Neuroscience Letters 543 (2013) 146–151

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

$GABA_B$ receptors resist acute desensitization in both postsynaptic and presynaptic compartments of periaqueductal gray neurons

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HIGHLIGHTS

- $GABA_BR$ activation resists desensitization in both pre- and postsynaptic compartments.
- Resisting desensitization at postsynaptic is independent of presynaptic alteration.
- Resisting desensitization at presynaptic is independent of postsynaptic alteration.

ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 17 March 2013
Accepted 18 March 2013

Keywords:
Periaqueductal gray
$GABA_B$ receptors
G-protein-coupled receptors
Baclofen
Desensitization
Pain

ABSTRACT

The ventrolateral midbrain periaqueductal gray (PAG) neurons have been intensively studied because of their pivotal role in the descending pain modulation system. Activation of $GABA_B$ receptors, one type of inhibitory G-protein-coupled receptors (GPCRs), in PAG neurons results in both presynaptic and postsynaptic inhibition. Acute desensitization is defined as rapid attenuation of receptor-mediated signaling. Recent studies report that multiple inhibitory GPCRs, including $GABA_B$ receptors, resist acute desensitization in the presynaptic but not postsynaptic compartments of certain neurons in mammal brains. In the present study, employing whole-cell voltage-clamp recordings on acute PAG slices from adult rats, we found that $GABA_B$ receptors resist acute desensitization to prolonged administration of baclofen ($GABA_B$ receptor agonist) in both presynaptic and postsynaptic compartments. The desensitization resistance of postsynaptic $GABA_B$ receptors was independent of presynaptic alteration and vice versa. The $GABA_B$ receptor-mediated inhibition at inhibitory presynaptic terminals also showed no desensitization. The results suggest that $GABA_B$ receptor-mediated inhibition remains functional in both postsynaptic and presynaptic compartments to sustained agonist administration in rat PAG neurons.

1. Introduction

γ-Aminobutyric acid (GABA) is the predominant inhibitory neurotransmitter in the mammalian central nervous system (CNS). GABA exerts its actions through two types of receptors: ionotropic and metabotropic receptors. While ionotropic $GABA_A$ and $GABA_C$ receptors are ligand-gated chloride channels involved in fast synaptic transmission, metabotropic $GABA_B$ receptors belong to the superfamily of G-protein-coupled receptors (GPCRs). Consisting of heterodimers of $GABA_{B1}$ and $GABA_{B2}$ subunits, $GABA_B$ receptors mediate both presynaptic and postsynaptic "slow" inhibitions [2].

$GABA_B$ receptors are distributed throughout mammalian CNS [14], including pain transmission and modulation circuits in spinal cord [7,19,24,25] and periaqueducatal gray (PAG) in the midbrain [14,24]. The ventrolateral part of PAG is a pivotal site for the descending pain modulation circuit and a major site for the

Abbreviations: aCSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; AP-5, D-2-amino-5-phosphonopentanoic acid; CGP52432, 3-[[[(3,4-dichlorophenyl)methyl]amino]propyl] (diethoxy-methyl) phosphinic acid; CNS, central nervous system; DNQX, 6,7-dinitroquinoxaline-2,3(1H,4H)-dione; EPSCs, excitatory postsynaptic currents; GABA, γ-aminobutyric acid; GDP-β-S, guanosine 5′-[β-thio]diphosphate trilithium salt; GPCRs, G-protein-coupled receptors; IPSCs, inhibitory postsynaptic currents; K–S test, Kolmogorov–Smirnov test; LC, locus ceruleus; PAG, periaqueductal gray; POMC, proopiomelanocortin; TTX, tedrodotoxin.

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http://dx.doi.org/10.1016/j.neulet.2013.03.035

analgesic actions of μ-opioids, cannabinoids and $GABA_B$ receptors [8,11,16]. Our previous data suggest that in ventrolateral PAG neurons, activation of $GABA_B$ receptors by a specific agonist, baclofen, results in both presynaptic and postsynaptic inhibition [22], where potassium channels play distinct roles in presynaptic and postsynaptic compartments [12].

$GABA_B$ receptors are one type of inhibitory GPCRs that play important roles in analgesia in ventrolateral PAG [11,16,21]. The sensitization and desensitization are important for the pharmacological actions mediated by these receptors. Recent studies in hypothalamic proopiomelanocortin (POMC) and locus ceruleus (LC) in pons suggest that multiple inhibitory GPCRs resist acute desensitization, which is a phenomenon of receptor-mediated signaling attenuation to sustained agonist exposure in POMC neurons [17] and LC neurons [5]. In specific, prolonged activation of $GABA_B$ receptors induces an acute desensitization at postsynaptic but not presynaptic compartments of POMC neurons in mouse brain [17]. Activation of μ-opioid receptors, another type of GPCRs, also induces postsynaptic receptor desensitization in LC neurons [5]. An unknown, however is whether $GABA_B$ receptor activation causes desensitization in rat PAG neurons, and subsequently, the features of desensitization at presynaptic and postsynaptic compartments. In the present study, employing conventional whole-cell voltage-clamp recordings on neurons of acute PAG slices, we demonstrate that prolonged exposure of $GABA_B$ receptors to baclofen, a selective agonist, induces no significant acute desensitization in either postsynaptic or presynaptic compartments in rat PAG neurons.

2. Materials and methods

2.1. Slice preparation and whole-cell recordings

Coronal midbrain slices containing PAG (400 μm thick) were dissected from adult (5–8 weeks old) male Sprague-Dawley rats, as described elsewhere [12,22]. Slices were incubated in oxygenated (95% O_2–5% CO_2) artificial cerebrospinal fluid (aCSF) containing (in mM) NaCl 124, KCl 3.6, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11 (pH 7.3, mOsm 300–305). The intrapipette solution for recording excitatory postsynaptic currents (EPSCs) contained (in mM) K-gluconate 135, KCl 5, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, Hepes 5 and Mg-ATP 3.6; for recording inhibitory postsynaptic currents (IPSCs) contained (in mM) CsCl 100, $MgCl_2$ 1, Hepes 10, EGTA 5, Mg-ATP 3.6, phosphocreatine disodium 14 and GTP 0.1. Neurons were visualized with a water-immersion objective on a standard upright microscope and whole-cell recordings were carried out on ventrolateral PAG neurons with glass pipette (5–8 MΩ) at room temperature [12,23]. All signals were amplified by an Axopatch 200B (Axon Instruments, CA, USA) and recorded by Clampex 8 or Clampex 9. Spontaneous EPSCs (sEPSCs) mediated by α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors were recorded at holding potential of −70 mV in the presence of NMDA receptor antagonist D-2-amino-5-phosphonopentanoci acid (AP-5; 50 μM) and $GABA_A$ receptor antagonist bicuculline (10 μM). Action potential-independent miniature EPSCs (mEPSCs) or miniature IPSCs (mIPSCs) were recorded in the presence of 0.5 μM tedrodotoxin (TTX) in bath solution. The evoked IPSCs (eIPSs) and mIPSCs were recorded in the presence of ionotropic glutamate receptor antagonists AP-5 (50 μM) and 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX; 10 μM) at a holding potential of −70 mV. Presynaptic focal-evoked glutamate or GABA release was stimulated by a bipolar tungsten electrode (0.1 ms rectangular wave at a frequency of 30 s^{-1}), by setting 100–500 μm away from the recording pipette. In the present study, 10 μM baclofen was used to reliably activate both presynaptic and postsynaptic $GABA_B$ receptors simultaneously [4,12,21,22].

2.2. Chemicals, drugs, data analysis and statistics

TTX, DNQX, guanosine 5′-[β-thio]diphosphate trilithium salt (GDP-β-S) and bicuculline were obtained from Sigma (MO, USA). 3-[[[(3,4-Dichlorophenyl)methyl]amino]propyl] (diethoxymethyl) phosphinic acid (CGP52432) was obtained from Tocris (Bristol, UK). Bath solution containing drugs was applied to custom-made recording chamber (volume 1.5 ml) with a rate of 8–10 ml/min, by switching a three-way tap, without changing perfusion rate for different solutions [23]. Data were analyzed off-line using Clampfit 9 (Axon Instruments). Average data values are presented as mean ± S.E.M. Cumulative frequency and amplitude distributions of mEPSCs and mIPSCs were compared with Kolmogorov–Smirnov test (K–S test), as shown in our previous studies [22]. Levels of significance were determined by ANOVA or Student's *t*-test. Significance was determined as $P < 0.05$.

3. Results

3.1. $GABA_B$ receptors resist acute desensitization to prolonged activation in postsynaptic compartments of PAG neurons

The postsynaptic actions of baclofen were measured by monitoring slow membrane current, a result of postsynaptic K^+ channel opening [12,23]. Bath application of 10 μM baclofen caused a sustained outward potassium current, which reached the peak within 2 min of wash-in under our recording conditions (Fig. 1A). In 13 of 15 neurons tested, prolonged baclofen exposure (up to 30 min) induced a sustained membrane current, while the other 2 cells showed an attenuation of the current beginning at ~3 min and ~5 min, respectively. In all 15 neurons tested, the initial peak amplitude taken at 3 min of wash-in was 27.6 ± 3.5 pA. The average peak of this membrane current was 24.5 ± 5.1 pA at 10 min, 23.5 ± 4.4 pA at 15 min and 22.5 ± 5.4 pA at 20 min wash-in, which showed no significant difference at different wash-in times ($n = 15$, $P > 0.05$ compared to the value at 3 min, two-way ANOVA) (Fig. 1C). This current of postsynaptic action decreased to 3.5 ± 2.1 pA after 15 min washout ($P < 0.01$ compared with the amplitude of baclofen wash-in at different time periods; one-way ANOVA). The results indicate that $GABA_B$ receptors on postsynaptic compartments of PAG neurons resist desensitization to prolonged receptor activation.

The change of postsynaptic membrane current depends on postsynaptic change (for example, K^+ channels open) and/or presynaptic neurotransmitter release [1]. In the above experiments, bath application of baclofen decreased presynaptic neurotransmitter release (see [22]), it is possible that this presynaptic release machinery alteration contributes to the resistance of postsynaptic desensitization. To address this issue, we blocked ionotropic glutamatergic neurotransmission with AP-5 (50 μM) and DNQX (10 μM) [12]. Under these conditions, administration of baclofen (10 μM) still induced a sustained outward current in 8 of 9 neurons tested. The average outward current was 26.1 ± 3.0 pA at 3 min, 24.4 ± 3.5 pA at 10 min, 24.7 ± 2.8 pA at 15 min and 23.3 ± 3.6 pA at 20 min of baclofen wash-in, indicating no significant desensitization ($n = 9$, $P > 0.05$, two-way ANOVA) (Fig. 1B and C). The amplitude of postsynaptic action was comparable to that induced without glutamate receptor blockade (Fig. 1C). The results suggest that prolonged exposure of $GABA_B$ receptors to the specific agonist shows no desensitization. The lack of postsynaptic $GABA_B$ receptor desensitization is not altered by presynaptic glutamate release. Pretreatment of a selective $GABA_B$ receptor antagonist, CGP52432 (1 μM; 5 min) largely prevented baclofen-induced membrane current in both aCSF ($n = 3$) and 10 μM DNQX bath ($n = 2$) (data not shown), indicating that the baclofen postsynaptic action was mediated by activating postsynaptic $GABA_B$ receptors.