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Research report

Activation of presynaptic $GABA_A$ receptors increases spontaneous glutamate release onto noradrenergic neurons of the rat locus coeruleus

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Abstract

In order to further explore how GABA can modulate the excitability of noradrenergic neurons of the locus coeruleus (LC), we investigated the presence of $GABA_A$ receptors on glutamatergic nerve terminals and the functional consequences of their activation. We used mechanically dissociated immature rat LC neurons with adherent nerve terminals and patch-clamp recordings of spontaneous excitatory postsynaptic currents. Activation of presynaptic $GABA_A$ receptors by muscimol facilitated spontaneous glutamate release by activating tetrodotoxin-sensitive Na^+ channels and high-threshold Ca^{2+} channels. Bumetanide (10 μM), a potent blocker of $Na^+-K^+-Cl^-$ cotransporter, diminished the muscimol-induced facilitatory action of glutamate release. Our results indicate that the $Na^+-K^+-Cl^-$ cotransporter accumulates Cl^- inside the nerve terminals so that activation of presynaptic $GABA_A$ receptors causes depolarization. This $GABA_A$-receptor-mediated modulation of spontaneous glutamatergic transmission is another mechanism by which GABA and its analogues can regulate the excitability and activity of noradrenergic neurons in the LC.

Theme: Excitable membranes and synaptic transmission
Topic: Mechanisms of neurotransmitter release

Keywords: Spontaneous EPSC; NKCC; Muscimol; Presynaptic Ca^{2+} channels

1. Introduction

The locus coeruleus (LC) contains large clusters of noradrenaline-containing neurons (A6 cell group) which project widely throughout the central nervous system (CNS) [49]. The LC plays important roles in the control of cognitive and emotional processes, including attention and anxiety [7,13]. LC neurons typically spontaneously fire action potentials [3,50] and this firing rate is altered by a variety of sensory stimuli and is particularly modulated by the sleep–wake cycle [5,21]. LC neurons show their highest level of activity during active waking, decrease their firing frequency during slow wave sleep, and are almost silent during rapid eye movement sleep [4]. At a cellular level, the excitability of LC neurons is regulated by both excitatory and inhibitory synaptic inputs. For instance, the in vivo iontophoretic application of glutamate receptor agonists increases the firing frequency [8], whereas iontophoretic application of γ-aminobutyric acid (GABA) suppresses the activity of LC neurons [12]. However, the systemic or intracerebroventricular administration of GABA-mimetics has a facilitatory effect on central noradrenergic neurons [2,11] and enhances the catecholamine current measured by in vivo voltammetry [40].

GABA is the primary inhibitory neurotransmitter throughout the mammalian CNS. Activation of ionotropic $GABA_A$ receptors increases the Cl^- conductance of membrane. This results in postsynaptic hyperpolarization

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in adult neurons, although in developing and injured neurons, Cl^- channel activation causes a depolarization because of the high intracellular Cl^- concentration [10,17,27,35]. These GABA-induced depolarizations can elevate the intracellular Ca^{2+} concentration via activation of voltage-dependent Ca^{2+} channels [29] and this is thought to contribute to synapse maturation. A $GABA_A$-receptor-mediated depolarization has also been observed in mature presynaptic nerve terminals [23,24,41,44]. In sensory afferent terminals, the activation of presynaptic $GABA_A$ receptors induces presynaptic inhibition of action-potential evoked transmitter release by inactivating Na^+ channels and/or shunting the presynaptic membrane potential [41,44]. In contrast, the presynaptic depolarization induced by $GABA_A$ receptor activation increases spontaneous transmitter release [23,24]. Such $GABA_A$-receptor-mediated presynaptic depolarization also results from a higher intraterminal Cl^- concentration, resulting from inwardly directed Cl^- transport mechanism such as the Na–K–Cl cotransporter (NKCC) [23,27,48].

Direct iontophoretic application of GABA to the LC in brain slices reduces the firing rate of rat LC neurons in a manner sensitive to the $GABA_A$ receptor blocker, bicuculline [45]. However, it is not clear whether this is mediated solely through postsynaptic $GABA_A$ receptors or whether presynaptic $GABA_A$ receptors also contribute. Capsaicin, for example, increases the activity of LC neurons by acting on presynaptic VR1 receptors to increase spontaneous glutamate release, without affecting either evoked release or without directly acting on LC neurons [31]. We therefore hypothesized that GABA might also affect LC noradrenergic neuron excitability by acting on glutamatergic presynaptic terminals. To test this, we studied the effects of the $GABA_A$ receptor agonist, muscimol, on spontaneous excitatory postsynaptic currents (sEPSCs) recorded in juvenile rat LC neurons which were mechanically isolated so as to retain adherent and functional presynaptic nerve terminals [1].

2. Experimental procedures

2.1. Preparation

Wistar rats (13–17 days old) were decapitated under pentobarbital sodium anesthesia (80 mg/kg, i.p.). The brain was quickly removed and sliced at a thickness of 380 μm using a microslicer (VT1000S; Leica, Nussloch, Germany). Slices were kept in the incubation medium (see below) saturated with 95% O_2 and 5% CO_2 at room temperature (21–24 °C) for at least 1 h before the mechanical dissociation. Slices were then transferred into a 35-mm culture dish (Primaria 3801; Becton Dickinson, Rutherford, NJ, USA), and the region of the LC was identified under a binocular microscope. Details of the mechanical dissociation have been recently reviewed [1].

All experiments were performed under guiding principles for care and use of animals approved by the Council to the Physiological Society of Japan.

2.2. Electrical measurements

The electrical measurements were performed using conventional whole-cell patch-clamp recordings at holding potentials of −60 to −65 mV. Membrane voltage was controlled, and currents recorded, with the use of a patch-clamp amplifier (EPC-7; List Medical, Darmstadt-Eberstadt, Germany). Patch pipettes were made from borosilicate capillary glass in two stages on a vertical pipette puller (PB-7, Narishige, Tokyo, Japan). The resistance between the recording pipettes filled with internal solution and the reference electrode was 4–6 MΩ. Neurons were visualized under phase contrast on an inverted microscope (Diaphot; Nikon, Tokyo, Japan). Current and voltage were continuously monitored on an oscilloscope and a pen recorder (WR3320, Graphtec, Tokyo, Japan). Membrane currents were filtered at 3 kHz (E-3201A; NF Electric Instruments, Tokyo, Japan), digitized at 6 kHz, and stored on a computer equipped with pCLAMP8.0 (Axon Instruments). All experiments were performed at room temperature (21–24 °C).

2.3. Immunostaining for tyrosine hydroxylase (TH) in dissociated LC neurons

We performed immunocytochemical experiments. Dissociated neurons were allowed to settle on to polyethlenimine (PEI)-coated, glass coverslips which were then transferred on to small strips of in a 35-mm culture dish. Coverslips were moved to parafilm sheet for immunocytochemistry. Neurons were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at 4 °C, and were then washed with PBS. After treatment with 0.2% Triton X-100 for 5 min at room temperature, neurons were incubated with PBS containing 5% normal bovine serum for 30 min and then incubated with 1% normal bovine serum for 10 min. Neurons were subsequently incubated with PBS containing rabbit anti-tyrosine hydroxylase (TH) antibody (1:1000; Chemicon International) and 1% normal bovine serum for 1.5 h, and then with fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit secondary antibody (1:150; Jackson ImmunoResearch Laboratories) for a further 1 h. Neurons were photographed through a microscope with a digital camera (Carl Zeiss, Germany).

2.4. Data analysis

Spontaneous EPSCs were detected and analyzed using the MiniAnalysis program (Synaptosoft, Decatur, GA). Spontaneous events were initially detected automatically, using an amplitude threshold of 3 pA, and then visually