Bradykinin inhibits the transient outward K⁺ current in mouse Schwann cells via the cAMP/PKA pathway

Man Zhang, Xiao-Wei Fei, Yan-Lin He, Guang Yang, and Yan-Ai Mei

Institutes of Brain Science, School of Life Sciences and State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, China

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Zhang M, Fei XW, He YL, Yang G, Mei YA. Bradykinin inhibits the transient outward K⁺ current in mouse Schwann cells via the cAMP/PKA pathway. Am J Physiol Cell Physiol 296: C1364–C1372, 2009. First published April 1, 2009; doi:10.1152/ajpcell.00014.2009.—Bradykinin (BK) is an endogenous peptide with diverse biological actions and is considered to be an important mediator of the inflammatory response in both the peripheral and the central nervous systems. BK has attracted recent interest as a potential mediator of K⁺ conductance, Cl⁻ channels, and Ca²⁺-activated K⁺ channels. However, few reports have associated BK with the voltage-gated K⁺ current. In this study, we demonstrated that BK suppressed the transient outward potassium current (Iₒ) in mouse Schwann cells using whole cell recording techniques. At a concentration of 0.1 μM to 5 μM, BK reversibly inhibited Iₒ in a dose-dependent manner with the modulation of steady-state activation and inactivation properties. The effect of BK on Iₒ current was abolished after preincubation with a B₂ receptor antagonist but could not be eliminated by B₁ receptor antagonist. Intracellular application of GTP-γ-S induced an irreversible decrease in Iₒ, and the inhibition of Gₛ using NP449 provoked a gradual augmentation in Iₒ and eliminated the BK-induced effect on Iₒ while the Gₛ/α₅ antagonist NF203 did not. The application of forskolin or dibutyryl-cAMP mimicked the inhibitory effect of BK on Iₒ and abolished the BK-induced effect on Iₒ. H-89, an inhibitor of PKA, augmented Iₒ amplitude and completely eliminated the BK-induced inhibitory effect on Iₒ. In contrast, activation of PKC by PMA augmented Iₒ amplitude. A cAMP assay revealed that BK significantly increased intracellular cAMP level. It is therefore concluded that BK inhibits the Iₒ current in Schwann cells by cAMP/PKA-dependent pathways via activation of the B₂ receptor.

In addition to being regarded as a mediator of inflammation and vasodilation, a number of studies have shown that BK can modulate membrane ion channels and membrane conductance. BK has been demonstrated to evoke a Ca²⁺-activated chloride current in nonneuronal cells isolated from neonatal rat DRG (6). BK also stimulates T- and L-type Ca²⁺ currents in heart cells via stimulation of B₂ receptors, inducing a positive chronotropic effect (5). In vagal afferent neurons (nodose ganglion neurons), BK increased membrane conductance by activating Ca²⁺-activated Cl⁻ conductance while reducing membrane conductance by decreasing K⁺ conductance (37). In addition, a recent study by Liu et al. (27) demonstrated that BK activated calcium-dependent K⁺ channels in cultured human airway smooth muscle cells via the release of inositol 1,4,5-trisphosphate-sensitive intracellular Ca²⁺ stores. However, there have been only a few studies concerning the possible role of BK in modifying voltage-gated K⁺ channels.

Schwann cells are responsible for myelination of nerve fibers in the peripheral nervous system. Schwann cells purified from mouse sciatic nerves are known to proliferate, exhibiting mostly a premyelinating phenotype (32). The whole cell recording technique has revealed that all SCs exhibit prominent voltage-gated outward K⁺ currents, including inactivating A-type (Iₐ), delayed-rectifier (Iₚ₅), and inward-rectifier (Iₐₕ) K⁺ channels, which constitute the main conductance found in SCs (38, 43). Molecular and physiological studies have previously shown that Iₖ channels may play an important role in SC proliferation and that they are modulated by Src (40). Moreover, recent studies have begun to address the molecular identity of K⁺ channel α-subunits in SCs (38). In the present study, we used the whole cell patch-clamp recording technique to examine the effects of BK on the outward K⁺ current, the BK receptors involved, and the relevant signaling pathway in SCs.

MATERIALS AND METHODS

Schwann cell culture. Isolated primary Schwann cell cultures were prepared from 4-day-old ICR mouse sciatic nerves according to Brocks et al. (2). Isolated cells were then plated onto poly-L-lysine-coated (10 μg/ml) 35-mm-diameter petri dishes (Costar, Corning, NY) at a density of 10⁶ cells per dish. Cultured cells were incubated at 37°C with 5% CO₂ in Dulbecco’s modified Eagle’s medium/ Nutrient Mixture F-12 Ham (DMEM/Ham’s F-12) supplemented with 10% fetal calf serum, insulin (5 μg/ml), and 1% antibiotic-antimycotic solution. After culturing for 24 h, cytosine 1-β-D-arabinofuranoside (5 μM/l) was added to the culture medium to eliminate actively proliferating fibroblasts for 48 h. On day 4, cultured cells were washed and maintained in fresh medium to stimulate Schwann cell proliferation. Within 7 days, Schwann cell purity could reach above 90%, which was confirmed by their bright, narrow, bipolar morphology under Nomarski image and positive immunoreactivity for S-100.
Cultured cells were expanded by passaging when confluent monolayers were obtained (every 7–10 days) and subcultured no more than five times. All experiments were carried out using Schwann cells from P3-P5 in culture.

**Patch-clamp recordings.** Whole cell currents of granule neurons were recorded using a patch-clamp technique. Before $I_A$ current recording, the culture medium was replaced with a bath solution containing (in mM) 120 NaCl, 5 KCl, 10 HEPES, 5 CaCl$_2$, 2 MgCl$_2$, and 20 tetraethylammonium chloride (TEA) (pH adjusted to 7.4 using NaOH). Soft glass recording pipettes were filled with an internal solution containing (in mM): 11 K gluconate, 164 KCl, 10 HEPES, 1 CaCl$_2$, 2 MgCl$_2$, and 11 EGTA (pH adjusted to 7.3 using KOH). The pipette resistance was 6–7 MΩ after filling with the internal solution. Bradykinin solutions were prepared extemporaneously, and gravity was ejected for 10–20 s from MSC-200 Manual Solution Changer (Bio-Logic-Science Instruments). All recordings were performed at room temperature (23–25°C).

**Data acquisition and analysis.** All currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) operated in voltage-clamp mode. A Pentium computer was connected to the recording equipment with a Digidata 1300 analog-to-digital (A/D) interface. Current was digitally sampled at 100 μs (10 kHz). The current signals were filtered by a 3-kHz, 3-pole Bessel filter. Currents were corrected online for leak and residual capacitance transients by a P/4 protocol. Data acquisition and analysis were performed with pClamp 8.01 software (Axon Instruments) and/or Origin6.1 (Microcal analysis software, Northampton, MA). Statistical analysis was performed using Student's $t$-test with nonpaired comparison or paired comparisons where it was relevant. Values are given as means ± SE, with n as the number of cell tested. $P < 0.05$ was used to denote the statistical difference between groups. When multiple comparisons were made, data were analyzed by a one-way ANOVA test.

**cAMP assay.** cAMP levels were measured as previously described (3). Briefly, $1 \times 10^5$ cells were plated in each 35-mm dish and grown to confluence. Cells were washed with 1 ml of media and preincubated at 37°C for 10 min in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (2.5 mM) (Sigma). Cells were incubated an additional 3 min at 37°C with or without BK with varying concentrations of 100 nM, 1 μM, and 5 μM. The media were removed, and 0.5 ml of 0.1 M HCl with 0.8% Triton X-100 (Sigma) was added to the plates. After 30 min of incubation at room temperature, the lysate was removed from the plates and centrifuged for 2 min. The supernatant was collected and assayed for cAMP levels using a direct cAMP enzyme immunoassay kit (Assay Designs) according to the manufacturer’s instructions. Protein concentrations in each sample were determined using a BCA Protein Assay Kit (Pierce).

**Chemicals.** BK, B6029 [N-alpha-adamantaneacetyl-o-Arg-(Hyp$^7$,Thi$^8$,d-Phe$^9$)-bradykinin], B6929 [Lys-(des-Arg$^7$,Leu$^8$)-bradykinin trifluoroacetate salt], GTP-$\gamma$S, dibutyryl (db)-cAMP, insulin, forskolin, and DMEM/Ham’s F-12 medium were purchased from Sigma-Aldrich (St. Louis, MO). NF449 (4,4',5,5'-[carbonylbis(imino-3,1-phenylene)] )tetrakis(azido-benzene-1,3-disulfonic acid; 8Na) and NF023 (8,8'-[carbonylbis(imino-3,1-phenylene)]bis(1,3,5-naphthalenetrisulfonic acid; 6Na) were purchased from A Brand of EMD Chemicals (Merck, Darmstadt, Germany). Fetal calf serum and antibiotic-antimycotic solution were obtained from Gibco Life Technologies (Grand Island, NY).

**RESULTS**

We investigated whether BK inhibited $I_A$ amplitude using a bath solution containing 20 mM TEA to eliminate $I_K$ currents. $I_A$ currents were evoked by 500-ms constant depolarizing pulses, ranging from −80 mV to 60 mV at 10-s intervals. Application of BK to the bath solution produced a clear reduction in $I_A$ current amplitude. The inhibitory effect of BK on $I_A$ was reversible, reached its maximum effect within 200–250 s, and recovered to control levels after 1–2 min. Moreover, the BK-induced effect on $I_A$ was concentration dependent. The inhibition of $I_A$ produced by BK at 100 nM, 500 nM, and 1 μM was 16.9 ± 3.9% (n = 6), 25.5 ± 3.3% (n = 5), and 29.6 ± 1.5% (n = 5), respectively ($P < 0.05$ using one-way ANOVA). When the concentration of BK was increased to 5 μM, $I_A$ decreased to 46 ± 3.9% (n = 6) of the control. Fig. 1A illustrates a series of typical experiments in which BK was applied at different concentrations. Statistical analysis of the concentration-response data are summarized in Fig. 1B.

The effect of BK on the activation and inactivation of $I_A$ was studied using the appropriate voltage protocols. In the voltage activation protocol, membrane potential was held at −80 mV, and $I_A$ was evoked by a 500-ms depolarizing pulse from a first pulse potential of −50 mV to +100 mV in 10-mV steps at 10-s intervals (Fig. 2A). When the depolarizing pulse was more positive than −10 mV, BK reduced the current amplitude throughout the activation voltage range (Fig. 2B). When the

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*Fig. 1. Bradykinin (BK) inhibited inactivating A-type outward K$^+$ current ($I_A$) in a concentration-dependent manner. A: superimposed K$^+$ current evoked by 500-ms depolarizing pulses from −80 mV to 60 mV. The current traces were obtained in the absence and presence of BK at concentrations of 0.1 μM to 5 μM. B: statistical analysis of the effect of various BK concentrations on $I_A$. The data represent means ± SE obtained from five to seven cells. *P < 0.05 compared with the control; #P < 0.05 compared with the data obtained from different concentrations, using one-way ANOVA.*
peak current evoked from each command potential was normalized to the maximal current amplitude, we obtained the $I_A$ activation curve. As shown in Fig. 2C, the activation curve was shifted to the left by the application of BK. The current was half-activated at $-10.6 \pm 1.9$ mV and $-24.1 \pm 4.0$ mV in the absence and presence of 500 nM BK, respectively ($n = 5, P < 0.05$ using Student’s $t$-test), suggesting that BK treatment significantly modified the voltage dependence of steady-state activation of $I_A$ channels.

To study the effect of BK on steady-state inactivation of $I_A$ currents, we elicited using 1-s conditioning prepulses from $-70$ mV to various membrane potentials before a 400-ms test pulse of $+60$ mV as shown by Sobko et al.'s report (43) (Fig. 3, A and B). After normalizing each current peak to the maximal current amplitude obtained from the $-70$-mV prepulses as a function of the conditioning prepulse potential, we obtained an inactivation curve of $I_A$ and calculated the $V_h$ (the voltage at which the current amplitude was half-inactivated; Fig. 3C). We found that BK did not affect the steady-state inactivation curve. In four cells studied, the half-maximal inactivation voltage was $-35.1 \pm 0.2$ mV and $-37.3 \pm 0.2$ mV in the absence and presence of BK (500 nM), respectively ($n = 4, P < 0.05$ using Student’s $t$-test).

Because previous studies in SCs of the DRG revealed that BK induced the release of glutamate via activation of B$_2$ receptors (39), experiments were carried out to identify whether and which BK receptors might mediate the BK-induced augmentation of $I_A$ currents. We first investigated whether B$_2$ receptors might be involved in the BK effect on the $I_A$ current by using a specific antagonist of the B$_2$ receptor, B6029. As shown in Fig. 4A, 100 nM B6029 alone did not change the current; however, in the presence of 100 nM B6029, the BK-induced effect on $I_A$ current was blocked (Fig. 4A). The 500 nM BK-induced effect on the $I_A$ current amplitude was $109 \pm 3.7\%$ of the control ($n = 4, P < 0.05$). In contrast, the B$_1$ receptor antagonist, B6929, did not abolish the BK-induced inhibitory effect on the $I_A$ current. In the presence of B6929, the inhibition of $I_A$ by 500 nM BK was $26.1 \pm 1.9\%$ ($n = 4$), not significantly different from the effect induced by BK alone (Fig. 4B). The statistical analysis of these experiments is shown in Fig. 4C.

BK receptors are coupled receptors known as the G protein-coupled receptors that mediate multiple signal transduction pathways (10, 36). This possible pathway was tested in the BK-induced effect on $I_A$ currents by adding GTP-$\gamma$S (100 $\mu$M) to the pipette solution. Internal infusion of GTP-$\gamma$S (100 $\mu$M) resulted in the gradual suppression of $I_A$ amplitude after membrane rupture (Fig. 5A). The amplitude declined significantly from $1,969.2 \pm 284.5$ pA at the control level to $1,234.7 \pm 225.9$ pA, a decrease of $34.9 \pm 8.6\%$ ($P < 0.05, n = 6$). Moreover, following the reduction in $I_A$ amplitude by internal application of GTP-$\gamma$S, BK induced average reductions in $I_A$ amplitude of $11.9 \pm 3.0\%$ ($n = 6$), which was significantly different from that induced by BK alone ($P < 0.05$). Since several G proteins could be involved in this process, we also investigated the role of G$_i$/$\alpha$ and G$_s$ proteins using the G$_i$ inhibitor NF449, as well as NF023, which inhibits both G$_i$ and G$_s$ activation with similar potency (7). As shown in Fig. 5B, intracellular application of NF449 (10 $\mu$M) induced a gradual augmentation of $I_A$ from $1,032.4 \pm 173.4$ pA to $1,484.2 \pm 224.5$ pA, an increase of $45.9 \pm 5.8\%$, and extracellular application of BK (500 nM) only provoked an $I_A$
inhibition of 5.9 ± 1.2%. Intracellular loading with NF023 (10 μM) also significantly increased $I_A$ amplitude from 1,416.8 ± 312.9 pA to 1,762.1 ± 423.5 pA after membrane rupture, an increase of 22.5 ± 12.0% of the control (Fig. 5C, $P < 0.05, n = 5$); however, BK decreased $I_A$ by 18.8 ± 3.5% (Fig. 5C), no significant difference was observed when compared with BK alone ($P > 0.05, n = 4$). This result suggested that $G_s$ was required for $I_A$ reduction induced by BK.

It is well known that the $G_s$ subtype directly enhances cAMP synthesis by activating adenylyl cyclase. To address whether the cAMP/PKA pathway contributed to the inhibitory effect of BK on $I_A$, the effect of BK was further investigated in the presence of an agonist of PKA. Perfusion of SCs with 1 μM forskolin provoked a gradual decrease in $I_A$ amplitude from 2,504.2 ± 319.2 pA to 1,584.3 ± 326.4 pA, a reduction of 36.7 ± 6.2%, similar with that induced by 500 nM BK. Moreover, in the presence of forskolin, BK only inhibited $I_A$ currents by 6.4 ± 1.2% (Fig. 6A, A and B). A uniform perfusion of 10 μM db-cAMP, a membrane-permeable cAMP analog, produced a significant reduction of 24.8 ± 4.6% in $I_A$ currents and abolished the BK-induced inhibitory effect on $I_A$ (Fig. 6C). In the presence of forskolin or cAMP, the BK-induced inhibitory effect on $I_A$ was 6.4 ± 1.2% and 6.0 ± 1.9%, respectively, with no significant differences compared with the control (Fig. 6D, $P < 0.05, n = 8$ and $n = 6$). To determine the mechanism by which the PKA pathway was involved in the BK-induced effect on $I_A$ currents, H-89, a selective PKA antagonist, was used. After addition of H-89 into the patch pipette solution to inhibit PKA, the $I_A$ current was significantly increased to 143.3 ± 7.1% of the control. Furthermore, H-89 significantly limited the effect of BK on the $I_A$ current to 125 ± 9.9% (Fig. 7A). In contrast, after the PKC pathway was activated by using PMA, the amplitude of the $I_A$ current was significantly increased after the establishment of the whole cell configuration and reached its maximum within 2–4 min. The percentage of the amplitude increased with intracellular PMA and was 154.4 ± 15% of the control ($n = 4, P < 0.05$ using Student’s $t$-test).

The effects of BK on the intracellular level of cAMP were detected by using a direct cAMP enzyme immunoassay in SCs exposed to BK. The results shown in Fig. 8 indicate that a basic cAMP level (15.1 ± 2.1 pmol, $n = 5$) was detected in SCs before the addition of BK. When the cultures were incubated with 100 nM BK for 3 min or 10 min, the level of cAMP activity was significantly increased to 24.8 ± 5.6 pmol and 33.8 ± 7.5 pmol, respectively ($P < 0.05$, compared with the control group and each other, $n = 5$).
obtained during 500-ms depolarizing step pulses from comparison with the corresponding controls. We showed that BK activated abolished the BK-induced effect on the coupled B2 receptor, and was mediated by cAMP/PKA-dependent signal transduction. The modulation of steady-state activation, via a G protein-

dent signal transduction.

also modified its gate character. The normalized activation and inactivation curves showed that the steady-state activation curve significantly left-shifted $-10$ mV. In fact, $I_A$ is fully available for activation at potentials near the resting potential, and the presence of a “window current” suggests that $I_A$ could greatly increase resting $K^+$ efflux, so that it may mediate proapoptotic $K^+$ efflux in some cases (11, 23, 33). Here, the left shift in the steady-state activation curve produced the resultant window current increasing toward the hyperpolarizing membrane potential, which may help to activate other types of $K^+$ channels such as the $I_K$ channel and then increase $K^+$ efflux.

In mammals, at least two subtypes of BK receptors have been identified: the bradykinin B1 receptor and B2 receptor (30). The kinin B1 receptors are not normally expressed in healthy tissues, whereas the kinin B2 receptors are constitutively expressed and are widely distributed throughout central and peripheral tissues in most organs, except the liver and spleen (28). However, although most of the physiological actions of BK are believed to be mediated by stimulation of the B2 receptors, the role of the B1 receptor in the control of cardiovascular function, modulation of pain, and activation of the $Na^+$/Ca$^{2+}$ exchanger has been reported recently (18, 24). In the present work, we showed that the selective B1 receptor antagonist failed to eliminate the BK-induced inhibitory effect on $I_A$ currents. In contrast, the selective B2 receptor antagonist significantly abolished the BK-induced inhibitory effect on $I_A$ currents. The results suggested that the B2 receptor but not the B1 receptor seemed to mediate the BK-induced inhibitory effect on $I_A$. These results were consistent with the findings of Parpura et al. (39) obtained from SCs in the DRG, in which BK induced the release of glutamate via the B2 receptor.

The BK receptor is known to be a G protein-coupled receptor and to transduce biological effects by multiple signal pathways (10, 36). Using a COS-7 cell expression system, Liebmann (26) revealed that stimulation of the B2 receptor led to mitogen-activated protein kinase signal transduction. In murine proximal tubule epithelial cells, a BK-induced rise in chloride current was mediated by $G_{\alpha_i}$ protein-coupled B2 receptors (45). By using inhibitors of $G_{\alpha_o}$, $G_{\alpha_i}$, and $G_{\alpha_o}$, we investigated the role of $G_{\alpha_i}$ and $G_{\alpha_o}$ proteins in this process. The results showed that the BK receptor and $G_{\alpha_i}$ proteins were associated in SCs because the BK-induced effect was fully mimicked by GTP-$\gamma$S and prevented by the $G_{\alpha_i}$ inhibitor. However, it was notable that both $G_{\alpha_i}$ and $G_{\alpha_o}$ inhibitors provoked a gradual increase in $I_A$ amplitude. This increase in $I_A$ by inhibition of $G_{\alpha_i}$ indicated that basic or unknown causal $G_{\alpha_i}$ activity may be associated with the basic G protein activity, whereas the results in which inhibition of $G_{\alpha_o}$ produced augmentation of $I_A$ indicated that, in mouse SCs, $G_{\alpha_o}$ was mediated by inhibition of adenylyl cyclase, rather than activation of PKC, because our results revealed that stimulation of the PKC pathway by PMA increased $I_A$ amplitude. However, $G_{\alpha_o}$ may interact with other second messengers, such as Ca$^{2+}$, PLC, and PLA$_2$, as suggested in recent reports (25).

Cyclic AMP is an important modulator of Schwann cell physiology in vitro (19, 20). Furthermore, it is known that a number of the voltage-dependent $K^+$ channels, including $I_A$, $I_K$, and Ca$^{2+}$-activated $K^+$ channels, are regulated by cAMP/ PKA or PKC pathways (1, 17, 29, 34), even though it is not
known whether the regulation of phosphorylation occurs directly or via intermediary proteins. The results obtained in this study demonstrated that the internal application of forskolin or cAMP completely mimicked the effect of BK on $I_A$, and when the cAMP/PKA pathway was activated by forskolin or cAMP, the BK-induced inhibitory effect on $I_A$ current did not occur. Furthermore, inhibiting the PKA pathway by intracellular H-89 produced a gradual increase in the amplitude of the $I_A$ current.

Fig. 5. G protein subtype Gs mediated the BK-induced effect on $I_A$. A: time course of changes in the amplitude of $I_A$ induced by application of 100 μM GTP-γS. Internal infusion of GTP-γS resulted in gradual suppression of $I_A$ amplitude after membrane ruptured, and eliminated the 500 nM BK-induced reductions in $I_A$ amplitude. B: time course of changes in the amplitude of $I_A$ induced by applying BK in the presence of 10 μM NF449. NF499 provoked a gradual decrease in $I_A$ amplitude and abolished the effect of 500 nM BK on $I_A$ amplitude. C: time course of changes in the amplitude of $I_A$ induced by BK in the presence of NF023. NF023 provoked a gradual decrease in $I_A$ amplitude, but did not abolish the effect of 500 nM BK on $I_A$ amplitude. D: statistical analysis of the effect of GTP-γS, NF499, and NF023 on $I_A$ amplitude and BK-induced $I_A$ decrease. Data are presented as means ± SE obtained from five to nine cells. *$P < 0.05$ compared with control group; #$P < 0.05$ compared with the corresponding group without BK.

Fig. 6. cAMP/PKA pathway involved in the BK-induced effect on $I_A$ of SCs. A: time course of the changes in the amplitude of $I_A$ induced by 500 nM BK alone. B: time course of changes in the amplitude of $I_A$ induced by BK in the presence of extracellular forskolin. Forskolin (1 μM) provoked a gradual decrease in $I_A$ amplitude and abolished the effect of 500 nM BK on $I_A$ amplitude. C: time course of the changes in the amplitude of $I_A$ induced by BK in the presence of dibutyryl (db)-cAMP. db-cAMP (10 μM) decreased $I_A$ amplitude and eliminated the effect of 500 nM BK on $I_A$ amplitude. In A–C, the lowercase letters (a, b, c and d) noted on the curves correspond to the superimposed $I_A$ current traces illustrated by insets. D: statistical analysis of the effects of forskolin (Fors) and db-cAMP on $I_A$ amplitude and the BK-induced $I_A$ increase. Data are presented as means ± SE obtained from five to nine cells. *$P < 0.05$ compared with control group; #$P < 0.05$ compared with the BK alone without forskolin or db-cAMP.
after the establishment of the whole cell configuration, and the BK-induced inhibitory effect on the \( I_A \) current was completely prevented by H-89, suggesting that the cAMP/PKA pathway mediated the BK-induced inhibitory effect on the \( I_A \) current. Our findings are different from those of Xu et al. (46), in which they revealed that activation of \( I_A \) was positively regulated by PKA in neurofibromatosis type-1 gene (\( \text{Nf1} \)) null mouse SCs. However, we note that the increase in \( I_A \) was long-term mediated via neurofibromatosis type-1 gene (\( \text{Nf1} \)) null mutations leading to activation of PKA by an increase in the expression of functional channels, rather than by the phosphorylation of channel subunits and drive-altered channel activation or inactivation properties by short-term activation of the cAMP/PKA pathway, as in the present study and in a previous report by Gallego et al. (8) involving \( \alpha_1 \)-adrenoceptor that induced a reduction in the amplitude of the transient outward current (\( I_o \)) by activating the cAMP/PKA signaling cascade, which in turn led to \( I_o \) channel phosphorylation (8). Whether long-term treatment of SCs by BK induces the upregulated expression of the \( I_A \) channel will require further investigation. Although previous investigations in human airway smooth muscle cells and astrocytes have revealed that the B2 receptor mediated the activation of the PKC pathway (12, 14), the PKC pathway may be excluded from the BK-induced inhibitory effect on \( I_A \) current in SCs because our results demonstrated that stimulation of the PKC pathway by PMA produced a significant increase in \( I_A \) currents, which was different from the BK-mediated inhibitory effect on \( I_A \) currents.

In excitable cells such as neurons or myocardial cells, the functional roles of \( I_A \) include influencing cell excitability, action-potential firing, controlling spike latency, and repetitive firing (41). \( I_A \) is also an excellent target for any modulatory mechanism influencing cell excitability and action potential firing (21, 41). We showed previously that apoptosis of cerebellar granular neurons induced by low potassium and free serum was associated with increased \( I_A \) (15–17), suggesting an important function of \( I_A \) channels. Although the precise function of \( I_A \) in SCs is not known, its aberrant expression may have important implications for tumorigenesis in SCs, because voltage-gated K+ channel activity in glia is clearly linked to proliferation and differentiation (22, 42, 43, 46). Therefore, the inhibitory effect on \( I_A \) mediated by activation of the BK receptor might reveal an important significance in modulating SCs proliferation and differentiation and open a valuable new avenue for investigation, in terms of therapeutic and/or basic research applications of BK.

In summary, the present study provides evidence that B2 receptor stimulation couples the receptor to a Go protein. This coupling to Go is functional and activates the cAMP/
PKA signaling cascade, which leads to $I_A$ amplitude reduction in mouse SCs. This appears to be the first example, in a physiological preparation, in which BK receptors were linked to the cAMP/PKA pathway and to the modulation of the $I_A$ current.

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