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Research article

Optoactivation of parvalbumin neurons in the spinal dorsal horn evokes GABA release that is regulated by presynaptic $GABA_B$ receptors

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HIGHLIGHTS

- PV interneurons in the spinal dorsal horn can be optogenetically labeled and activated.
- Selective activation of PV neurons induces GABA release.
- Activation of $GABA_B$ receptors on PV neuron terminals depresses GABA release.
- Baclofen activity on GABA release is mainly mediated by P/Q type Ca^{2+} channels.

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ABSTRACT

Among heterogeneous neural cells in the spinal dorsal horn, parvalbumin (PV)-positive neurons are one subtype of GABA (γ-aminobutyric acid)-containing interneurons. Using an optogenetic approach, we expressed blue light-sensitive cation channel channelrhodopsin-2 (ChR2) via a viral vector on PV neurons in the spinal dorsal horn. Combined with in vitro whole-cell recordings, we activated ChR2 expressed on PV neurons by blue light and recorded GABAA receptor-mediated light-evoked inhibitory postsynaptic currents (L-IPSCs). The L-IPSCs were action potential-dependent and abolished by the $GABA_A$ receptor antagonist picrotoxin, indicating a synchronic GABA release from presynaptic terminals. Activation of $GABA_B$ receptors (the metabotropic receptors of GABA) on presynaptic terminals by a putative agonist, baclofen, depressed the amplitude of L-IPSCs. This depression was largely occluded by pretreatment with the highly selective Cav2.1 (P/Q-type) Ca^{2+} channel blocker ω-agatoxin IVA. N-type Ca^{2+} channel blocker ω-conotoxin GVIA showed less effects on either L-IPSCs or baclofen depression. We conclude that optoactivation of PV-ChR2 neurons in the spinal dorsal horn induces GABA release from presynaptic terminals, which is modulated by presynaptic $GABA_B$ receptors that are coupled to P/Q-type Ca^{2+} channels. Importantly, our studies provide a simple and reliable optogenetic approach to study dorsal horn neural circuits.

1. Introduction

Gray matter in the spinal dorsal horn consists of heterogeneous projection neurons and interneurons, as well as glial cells. Dorsal horn neurons receive nociceptive information from peripheral nociceptors, which relay sensory information to multiple supraspinal structures [6,24]. This ascending pathway is mainly mediated by glutamatergic excitatory projection neurons. On the other hand, inhibitory interneurons containing GABA (γ-aminobutyric acid) or glycine also play important roles in modulating projections, maintaining proper function and contributing to plasticity under specific pathological conditions [17]. Among these diverse cells, parvalbumin (PV)-positive neurons are one subtype of

Abbreviations: aCSF, artificial cerebrospinal fluid; AAV, adeno-associated virus; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; AP-5, D-2-amino-5-phosphonopentanoic acid; ChR2, channelrhodopsin-2; DNQX, 6,7-dinitroquinoxaline-2,3(1H,4H)-dione; GABA, γ-aminobutyric acid; GABA, γ-aminobutyric acid; GDP-β-S, guanosine 5′-(β-thio)diphosphate trilithium salt; IPSCs, inhibitory postsynaptic currents; NMDA receptors, *N*-methyl-D-aspartic acid receptors; TTX, tetrodotoxin.

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inhibitory interneurons which play important roles in processing nociception from peripheral to the central structures [9]. Several lines of evidence show that diverse interneurons have specific morphological and physiological features [12], and interneurons in the dorsal horn superficial laminae (I–III) play distinct roles [9,20,22]. However, it was impossible to causally implicate the contribution of specific neurons. Since the introduction of optogenetics in neuroscience [2,5], much scientific effort has been invested in the study of neural circuits and function, and the incorporation of optogenetics in pain research has resulted in many scientific advancements [3,21]. However, the roles of PV neurons in the spinal cord dorsal horn have not been well elucidated. In the present study, we employed optogenetic methods to express a blue light-sensitive cation channel channelrhodopsin-2 (ChR2) in PV neurons (including axons and dendrites), and using in vitro electrophysiological recording techniques, we analyzed optoactivation-induced GABA release. Finally, we examined the modulation of GABA release by presynaptic $GABA_B$ receptor activation. Because lamina II of the dorsal horn, which is also known as the substantia gelatinosa (SG), mainly consists heterogeneous populations of cells, including PV interneurons [1,9,14,20], our present data were predominantly collected from neurons within the SG.

2. Materials and methods

All of the experiment procedures were approved by the local Animal Care and Use Committees. We employed a strategy to use transgenic mice, which expressed Cre recombinase in PV neurons. Briefly, adult male and female PV-cre mice (5–7 weeks of age, The Jackson Laboratory) were anesthetized with ketamine and xylazine (60 mg/kg and 16 mg/kg bodyweight, respectively) until a loss of the paw withdrawal reflex was observed. To expose the lumbar enlargement of the spinal cord, a laminectomy was performed at the level of T10 to L4 [28], and the dura matter and membrane were carefully opened. The surface of the exposed spinal cord was moistened with warm saline (~36 °C). The animal was then placed in a stereotaxic holder (Kopf 900LS) with the head fixed and the spinal cord secured by additional clamps along the vertebrae [7]. The body was free to the bottom to reduce vibration from either respiration or heartbeat. A pulled calibrated micropipette (Drummond Scientific Company) with a tip diameter of ~15 μm was used for injection. To infect PV neurons with ChR2, an AAV vector (University of Pennsylvania Vector Core) containing a flexed ChR2 and mCherry was pressure-injected into the dorsal horn. The injection was performed unilaterally at 3 points along the caudal-rostral line, which was 400 μm lateral to the midline at the lumbar enlargement, and 30–120 μm below the surface. Each point was injected with 1 μL by a motorized syringe pump (KD Scientific) over a course of 5 min. Aseptic surgical technique was performed and the animals were allowed to recover for 18–25 d for ChR2 expression as previously described [18]. The animals were deeply anesthetized with urethane (1.5 g/kg bodyweight), and transverse spinal cord slices (400 μm thickness) were prepared as previously described in detail [28], using ice-cold aCSF (in mM): NaCl 125, KCl 3, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25 and glucose 11. The solution was saturated with mixed gas (95% O_2 and 5% CO_2). After a 40 min pre-incubation at room temperature (22–24 °C) in an interface chamber, one slice was transferred to a custom-made recording chamber (~1 mL) under a microscope (Olympus BX51WI). In a set of experiments for investigating the role of Ca^{2+} channels, some slices were further incubated with aCSF containing ω-agatoxin IVA (agatoxin; 250 nM) or ω-conotoxin GVIA (conotoxin; 250 nM) (both from Tocris) for at least 1 h. ChR2 expression on PV neurons was confirmed using yellow light of an ~589 nm wavelength. Optogenetic evocation of ChR2 was performed using a blue light pulse (~473 nm wavelength, 5 ms, 0.05 Hz, ~2.0 mw), delivered from Olympus 100 W mercury lamp through microscope objective, achieved by fluorescence filter cubes (Olympus). The objective on the upright fluorescent microscope was 40 X water-immersion 0.75 NA (numerical aperture) type with a flash spot size of ~200 μm diameter in aCSF. The blue light and electrophysiological recordings were simultaneously manipulated by pClamp 9 (Molecular Devises), in which the presence and absence of light was controlled using a pulse generator (PulseMaster A300, WPI). The recording configuration was established under a differential interference contrast system. Whole-cell recordings were obtained from randomly selected ChR2-negative neurons. To rule out the possibility of patching a ChR2-exprssion neuron to clarify the presynaptic analysis, after establishment of configurations, a 10 ms blue light was delivered to test the photocurrent, similar to that we did before (Nagode et al., 2014). If a detectable photocurrent was observed, the recoding was discarded. The light-evoked IPSCs (L-IPSCs) had an average time delay of 9.4 ± 0.7 ms (n = 20; calculated from the end of blue light to L-IPSCs onset). The latency of a single neuron was stable with fluctuation less than 10% throughout the recordings. Signals were amplified by Axopatch 200B (Molecular Devices), filtered at 5 k Hz and sampled at 10 k Hz. The internal solution for the recording glass pipette was (in mM): Cs-methanesulfonate115, CsCl 20, HEPES 10, $MgCl_2$ 2.5, Na_2-ATP 4, Na_3-GTP 0.4, Na-phosphocreatine 10, GTP-β-S 1 and EGTA 0.6 (pH 7.2 adjusted by CsOH, mOsm ~290). The pipette had a resistance of 5–8 MΩ when filled by internal solution. After establishing the whole-cell configuration, the holding potential was maintained at −70 mV and $GABA_A$ receptor-mediated L-IPSCs were pharmacologically isolated using an antagonist cocktail (AP-5 50 μM for NMDA receptors; strychnine 1 μM for glycine receptors and DNQX 20 μM for AMPA receptors) [29].

Baclofen (a putative $GABA_B$ receptor agonist), AP-5, strychnine and DNQX were obtained from Sigma–Aldrich. Data were expressed as the mean ± SEM. Statistical comparisons of drug effects were performed using Student's t-test. Significance was determined at $P < 0.05$.

3. Results

We first confirmed ChR2 expression on PV neural structures using yellow light epifluorescence illumination. Injection of AAV-containing ChR2 resulted in the expression of ChR2-mCherry, which was detected in a population of cells throughout the dorsal horn. At higher magnification, ChR2-positive neurons exhibited the typical characteristics of interneurons (Fig. 1A), with rounded or oval cell bodies and a major diameter of ~10 μm. In ChR2-expressing slices, we subsequently performed whole-cell recordings. After establishing a whole-cell configuration, a blue light pulse was delivered to the entire visual field to activate ChR2. In 19% of the neurons recorded (6 of 32) in PV-cre mice, maximal intensity blue light failed to evoke any detectable L-IPSCs. However, in the remaining neurons, 0.05 Hz blue light pulse reliably activated reproducible L-IPSCs from the same cell, although the amplitudes varied between neurons (158–1865 pA). The optoactivation yielded L-IPSCs in the presence of antagonist cocktail, and pretreatment of TTX (300 nM) abolished the L-IPSCs by 94.8 ± 2.4% (n = 5), indicating synchronic GABA release from presynaptic PV neurons (Fig. 1B and D). The L-IPSCs were sensitive to a selective $GABA_A$ receptor antagonist, picrotoxin (100 μM; 8.2 ± 3.5% of control, n = 5), indicating that the $GABA_A$ receptors mediated L-IPSCs (Fig. 1B and D). Bath application of baclofen, a selective $GABA_B$ receptor agonist (10 μM; 4 min), reduced the amplitude of L-IPSCs in all 17 neurons tested by 27.7 ± 3.5% (control: 991 ± 138 pA; in the presence