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Pregnenolone sulfate modulates glycinergic transmission in rat medullary dorsal horn neurons

Jung-Su Hong [a], Jin-Hwa Cho [a], In-Sun Choi [a], Maan-Gee Lee [b,c], Il-Sung Jang [a,c,*]

[a] Department of Pharmacology, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea
[b] Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu 700-422, Republic of Korea
[c] Brain Science and Engineering Institute, Kyungpook National University, Daegu 700-422, Republic of Korea

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ABSTRACT

The neurosteroid pregnenolone sulfate (PS), a representative excitatory neuromodulator, has a variety of neuropharmacological actions, such as memory enhancement and convulsant effects. In this study, the effects of PS on glycinergic transmission, such as glycinergic spontaneous miniature inhibitory postsynaptic currents (mIPSCs), were investigated in acutely isolated medullary dorsal horn neurons by use of a conventional whole-cell patch-clamp technique. PS significantly increased the frequency but decreased the amplitude of glycinergic mIPSCs in a concentration-dependent manner. PS also accelerated the decay time constant of glycinergic mIPSCs. The PS-induced decrease in mIPSC amplitude was due to the direct postsynaptic inhibition of glycine receptors because PS inhibited the glycine-induced Cl^- currents in a noncompetitive manner. The PS-induced increase in mIPSC frequency was not due to the activation of α7 nicotinic acetylcholine, NMDA, σ1 receptors and voltage-dependent Ca^{2+} channels, which are known to be molecular targets of PS. On the other hand, the PS-induced increase in mIPSC frequency was completely attenuated either in the Ca^{2+}-free external solution or in the presence of transient receptor potential (TRP) channel blockers, suggesting that PS elicits an increase in Ca^{2+} concentration within glycinergic nerve terminals via the activation of putative TRP channels. The PS-mediated modulation of glycinergic synaptic transmission, such as the enhancement of presynaptic glycine release and direct inhibition of postsynaptic glycine receptors, might have a broad impact on the excitability of medullary dorsal horn neurons and therefore affect the processing of nociceptive transmission from orofacial tissues.

1. Introduction

Neurons within the trigeminal subnucleus caudalis (Vc) receive sensory fibers from orofacial tissues and processes orofacial sensory transmission (Jacquin et al., 1986; Ambalavanar and Morris, 1992; Crissman et al., 1996). In particular, medullary dorsal horn neurons in the Vc also receive Aδ- and C-fibers, which mediate nociceptive transmission from orofacial tissues (Li et al., 1999). These neurons are either excitatory or inhibitory interneurons and project their excitatory and inhibitory axon terminals to the adjacent laminae (Li et al., 1999). Since glycine and GABA are the primary inhibitory neurotransmitters in the brain stem and spinal cord, glycinergic and GABAergic transmission plays a pivotal role in the modulation of pain signals from peripheral tissues (for a review, Furue et al. (2004)). For example, intrathecal co-application of strychnine and bicuculline, selective glycine and $GABA_A$ receptor antagonists, elicits synergistic allodynia in the rat (Loomis et al., 2001). In contrast, intrathecal administration of glycine reduces mechanonociceptive responses or thermal hyperalgesia (Simpson et al., 1996, 1997). Recently, a long-lasting inhibition of glycine receptors containing α3 subunits has been implicated in prostaglandin E_2-mediated central sensitization (Ahmadi et al., 2002; Harvey et al., 2004). These results suggest that glycinergic inhibitory transmission within the dorsal horn plays a pivotal role in the processing of pain signals from peripheral tissues.

Neurosteroids have a lot of physiological and pathological functions in the central nervous system, as neurosteroids and their synthesizing enzymes are found in most of brain area (Mensah-Nyagan et al., 1999; Mellon and Griffin, 2002). According to their chemical structures, neurosteroids act as either inhibitory or excitatory modulators in the neural tissues. For example, 5α-reduced neurosteroids, such as allopregnanolone and tetrahydrodeoxycorticosterone, are positive modulators of $GABA_A$ receptors (Zhu and Vicini, 1997; Stell et al., 2003; Akk et al., 2005), and they play inhibitory roles in the neuronal excitability (Wieland et al.,

* Corresponding author at: Kyungpook National University, Department of Pharmacology, School of Dentistry, 188-1, Samduk 2 ga-dong, Jung-gu, Daegu 700-412, Republic of Korea. Tel.: +82 53 660 6887; fax: +82 53 424 5130.
E-mail address: jis7619@mail.knu.ac.kr (I.-S. Jang).

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1991; Kokate et al., 1994). 5α-Reduced neurosteroids can activate $GABA_A$ receptors even in the presence of a competitive $GABA_A$ receptor antagonist (Kim et al., 2011; Park et al., 2011). In contrast, sulfated neurosteroids, such as pregnenolone sulfate (PS) and dehydroepiandrosterone sulfate (DHEAS), are known to increase the neuronal excitability by acting on a number of ligand-gated and voltage-gated ion channels, and G-protein coupled receptors. For example, sulfated neurosteroids directly inhibit inhibitory $GABA_A$ and glycine receptors (Majewska et al., 1988; Park-Chung et al., 1999; Fodor et al., 2006) and potentiate excitatory NMDA receptors (Wu et al., 1991; Park-Chung et al., 1997). In addition to their direct action on ion channels and/or receptors, sulfated neurosteroids act presynaptically to modulate neurotransmitter release. In coincide with their excitatory action on the neuronal excitability, sulfated neurosteroids reduce inhibitory GABAergic transmission, but enhance excitatory glutamatergic transmission (Teschemacher et al., 1997; Mtchedlishvili and Kapur, 2003; Lee et al., 2010; Zamudio-Bulcock and Valenzuela, 2011). However, it is still unknown whether sulfated neurosteroids modulate glycinergic transmission. In the present study, therefore, we have investigated the functional roles of PS on glycinergic transmission in mechanically isolated rat medullary dorsal horn neurons.

2. Materials and methods

2.1. Preparation

All experiments confirmed the guiding principles for the care and use of animals approved by the Council of the Physiological Society of Korea and the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all efforts were made to minimize both the number of animals used and their suffering.

Sprague Dawley rats (12–16 d old) were decapitated under pentobarbital anesthesia (50 mg/kg, i.p.). The brain stem was dissected and transversely sliced at a thickness of 400 μm using a microslicer (VT1000S; Leica, Nussloch, Germany). Slices containing the medullary dorsal horn region were kept in an incubation medium (see Section 2.4) saturated with 95% O_2 and 5% CO_2 at room temperature (22–24 °C) for at least 1 h before the mechanical dissociation. For dissociation, slices were transferred into a 35 mm culture dish (Primaria 3801; Becton Dickinson, Rutherford, NJ) containing a standard external solution (see Section 2.4), and the medullary dorsal horn was identified under a binocular microscope (SMZ-1; Nikon, Tokyo, Japan). Details of the mechanical dissociation have been described previously (Rhee et al., 1999). Briefly, mechanical dissociation was accomplished using a custom-built vibration device and a fire-polished glass pipette oscillating at about 50–60 Hz (0.3–0.5 mm) on the surface of the superficial dorsal horn region. The slices were removed and the mechanically dissociated neurons were allowed to settle and adhere to the bottom of the dish for 15 min. These dissociated neurons retained a short portion (~50 μM in length) of their proximal dendrites.

2.2. Electrical measurements

All electrical measurements were performed using conventional whole-cell patch recordings and a standard patch-clamp amplifier (Axopatch 200B; Molecular Devices, Union City, CA, USA). Neurons were voltage-clamped at a holding potential (V_H) of 0 mV, except where indicated. Patch pipettes were made from borosilicate capillary glass (G-1.5; Narishige, Tokyo, Japan) by use of a pipette puller (P-97; Sutter Instrument Co., Novato, CA, USA). The resistance of the recording pipettes filled with an internal (pipette) solution was 4–6 MΩ. The liquid junction potential (~−11 mV, measured by exchanging bath solution from internal solution to standard external solution) and pipette capacitance were compensated for. Neurons were viewed under phase contrast on an inverted microscope (TE-2000; Nikon). Membrane currents were filtered at 1 kHz (Axopatch 200B), digitized at 4 kHz, and stored on a computer equipped with pCLAMP 10 (Molecular Devices). When recording, 10 mV hyperpolarizing step pulses (30 ms in duration) were periodically delivered to monitor the access resistance. All experiments were performed at room temperature (22–24 °C) for stable electrophysiological recordings over 1 h.

2.3. Data analysis

Spontaneous miniature IPSCs (mIPSCs) were counted and analyzed using the MiniAnalysis program (Synaptosoft, Inc., Decatur, GA), as described previously (Jang et al., 2002). Briefly, glycinergic mIPSCs were screened automatically using an amplitude threshold of 10 pA and then visually accepted or rejected based upon the rise and decay times. The average values of both the frequency, amplitude and decay time constant (90–37%) of sEPSCs during the control period (5–10 min) were calculated for each recording, and the frequency, amplitude and decay time constant of all the events during the PS application (1–2 min) were normalized to these values. The inter-event intervals and amplitudes of a large number of synaptic events obtained from the same neuron were examined by constructing cumulative probability distributions and then compared using the Kolmogorov–Smirnov (K–S) test with Stat View software (SAS Institute, Inc., Cary, NC, USA). The effects of PS were quantified as a percentage increase in mIPSC frequency compared to the control values. The continuous curve for the concentration-response relationship of PS was fitted using a least-squares fit to the following equation:

$$F = F_{max} \times C^n/(C^n + EC_{50}{}^n),$$

where F is the PS-induced facilitation ratio of sEPSC frequency and C is the corresponding PS concentration. EC_{50} and n denote the half-effective concentration and the Hill coefficient, respectively. The continuous curves for the concentration–inhibition relationship of PS were fitted using a least-squares fit to the following equation:

$$I = 1-[C^n/(C^n + IC_{50}{}^n)],$$

where I is the amplitude of glycine-induced currents, C is the concentration of PS, IC_{50} are the concentrations for the half-inhibitory response, and n is the Hill coefficient. Numerical values are provided as the mean ± standard error of the mean (S.E.M.) using values normalized to the control. Significant differences in the mean amplitude and frequency were tested using a Student's paired two-tailed t-test, except where indicated, with absolute values rather than normalized ones. Values of $P < 0.05$ were considered significant.

2.4. Solutions

The ionic composition of the incubation medium (bath solution) consisted of (in mM) 124 NaCl, 3 KCl, 1.5 KH_2PO_4, 24 $NaHCO_3$, 2 $CaCl_2$, 1.3 $MgSO_4$ and 10 glucose saturated with 95% O_2 and 5% CO_2. The pH was about 7.45. The standard external solution was (in mM) 150 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 Hepes, and was adjusted to a pH of 7.4 with Tris-base. The Ca^{2+}-free external solution was (in mM) 150 NaCl, 3 KCl, 3 $MgCl_2$, 2 EGTA, 10 glucose and 10 Hepes, and was adjusted to a pH of 7.4 with Tris-base. The Na^+-free external solution was (in mM) 150 *N*-methyl-D-glucamine-Cl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 Hepes, and was adjusted to a pH of 7.4 with Tris-base. For recording glycinergic mIPSCs, these standard external solutions routinely