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$GABA_B$ receptors inhibit low-voltage activated and high-voltage activated Ca^{2+} channels in sensory neurons via distinct mechanisms

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ABSTRACT

Growing evidence suggests that mammalian peripheral somatosensory neurons express functional receptors for gamma-aminobutyric acid, $GABA_A$ and $GABA_B$. Moreover, local release of GABA by pain-sensing (nociceptive) nerve fibres has also been suggested. Yet, the functional significance of GABA receptor triggering in nociceptive neurons is not fully understood. Here we used patch-clamp recordings from small-diameter cultured DRG neurons to investigate effects of $GABA_B$ receptor agonist baclofen on voltage-gated Ca^{2+} currents. We found that baclofen inhibited both low-voltage activated (LVA, T-type) and high-voltage activated (HVA) Ca^{2+} currents in a proportion of DRG neurons by 22% and 32% respectively; both effects were sensitive to $G_{i/o}$ inhibitor pertussis toxin. Inhibitory effect of baclofen on both current types was about twice less efficacious as compared to that of the μ-opioid receptor agonist DAMGO. Surprisingly, only HVA but not LVA current modulation by baclofen was partially prevented by G protein inhibitor GDP-β-S. In contrast, only LVA but not HVA current modulation was reversed by the application of a reducing agent dithiothreitol (DTT). Inhibition of T-type Ca^{2+} current by baclofen and the recovery of such inhibition by DTT were successfully reconstituted in the expression system. Our data suggest that inhibition of LVA current in DRG neurons by baclofen is partially mediated by an unconventional signaling pathway that involves a redox mechanism. These findings reinforce the idea of targeting peripheral GABA receptors for pain relief.

1. Introduction

Mammalian peripheral somatosensory neurons detect and transmit to CNS versatile information about body's environment; a large population of these neurons ("pain" or nociceptive neurons) responds specifically to tissue damage. Cell bodies of these neurons reside in peripheral ganglia (e.g. dorsal root ganglia, DRG) while their long, T-shaped axons form afferent sensory fibres innervating skin, muscles, joints and other bodily organs. Excitation of peripheral terminals of somatosensory fibres initiate physiological somatosensory transmission, in addition, action potentials produced elsewhere along the fibre (i.e. ectopically) can result in pathological sensations (e.g. chronic pain or 'phantom' itch) [1]. Although main somatosensory integration and processing is performed by CNS, growing evidence suggest that peripheral neurons themselves can communicate with each other, both electrically and chemically [2–4]. Accordingly, cell bodies of sensory neurons express various receptors for classical neurotransmitters such as acetylcholine, glutamate and GABA [5]. Particularly, both $GABA_A$ [6–9] and $GABA_B$ [10,11] receptors are abundantly expressed in somatosensory neurons, including nociceptors. A recent study demonstrated that peripheral nociceptive terminals are capable of releasing GABA, which acts in an autocrine fashion at the

Abbreviations: DRG, dorsal root ganglion; DTT, dithiothreitol; GABA, gamma-aminobutyric acid; GFP, green fluorescent protein; HEK293, human embryonic kidney 293 cells; HVA, high-voltage activated Ca^{2+} channels; LVA, low-voltage activated Ca^{2+} channels; NK1, neurokinin receptor isoform 1; ROS, reactive oxygen species; PTX, pertussis toxin; VGCC, voltage-gated Ca^{2+} channels.

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endogenous $GABA_{B1}$ receptors to attenuate sensitization of heat- and inflammation-activated TRPV1 channels thus limiting hyperalgesia in rats [10]. Hence, emerging data demonstrate functional significance of peripheral GABA receptors in nociceptive neurons. Yet, the mechanisms of inhibitory action of $GABA_B$ receptors in the periphery are only beginning to emerge.

$GABA_B$ receptors belong to $G_{i/o}$ type of G-protein coupled receptors (GPCR) [12] which are known to exert inhibitory action in CNS via the inhibition of voltage-gated Ca^{2+} channels (VGCC) and activation of GIRK K^+ channels [13]. Therefore, we investigated effects of $GABA_B$ agonist baclofen on voltage-gated Ca^{2+} currents in cultured small-diameter DRG neurons. DRG neurons express variety of VGCC subtypes, including high-voltage activated (HVA) N, P/Q and L-type channels [14–18] as well as low-voltage activated (LVA) T-type Ca^{2+} channels [19–21]. Using patch-clamp recordings we analysed effects of baclofen on both LVA and HVA currents; we found that both currents are inhibited by baclofen in subpopulations of DRG neurons. Interestingly, we found that inhibition of LVA and HVA currents was mediated by distinct mechanisms. These findings extend our understanding of $GABA_B$ receptor action in peripheral somatosensory system and suggest a possibility of targeting these receptors for analgesia.

2. Materials and methods

2.1. Cell cultures and transfection

DRG neurons were cultured as described previously [22–24]. Briefly, adult Sprague Dawley rats (170 g–180 g) were humanly euthanized by isoflurane overdose. DRGs from all spinal levels were extracted and dissociated using collagenase/dispase method as described [22–24]. Dissociated cells we cultured in DMEM supplemented with GlutaMax I, 10% fetal calf serum, penicillin (50 U/ml) and streptomycin (50 μg/ml) on glass coverslips coated with poly-D-lysine for 2–5 days in a humidified incubator (37 °C, 5% CO_2). No growth factors were added to the culture media.

Hunan embrionic kidney cells 293 (HEK293) cells were cultured in the same DMEM supplemented with GlutaMax I, 10% fetal calf serum, penicillin (50 U/ml) and streptomycin (50 μg/ml) and passaged every 2–3 days. Human $GABA_{B2}$ receptor (GenBank accession number NM_005458) cDNA was purchased from the Missouri Science and Technology cDNA Resource Center. Human Cav3.2 (GenBank accession number AF051946) cDNA was kindly provided by Dr E. Perez-Reyes, (University of Virginia, USA). HEK293 cells were transfected using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions.

2.2. Electrophysiology

All recordings were made using Multiclamp 700B amplifier in combination with pCLAMP 10.4 software (Axon Instruments, Union City, CA, USA). Voltage-clamp recordings were sampled at 4 kHz. A Whole-cell configuration of the patch clamp technique was used throughout. The standard bath solution contained (in mM): 150 TEA-Cl; 2 $CaCl_2$; 10 HEPES; 10 glucose (pH 7.4 adjusted with CsOH). The standard pipette solution contained (in mM): 135 CsCl; 3 $MgCl_2$; 10 EGTA; 10 HEPES; 3 Mg-ATP; 0.6 GTP (PH 7.4 adjusted with CsOH). LVA currents were measured by 50 ms square voltage pulses to −40 mV from a holding potential of −90 mV. HVA currents were measured by 100 ms square voltage pulses to 0 mV from a holding potential of −90 mV. A 3% agar-salt (3 M KCl) bridges were used in order to avoid possible redox-induced offset potentials.

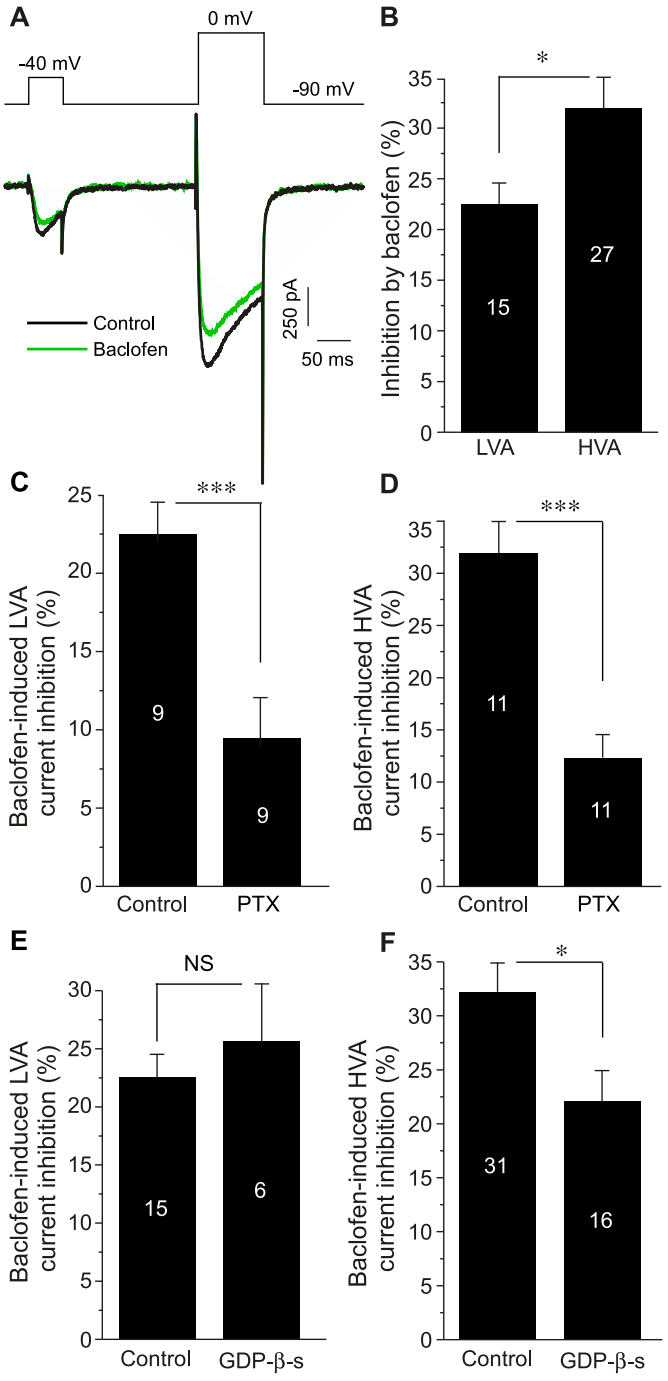

Fig. 1. Baclofen inhibits LVA and HVA calcium currents in DRG neurons. (A) Voltage protocol (top) and exemplary current traces showing inhibition of LVA and HVA Ca^{2+} currents in DRG neurons by 10 μM baclofen recorded using whole-cell patch clamp. Panel (B) shows summary of the effects of baclofen on peak LVA and HVA current amplitudes, number of recordings is indicated within the bars. (C, D) Pre-treatment of DRG cultures with $G_{i/o}$ inhibitor pertussis toxin (PTX, 400 ng/ml) reduced baclofen-induced inhibition of LVA (C) and HVA (D) currents in DRG neurons. (E, F) Inclusion of the non-hydrollyzable GDP analog GDP-β-s (2 mM) into the intracellular pipette solution reduced baclofen-induced inhibition of HVA (F) but not LVA (E) Ca^{2+} currents. The number of experiments is shown within the bars. *, *** Significantly different from the group indicated by the line connector with $P < 0.05$ or $P < 0.001$ (paired or unpaired t-test, as appropriate).