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The endogenous agonist, β-alanine, activates glycine receptors in rat spinal dorsal neurons

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ABSTRACT

β-alanine is a structural analog of glycine and γ-aminobutyric acid (GABA) and is thought to be involved in the modulation of nociceptive information at the spinal cord. However, it is not known whether β-alanine exerts its effect in substantia gelatinosa (SG) neurons of the spinal dorsal horn, where glycine and GABA play an important role in regulating nociceptive transmission from the periphery. Here, we investigated the effects of β-alanine on inhibitory synaptic transmission in adult rat SG neurons using whole-cell patch-clamp. β-alanine dose-dependently induced outward currents in SG neurons. Current-voltage plots revealed a reversal potential at approximately −70 mV, which was close to the equilibrium potential of Cl^-. Pharmacological analysis revealed that β-alanine activates glycine receptors, but not $GABA_A$ receptors. These results suggest that β-alanine hyperpolarizes the membrane potential of SG neurons by activating Cl^- channels through glycine receptors. Our findings raise the possibility that β-alanine may modulate pain sensation through glycine receptors.

1. Introduction

There are many amino acids in the human body, some of which function as neurotransmitters in the central nervous system (CNS). β-alanine (β-amino acid) is an endogenous small amino acid and a structural analog of the major inhibitory neurotransmitters, glycine (α-amino acid) and γ-gamma aminobutyric acid (GABA, γ-amino acid) [1]. Fig. 1 shows that the structural formulae of these three amino acids are very simple and similar, with only differences in the number of carbon chains. β-alanine is present in blood, organs, muscles, the CNS [1–3]. β-alanine can act as a neuromodulator [3] or neurotransmitter, like glycine and GABA [4], in the CNS. β-alanine is released following electrical stimulation in the rat neocortex [5] and medulla oblongata [6], and rabbit superior colliculus [4]. β-alanine pharmacologically activates glycine and $GABA_A$ receptors with less efficacy than their native ligands in the brain [7,8]. Additionally, β-alanine decreases glutamatergic excitation by binding to the glycine co-agonist site on the *N*-methyl-D-aspartate receptor [9,10]. These dual effects of β-alanine are unique, and contribute to neuroprotective action in the hippocampus [11] and visual information transmission in the retina [4,12].

Autoradiography studies show that β-alanine is localized in neurons and glial cells in the spinal cord [13]. Studies indicate that β-alanine plays an important role in the spinal cord [14,15]. Intrathecal β-alanine decreased vocalizations and skin hyperalgesia triggered by intrathecal administration of strychnine in rats [14]; however, another study reported that β-alanine did not reduce allodynia and hyperalgesia caused by strychnine in mice [15]. β-alanine inhibits the firing of spinal cord neurons in frogs, chicks, mice, and cat, which has been attributed to an increase in Cl^- and/or K^+ conductance in the neuronal membrane of spinal cord [16–19].

Lamina II (SG) spinal cord neurons play an important role in regulating nociceptive input from the periphery [20,21]. Moreover, lamina II neurons are concentrated with glycinergic or GABAergic inhibitory interneurons. Therefore, it is possible that β-alanine might modulate nociceptive transmission in the spinal cord. This idea is supported by our previous report that taurine (β-amino acid), a structural analog of β-alanine, activates glycine and $GABA_A$ receptors in rat SG neurons [22]. In the present study, we aimed to examine whether β-alanine affects SG neurons by using whole-cell patch-clamp technique.

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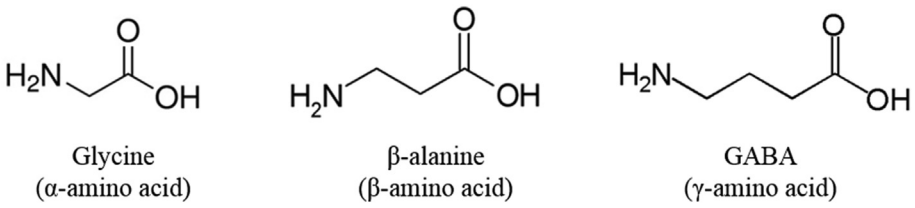

Fig. 1. β-alanine is structurally intermediate between the main inhibitory neurotransmitters, glycine and GABA.

2. Materials and methods

2.1. Preparation of the spinal cord slices

This study was approved by the Institutional Animal Care and Use Committee of Niigata University Graduate School of Medical and Dental Sciences in Niigata, Japan. Adult male Wistar rats (6–8 weeks old, 120–200 g) were anesthetized with urethane (1.5 g kg^{-1}, intraperitoneally). A dorsal laminectomy was performed and a lumbosacral segment of the spinal cord with ventral and dorsal roots attached was removed. The rats were then immediately killed by exsanguination. The isolated spinal cord was placed in pre-oxygenated ice-cold Krebs solution. After the arachnoid membrane and all ventral and dorsal roots were removed, the spinal cord was placed on an agar block, mounted on a metal stage of a microslicer (Linear Slicer PRO 7; Dosaka, Kyoto, Japan), and 650 μm transverse slices were cut [20]. Slices were placed on a nylon mesh in a recording chamber, perfused with 36 °C Krebs solution at a rate of 10–15 ml min^{-1}, and bubbled with a gas mixture of 95% O_2 and 5% CO_2. The Krebs solution contained (in mM): NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, and D-glucose 11.5.

2.2. Patch-clump recordings from dorsal horn neurons

Whole-cell patch-clamp recordings were made from SG neurons in voltage-clamp mode. The membrane potential was held at 0 mV throughout the experiment, except when the reversal potential was examined. The resistance of the patch pipette was 5–10 MΩ. The patch pipette solution contained (in mM): Cs_2SO_4 110, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5, tetraethyl ammonium chloride 5, and ATP-Mg 5. Signals were amplified using an Axopatch 200B amplifier (Molecular Devices, Union City, CA, USA), filtered at 2 kHz, and digitized at 5 kHz. Data were collected and analyzed with a personal computer using the pCLAMP 10.4 software suite (Molecular Devices).

2.3. Drug application

Drugs were applied by superfusion with no alteration of perfusion rate and temperature. The drugs used in this study were β-alanine, bicuculline, and strychnine (Sigma-Aldrich, St. Louis, MO, USA). β-alanine was applied to the slices for 30 s. Peak currents were measured before and after each treatment and expressed as

$$\frac{Pretreatment\ current}{Postreatment\ current} \times 100$$

Bicuculline was stored in 1000 × dimethyl sulfoxide (DMSO). The other drugs were stored in 1000 × distilled water. Stock solutions were diluted to the final concentration in Krebs solution immediately before use.

2.4. Statistical analysis

Data are expressed as mean ± SEM. Statistical significance was determined as $P < 0.05$ using paired Student's *t* tests. In all cases, *n* refers to the number of neurons studied.

3. Results

3.1. β-alanine produces outward currents in SG neurons

Bath-applied β-alanine (0.3 mM) induced an outward current in all SG neurons tested; the average peak amplitude was 62 ± 9 pA at 0 mV ($n = 20$). There was a concentration dependent increase in peak amplitude (Fig. 2A). Fig. 2B demonstrates the dose-response curve of β-alanine-induced current. Analysis of the curve based on the Hill plot showed that the effective concentration producing a half-maximal response (EC_{50}) was 0.97 mM with a Hill coefficient of 1.21.

3.2. Reversal potential of β-alanine-induced outward current

In order to identify the ion channel involved in the β-alanine-induced current, we investigated the reversal potential of β-alanine-induced current by application of β-alanine (0.3 mM) at different holding potentials (Fig. 3A). Fig. 3B shows the current-voltage relationship of the β-alanine-induced current. Analysis of the curve revealed a reversal potential at approximately −70 mV, which was close to the theoretical Cl^- equilibrium potential (−68 mV), based on the Nernst equation of our experimental conditions (Cl^- concentrations: 10 mM and 128 mM, respectively).

3.3. Pharmacological analysis of β-alanine-induced responses

To determine which receptors are involved in β-alanine-induced current in SG neurons, we investigated the pharmacological properties using the selective $GABA_A$ and glycine receptor antagonists, bicuculline and strychnine, respectively. First, we examined the β-alanine-induced outward current at 0.3 mM, which is lower than the EC_{50}. This induced current was inhibited to 6.5% ± 2.9 of control by 2 μM strychnine ($n = 8$, $P < 0.01$), but was not affected by 20 μM bicuculline (98.0% ± 4.2 of control, $n = 8$, $P = 0.13$; Fig. 4A). Next, we examined β-alanine-induced outward current at a high concentration of 3 mM, because we previously demonstrated that taurine, a structural analog of β-alanine, activates both glycine and $GABA_A$ receptors at high concentrations [22]. Nevertheless, 3 mM β-alanine-induced current was also inhibited by 10 μM strychnine (9.3% ± 3.1 of control, $n = 11$, $P < 0.01$), but not affected by 20 μM bicuculline (94.7% ± 3.3 of control, $n = 11$, $P = 0.11$; Fig. 4B).

4. Discussion

Several lines of evidence suggest that β-alanine pharmacologically activates glycine and $GABA_A$ receptors in the CNS [1,7,8]. However, function of β-alanine on spinal dorsal horn neurons has

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