the University of Virginia Animal Care and Use Committee.

Histochemistry

Sprague–Dawley rats [postnatal day 10 (P10) to P18] were perfused transcardially with heparin-containing saline and then 4% paraformaldehyde (PFA) in phosphate buffer (pH 7.4). The brains were removed, postfixed overnight in 4% PFA, and cut (30 μm) on a vibrating microtome (Leica VT1000S). Sections were stored until use at −20°C in cryoprotectant (30% RNase free sucrose, 30% ethylene glycol, 1% polyvinylpyrrolidone, 100 mM sodium phosphate, pH 7.4).

Nonisotopic in situ hybridization combined with immunohistochemistry

Sections were rinsed in sterile phosphate-buffered saline (PBS) and placed free-floating into prehybridization mixture (0.6 M NaCl, 0.1 M Tris–Cl, pH 7.5, 0.002 M EDTA, 0.05% NaPPI, 0.5 mg/ml yeast total RNA, 0.05 mg/ml yeast tRNA, 1× Denhardt’s BSA, 50% formamide, 10% dextran sulfate, 0.05 mg/ml oligo-dA, 10 μM of the four deoxynucleoside triphosphates, 0.5 mg/ml herring sperm DNA, and 10 mM DTT) at room temperature for 30 min and then at 37°C for 1 h. The TASK-3 DNA template was linearized in antisense and sense orientations and in vitro transcription performed using digoxigenin-11-UTP (Boehringer Mannheim, Indianapolis, IN) in the labeling solution.
reaction with orientation appropriate RNA polymerases. Labeled riboprobes were purified on ProbeQuant G-50 Microcolumns (GE Healthcare, Piscataway, NJ). Digoxigenin-labeled probes were added directly to the prehybridization solution and sections were incubated at 55–60°C for 16–20 h. Sections were rinsed through decreasing concentrations of salt solutions, treated with RNase A at 37°C, and then subjected to a final high-stringency wash (0.1× SSC at 55°C for 60 min).

Sections were incubated in blocking solution [0.1% Triton X-100, 10% horse serum and 100 mM Tris-buffered saline (TS)] for 30 min. Immunohistochemical detection of digoxigenin and choline acetyltransferase (ChAT) was achieved by incubating sections with alkaline phosphatase-conjugated sheep anti-digoxigenin antibody (1:1,000; Boehringer Mannheim) and goat anti-ChAT (1:100; Chemicon, Temecula, CA) for 36–48 h at 4°C. After rinsing, alkaline phosphatase was reacted with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt in colorization buffer (50 mM MgCl₂, 100 mM Tris pH 9.5, 100 mM NaCl) while protected from light. The reaction was quenched by rinsing in 10 mM Tris and 1 mM EDTA, pH 8.5 before sections were incubated for 1 h with Cy3-conjugated anti-goat antibody (1:400; Jackson ImmunoResearch, West Grove, PA).

**ChAT and TASK-3 double-labeling immunohistochemistry**

For all immunohistochemistry, sections were washed extensively in phosphate and TS solutions, blocked for 1 h in 3% horse serum/0.1% Triton X-100/TS, and then incubated at 4°C for 48 h with goat anti-ChAT (1:100) and rabbit anti-TASK-3 (Berg et al. 2004). For detection, sections were incubated (1 h) with Alexa-488–conjugated donkey anti-goat (1:500; Invitrogen–Molecular Probes) and Cy3-conjugated goat anti-rabbit (1:500; Jackson ImmunoResearch).

**Immunohistochemical identification of interneurons recorded in brain slices**

Immediately after recording, brain slices containing biocytin-filled neurons were immersed in 4% PFA. The tissue was washed in phosphate and TS solutions and blocked for 1 h in 5% horse serum/0.5% Triton X-100/TS. Immunohistochemistry for ChAT and TASK-3 was performed as described above; biocytin was detected with Alexa-488–conjugated streptavidin (1:400; Invitrogen–Molecular Probes, Carlsbad, CA). For all immunohistochemistry, sections were wet-mounted onto gelatin-subbed microscope slides and coverslipped with ProLong Antifade (Invitrogen–Molecular Probes). Sections were visualized and computer-assisted mapping was performed on a Zeiss FS fluorescence microscope equipped with a digital camera and motor-driven stage using IPLab (Scanyanetics) and Neurulocida (MicroBrightfield) software.

**Brain slice preparation**

Whole cell recording using brain slices was performed as previously described (Berg et al. 2004). Rats (P10–P18) were anesthetized with ketamine/xylazine and rapidly decapitated. Brains were removed, blocked, and glued to the stage of the microslicer (DSK 1500E, Dosaka, Kyoto, Japan) and striatal slices were cut (300 μm) while immersed in ice-cold substituted Ringer solution consisting of (in mM): 260 sucrose, 3 KCl, 5 MgCl₂, 1 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose, and 1 kynurenic acid. Slices were incubated for 1 h at 37°C and subsequently at room temperature in normal Ringer solution containing (in mM): 130 NaCl, 3 KCl, 2 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 glucose. Substituted and normal Ringer’s solutions were bubbled with 95% O₂-5% CO₂.

**Electrophysiology**

Slices were placed in a tissue chamber on a Zeiss Axioskop FS microscope and visualized with infrared differential interference contrast microscopy; cholinergic interneurons were identified in the neostriatum based on their distinctive morphology (large neurons, about 25–50 μm in diameter) (Calabresi et al. 2000). Recordings were performed at room temperature using Sylgard-coated borsilicate glass-pipette capillaries (3–5 MΩ). Pipette solution contained: (for voltage clamp, in mM) 120 KCH₃SO₄, 4 NaCl, 1 MgCl₂, 0.5 CaCl₂, 10 HEPES Na, 10 EGTA, 3 ATP, 0.3 GTP; (for current clamp, in mM) 17.5 KCl, 122.5 K-glucuronate, 9 NaCl, 1 MgCl₂, 10 HEPES Na, 0.2 EGTA, 3 ATP, and 0.3 GTP. To block Ih, the pipette solution contained 50 μM 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyridinium chloride (ZD7288, Tocris Bioscience, Ellenville, MO). For some experiments, biocytin (0.2%) was added to the internal solution for post hoc identification of recorded cells. We obtained perforated patch recordings by including g ancimidicin at 65 μg/ml in the KCH₃SO₄-based internal solution (prepared by sonication from a 10 mg/ml stock in DMSO and used within 2 h; Sigma–Aldrich, St. Louis, MO); for these experiments, we used capillary glass without filaments and omitted ZD7288 from the pipette solution. Bath solution contained (in mM): 140 NaCl, 3 KCl, 10 HEPES Na, 10 glucose, 2 CaCl₂, and 2 MgCl₂. We added tetrodotoxin (TTX, 0.5 μM) to block action potentials and included glibenclamide (10 μM) and barium (200 μM) to block K ATP and Kir channels. For current-clamp experiments TTX was excluded from the bath solution described above and we included 10 μM bicuculline, 30 μM strychnine, and 10 μM CNQX (6-cyano-7-nitroquinazoline-2,3-dione). Where noted, zinc was added to the bath at 100–150 μM and halothane was bubbled into bath solutions through a calibrated vaporizer.

**Data acquisition and analysis**

Voltage commands were applied and currents recorded using pCLAMP software interfaced with an Axopatch 200A amplifier by a Digidata 1322A digitizer (all Axon Instruments). Series resistance was compensated by 60–75% and monitored throughout the recordings to ensure adequate compensation. Cells were held at −60 mV and hyperpolarized from −60 to −130 mV by applying voltage steps (Δ −10 mV) or a voltage-ramp protocol (−0.05 V/s). Values are expressed as averages ± SE and statistical comparisons were by t-test with P < 0.05 considered significant.

**RESULTS**

Stereotial cholinergic interneurons express TASK-3

We previously described a prominent and punctate pattern of TASK-3 mRNA expression in the striatum strikingly reminiscent of the distribution expected for large aspiny cholinergic interneurons (Talley et al. 2001). To demonstrate directly that TASK-3 is expressed in cholinergic interneurons, we used nonisotopic in situ hybridization combined with immunohistochemistry; as illustrated in Fig. 1A (top), TASK-3 transcripts were detected in ChAT-immunoreactive (-ir) cells. In addition, we performed double-labeling immunohistochemistry with an affinity-purified α-TASK-3 antibody (Berg et al. 2004) to show that ChAT-ir cells also express TASK-3 protein (Fig. 1A, bottom). We counted labeled cells in 1-mm² areas of six coronal sections of striatum stained with α-ChAT and α-TASK-3 antibodies (three rats, two sections each), as exemplified for a representative section in Fig. 1B. By this analysis, we found that 100% of ChAT-ir cells were TASK-3 positive; similarly, all TASK-3-ir neurons in striatum were cholinergic (212/212 cells counted).
TASK-like open rectifier $K^+$ currents are evident in striatal cholinergic interneurons.

In light of the seemingly universal expression of TASK-3 in striatal cholinergic interneurons, we sought to isolate a TASK-like current by recording from these neurons in brain slices. We obtained whole cell voltage-clamp recordings from large aspiny interneurons that could be distinguished easily from the smaller, more abundant medium spiny neurons in the striatal slice preparation (Calabresi et al. 2000). To verify that we indeed recorded from cholinergic TASK-3–expressing interneurons, we filled a subset of cells with biocytin and immunostained the slices for either ChAT or TASK-3 (Fig. 1C). As expected, we could detect ChAT-ir or TASK-3-ir in nearly all biocytin-filled neurons that were recovered ($n = 23/27$ and 5/5, respectively).

There are no known blockers selective for TASK channels. To obtain functional evidence for TASK-like currents in striatal interneurons we took advantage of their characteristic physiological properties (voltage independence and $K^+$ selectivity) and sensitivity to extracellular pH and inhalation anesthetics (Goldstein et al. 2001; Lesage 2003; Talley et al. 2003). We blocked other potentially confounding currents present in striatal interneurons, including the pH- and anesthetic-sensitive $I_h$, a $K_{ATP}$ current that occasionally activated over the course of recording, and the prominent inwardly rectifying $K^+$ currents that were evident during hyperpolarizing voltage steps.

TASK channels are sensitive to extracellular pH, with channel activity inhibited by acidification and augmented by alkalization. As shown in the time series from a representative cell (Fig. 1D), bath acidification from pH 7.3 to 5.9 decreased outward current at a holding potential of $-60 \pm 10 \text{ mV}$ ($n = 8$) but bath alkalization to pH 8.4 induced essentially no increase in holding current, relative to that measured under control conditions. $E$: averaged current–voltage ($I-V$) relationship (filled squares; $\pm SE$) for the joint halothane- and pH-sensitive current was obtained by subtraction; the current reverses near $E_C$ and was well fitted with the GHK (Goldman–Hodgkin–Katz) equation, as expected for a TASK-like open rectifier current.

**FIG. 1.** TASK-3 is expressed in striatal cholinergic interneurons. A: immunohistochemistry and nonradioactive in situ hybridization (top) or double-labeling immunohistochemistry (bottom) was performed on coronal sections through rat forebrain using an antibody against choline acetyltransferase (ChAT) (left) with either digoxigenin-labeled RNA probes complementary to TASK-3 (top right) or an antibody against TASK-3 (bottom right). Striatal cholinergic interneurons identified by ChAT immunoreactivity (ChAT-ir) contain TASK-3 mRNA and TASK-3 immunoreactivity (TASK-3-ir). B: camera lucida drawing of a representative coronal section in which neurons expressing ChAT and TASK-3 were mapped within a 1-mm$^2$ area of striatum; all ChAT-ir neurons were also TASK-3-ir. Acb, nucleus accumbens; cc, corpus callosum; Cpu, caudate putamen; LV, lateral ventricle. C: large neurons were recorded in the striatum and filled with biocytin (left); post hoc immunostaining revealed that recorded neurons were ChAT-ir (top right) or TASK-3-ir (bottom right). D: holding current (at $-60 \text{ mV}$) from a representative striatal interneuron under control conditions (pH 7.3), in an acidified bath (pH 5.9) and in an alkalized bath (pH 8.4); the cell was exposed to 3% halothane, as indicated. Note that bath acidification decreased outward current and reversed the halothane-enhanced outward current; bath alkalization had little effect on holding current, relative to that measured under control conditions. E: averaged current–voltage ($I-V$) relationship (filled squares; $\pm SE$) for the joint halothane- and pH-sensitive current was obtained by subtraction; the current reverses near $E_C$ and was well fitted with the GHK (Goldman–Hodgkin–Katz) equation, as expected for a TASK-like open rectifier current.
We took advantage of the relative specificity of Zn$^{2+}$ and ruthenium red among TASK channels to test the hypothesis that the TASK-like background current in striatal cholinergic interneurons was attributable to homomeric TASK-3 channels; at concentrations of 100 and 5–10 μM, respectively, Zn$^{2+}$ and ruthenium red potently inhibit only TASK-3 homomeric channels, with little to no effect on TASK-1 homomeric or TASK-1/TASK-3 heteromeric channels (Clarke et al. 2004; Czirjak and Eyjolfsson 2002). In the experiment illustrated in Fig. 2A (left), a TASK–3–like current was indeed evident as a halothane-activated and Zn$^{2+}$- or H$^+$-inhibited open-rectifier K$^+$ current. Consistent with a predominant contribution from TASK-3 subunits, note that the current inhibited by bath acidification was also nearly entirely eliminated by 100 μM Zn$^{2+}$. The averaged $I$–$V$ relationship for the Zn$^{2+}$–sensitive current in these striatal interneurons was well fitted by the GHK equation (Fig. 2A, right), as expected for inhibition of TASK-3 channel currents. Together, these data provide excellent evidence that TASK-3 homomeric channels contribute to background K$^+$ currents in rat striatal cholinergic neurons.

We also tested effects of ruthenium red in cholinergic interneurons. Results from those experiments were difficult to interpret because that compound appeared to modulate multiple conductances; the current inhibited by ruthenium red was weakly rectifying but reversed at about −80 mV, a point some 10 mV depolarized to the pH–, zinc–, and anesthetic-sensitive current ($n = 5$, data not shown).

**Zn$^{2+}$–sensitive TASK-3–like currents influence interneuron membrane potential**

We tested the contribution of TASK-3 currents to striatal cholinergic interneuron membrane potential under whole cell current clamp, using zinc as the preferred blocker because we found that the Zn$^{2+}$–sensitive background current most strongly approximated a TASK-like current (i.e., acidification appeared to modulate additional non-TASK currents). To replicate conditions used for voltage-clamp recording, we initially recorded cells without the addition of TTX, but with barium and glibenclamide in the bath and ZD7288 in the pipette (Fig. 2B). Membrane potential on whole cell access ranged from −55 to −74 mV (−65.8 ± 5.1 mV, $n = 4$); some cells were spontaneously active whereas others were not. The addition of Zn$^{2+}$ to the bath increased firing rate in spontaneously active cells and led to action potential discharge in silent cells (Fig. 2B). In the presence of TTX, we found that Zn$^{2+}$ caused a 6.0 ± 0.3 mV membrane depolarization from a starting membrane potential of −61.8 ± 3.3 mV ($n = 4$; $P < 0.001$ by paired $t$-test). We performed similar experiments under conditions when inwardly rectifying K$^+$ currents and $I_h$ were intact (i.e., in the absence of barium and ZD7288). As shown in Fig. 2C, even in the continued presence of those prominent background conductances, we found that Zn$^{2+}$ increased firing rate and caused a strong membrane depolarization (8.2 ± 0.7 mV in TTX, $n = 3$). Inasmuch as Zn$^{2+}$ inhibits a K$^+$ current with properties expected of TASK channel currents and is relatively selective among TASK channels for TASK-3 homodimers, these data imply that homodimeric TASK-3 channels contribute to establishing the prevailing membrane potential in striatal cholinergic neurons.
Interneuron TASK-3–like currents are resistant to neurotransmitter modulation

It was previously reported that activation of Goq-coupled receptors (H1 histamine and type I metabotropic glutamate, mGluR1/5) in striatal interneurons leads to increased excitability by inhibition of background K⁺ currents (Bell et al. 2000; Munakata and Akaike 1994; Takeshita et al. 1996). Moreover, native TASK-like currents and cloned TASK channels can be inhibited downstream of multiple Goq-coupled receptors (Goldstein et al. 2001; Lesage 2003; Talley et al. 2003), including mGluRs (Chemin et al. 2003). Therefore we tested whether TASK-3–like currents in striatal interneurons are subject to modulation by histamine or mGluR1/5 receptors.

Surprisingly, striatal interneuron TASK-3 channel currents do not appear to be targeted by these receptors. As illustrated in Fig. 3A, when challenged with either the mGluR1/5 agonist 3,5-dihydroxyphenylglycine (DHPG, 100 μM) or histamine (10 μM), a large and rapidly desensitizing inward current developed in striatal interneurons. The agonist-induced current, measured at the peak (Fig. 3B) or at steady state (data not shown), was associated with an increase in conductance and did not reverse over the voltage range tested. By comparison with the first response to a given agonist, currents evoked by a second exposure to the same drug were smaller in amplitude (i.e., they activated quickly and were associated with an increase in conductance and an extrapolated reversal well depolarized to the holding potential).

As shown in Fig. 3A, there was no evidence of cross-desensitization between agonists because histamine effects were apparently undiminished after DHPG.

We exploited the halothane- and Zn²⁺ sensitivity of TASK-3–like currents to rule out the possibility that some small receptor-mediated inhibition of TASK channels was masked by the large, desensitizing agonist-induced current. First, we compared the effect of DHPG or histamine in control cells and in cells treated with halothane. In the presence of halothane, agonists activated an inward current that inactivated quickly and reversed at depolarized potentials, similar to the agonist-induced current without halothane. Moreover, there was no difference in amplitude of agonist-activated current in these two groups of cells (histamine: control, 157 ± 51 pA, n = 4; in halothane, 131 ± 34 pA, n = 4; DHPG: control 89 ± 25 pA, n = 6; in halothane, 67 ± 9 pA, n = 4), despite clear activation of halothane-sensitive TASK-3-like current (see Fig. 3D).

As a second test, we reasoned that if the agonist acted to inhibit a component of TASK-like current, the Zn²⁺-sensitive component of halothane-activated current would be correspondingly reduced. This was not the case. In the absence of agonist (Fig. 3C), halothane evoked a large outward current that was strongly inhibited by Zn²⁺. In a separate group of cells in which we interjected a histamine exposure between halothane and Zn²⁺ applications (Fig. 3D), zinc inhibited a

![Image](https://example.com/image.png)

**FIG. 3.** TASK-3–like currents are not inhibited by histamine in striatal cholinergic interneurons. A: holding current from a representative striatal interneuron under control conditions and during exposure to 3,5-dihydroxyphenylglycine (DHPG, 100 μM) and histamine (10 μM), as indicated; both DHPG and histamine caused a strong, transient inward shift in holding current. B: I–V of the peak DHPG- and histamine-evoked currents (from cell in A), obtained by digital subtraction of ramp currents. C: effect of halothane and zinc (100 μM) on holding current. D: effect of zinc on holding current during continued exposure to halothane and histamine. E: averaged I–V relationship for the Zn²⁺-sensitive current in 2 groups of interneurons, obtained either in the presence of halothane (n = 6) or in the presence of halothane and histamine (n = 5). F: amplitude of Zn²⁺-sensitive current was normalized to halothane-activated current in individual cells and averaged, under control conditions or during exposure to histamine. Properties and relative amplitude of Zn²⁺-sensitive current were unaffected by histamine.
similar, weakly rectifying TASK-like K⁺ current (Fig. 3E) that accounted for an identical, major fraction of the halothane-activated current (Fig. 3F; control: 91.6 ± 3.0%, n = 6; histamine: 86.7 ± 2.7%, n = 5). We considered the possibility that intracellular dialysis from the whole cell pipette might disrupt the signaling pathway in these neurons and repeated these experiments using gramicidin perforated-patch recordings. As observed with whole cell recordings, the Zn²⁺-sensitive component of the halothane-activated current remained unaffected by histamine in the perforated-patch configuration (i.e., Zn²⁺ accounted for 93.9 ± 27.5% of halothane-sensitive current, n = 3; data not shown). These data indicate that the TASK-3-like current (i.e., the halothane- and Zn²⁺-sensitive current) is unaffected by prior activation of histamine receptors present in these cells.

DISCUSSION

In this study, we provide histochemical and electrophysiological evidence for expression of homomeric TASK-3 channels in striatal cholinergic interneurons, where they contribute to the background K⁺ currents that establish neuronal membrane potential. Inasmuch as the distinct firing patterns observed in striatal cholinergic interneurons are influenced by the prevailing membrane potential, these data suggest that activity of TASK-3 channels can affect striatal cholinergic tone and basal ganglia function.

A number of observations support our conclusion that homomeric TASK-3 channels account for a background K⁺ current in striatal cholinergic neurons. By using histochemical approaches, we directly demonstrated that TASK-3 expression is a feature common to all striatal cholinergic interneurons. Moreover, we were also able to identify a TASK-like current (i.e., an anesthetic- and pH-sensitive open-rectifier K⁺ current) by voltage-clamp recording from cholinergic interneurons directly visualized in rat striatal slice preparations. We could attribute this background K⁺ current to homomeric TASK-3 channels because: 1) it was strongly inhibited by acidification but unaffected by alkalization (from pH 7.3), as expected for TASK-3 but not other TASK channels; and 2) it was potently inhibited by Zn²⁺ at concentrations selective among TASK channels for TASK-3 homomers. These cholinergic striatal interneurons are thus distinct from other neurons studied to date, where TASK-like currents appear to represent an admixture of homomeric TASK-1 and TASK-3 as well as heteromeric TASK-1/TASK-3 channels (Berg et al. 2004; Kang et al. 2003; Meuth et al. 2006).

In heterologous expression and native neuronal systems, TASK channels are strongly inhibited by activation of receptors that couple by heterotrimeric G proteins containing the Goq/11 subunits (Chen et al. 2006; Goldstein et al. 2001; Lesage 2003; Talley et al. 2003). Thus it was surprising that we could find no evidence for receptor modulation of the TASK-3–like current in striatal interneurons, despite clearly functional mGluR1/5 and histamine receptors and previous reports of receptor-modulated background K⁺ channels in those cells in animals of similar age (Bell et al. 2000; Munakata and Akaike 1994; Takeshita et al. 1996). Indeed, rather than observing receptor-mediated inhibition of a K⁺ current, we found that DHPG and histamine activate a large, rapidly desensitizing inward current. Our experiments were designed specifically to test a TASK channel contribution to receptor-activated currents under conditions that support clear and robust TASK channel modulation in other systems; they did not explore potential contributions from other background K⁺ channels. Likewise, the molecular basis for the previously unreported, receptor-activated cationic current remains to be determined, although it seems likely that it could be mediated by TrpC cationic channels that are known to be targeted by Goq-linked receptors in other systems (Clapham et al. 2001). In any case, these data imply a heretofore unrecognized context dependence of TASK channel modulation. In light of our recent report indicating that activated Goq inhibits TASK channels through a close interaction of the G-protein subunit with the channels (Chen et al. 2006), it is tempting to speculate that cell-type–specific factors may be required for functionally appropriate localization of receptor, G protein, and TASK channels. In this respect, neither our immunohistochemical detection of TASK-3 (see Fig. 1A; Berg et al. 2004) nor earlier work from others on immunolocalization of mGluts (Paquet and Smith 2003; Testa et al. 1998) suggest any discrete and/or distinct subcellular localization of channel and receptor in striatal neurons. Of course, the resolution afforded by light microscopy cannot rule out the possibility that, despite widespread (and likely overlapping) somatodendritic localization, the receptor and channel fail to interact in a functionally meaningful way.

In summary, these data highlight unique features of the TASK-like background K⁺ currents in striatal cholinergic interneurons: they are mediated by predominantly homomeric TASK-3 channels, which appear to be insensitive to modulation by Goq-linked receptors. Nevertheless, these native TASK-3 homomorphic channels contribute to background conductances that determine membrane potential and firing activity in striatal cholinergic interneurons and their activity may be regulated by endogenous TASK-3 channel modulators, such as protons or zinc, or by the actions of inhaled anesthetics.

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