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Presynaptic nicotinic acetylcholine receptors enhance GABAergic synaptic transmission in rat periaqueductal gray neurons

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ABSTRACT

The periaqueductal gray (PAG) is a major component of the descending pain inhibitory pathway, which is related to central analgesia. In the present study, we have investigated the possible roles of presynaptic nicotinic acetylcholine receptors in GABAergic transmission onto PAG neurons. In acutely isolated rat PAG neurons, GABAergic miniature inhibitory postsynaptic currents (mIPSCs) were recorded by use of a whole-cell patch clamp technique. Acetylcholine (30 μM) transiently increased both the frequency and amplitude of GABAergic mIPSCs. However, acetylcholine did not affect the GABA-induced membrane currents. This facilitatory action of acetylcholine disappeared in the presence of mecamylamine, a nonselective nicotinic receptor antagonist, and mimicked by nicotine, a nicotinic receptor agonist. The nicotine-induced increase in mIPSC frequency was completely blocked by dihydro-β-erythroidine, a selective β2-containing nicotinic receptor antagonist, but not methyllycaconitine or α-bungarotoxin, selective α7 nicotinic receptor antagonists. The results suggest that acetylcholine or nicotine acts presynaptic β2-containing nicotinic receptors, presumably α4β2 nicotinic receptors, to enhance spontaneous GABA release onto PAG neurons. The nicotine-induced increase in mIPSC frequency was completely occluded in the presence of Cd^{2+}, a general voltage-dependent Ca^{2+} channels blocker, and in the absence of extracellular Ca^{2+} or Na^{+}. The results suggest that presynaptic nicotinic receptors are less permeable to Ca^{2+}, and that the activation of these receptors depolarizes GABAergic nerve terminals. In conclusion, presynaptic nicotinic receptors would temporally regulate the excitability of PAG neurons being not overexcited and eventually contribute to the cholinergic modulation of output from the PAG.

1. Introduction

Nicotinic acetylcholine receptors are nonselective cation channels triggered by the binding of endogenous neurotransmitter acetylcholine. Nicotinic receptors have pentameric structures, which are homomeric or heteromeric combinations composed of α (α2–α10) and/or β (β2–β4) subunits, and they have different pharmacological and physiological properties based on the subunit composition. Although the subunit composition of nicotinic receptors varies among the brain region, both heteromeric α4β2 and homomeric α7 nicotinic receptors are abundantly distributed in the CNS (for review, Gotti et al., 2006). While nicotinic receptors are expressed at postsynaptic sides and contribute to the fast excitatory transmission via the influx of Na^{+} and Ca^{2+} in neuromuscular junction and ganglionic synapse, they are also widely expressed on presynaptic terminals in the CNS (Wonnacott, 1997; Vizi and Lendvai, 1999). The activation of presynaptic nicotinic receptors increases the release probability of various neurotransmitters, such as GABA, glycine, glutamate, dopamine, noradrenalin and acetylcholine itself (Clarke and Reuben, 1996; Fu et al., 1998; Genzen and McGehee, 2003; Guo et al., 1998; Kiyosawa et al., 2001). Therefore, it has been suggested that presynaptic nicotinic receptors play a modulatory role in synaptic transmission.

The midbrain periaqueductal gray (PAG) is involved in the various physiological functions including pain, fear and anxiety, vocalization, lordosis and cardiovascular control (for review, Behbehani, 1995; Millan, 2002). The PAG is also a major component of the descending pain inhibitory pathway, which is related to central analgesia, and is one of major target sites for the action of analgesics, such as opioids and cannnabinoids (Yaksh, 1997; Lichtman et al., 1996; Finn et al., 2003). The excitability of PAG neurons would be regulated by various neurotransmitters, such as GABA, glutamate, acetylcholine and so on, released from surrounding synapses projecting to the PAG. Among them, GABAergic input seems to be a pivotal regulating factor to maintain the excitability of PAG neurons, as the major intrinsic neural circuit within the PAG is a tonically active spontaneous GABAergic network and the inhibition of this network changes the intrinsic

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excitability of PAG neurons to modulate the output of the PAG (Behbehani et al., 1990; Ogawa et al., 1994). Therefore, the modulation of spontaneous GABAergic activity within the PAG would play a crucial role in the regulation of various functions. On the other hand, an immunohistochemical study has revealed that the PAG receives a dense projection of cholinergic fibers that arise from choline acetyltransferase-containing cells in the pontine tegmentum (Woolf et al., 1990). Although a recent study has shown that muscarinic receptors modulate GABAergic transmission onto PAG neurons (Lau and Vaughan, 2008), it is still unknown whether nicotinic receptors are expressed on GABAergic nerve terminals projecting to PAG neurons and whether their activation can regulate GABAergic transmission. In the present study, therefore, we have investigated the functional roles of nicotinic receptors in spontaneous GABAergic transmission in acutely isolated rat PAG neurons.

2. Materials and methods

2.1. Preparation

All experiments complied with 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 every effort was made to minimize both the number of animals used and their suffering.

Sprague Dawley rats (11–14 d old) were decapitated under ketamine anesthesia (100 mg/kg, i. p.). The brain was dissected and transversely sliced at a thickness of 400 μm using a microslicer (VT1000S; Leica, Nussloch, Germany). Midbrain slices containing the PAG were kept in an incubation medium (see Solutions) saturated with 95% O_2 and 5% CO_2 at room temperature (22–24 ºC) 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, USA) containing a standard external solution (see Solutions), and the PAG region 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 PAG region. Slices were removed and the mechanically dissociated neurons were left for 15 min to allow the neurons to adhere to the bottom of the culture dish.

2.2. Electrical measurements

All electrophysiological measurements were performed using conventional whole-cell patch recording mode at a holding potential (V_H) of 0 mV (Axopatch 200B; Molecular Devices, Union City, CA, USA). Patch pipettes were made from borosilicate capillary glass (1.5 mm outer diameter, 0.9 mm inner diameter; 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 internal solution was 4–6 MΩ. The liquid junction potential and pipette capacitance were compensated for. Neurons were viewed under phase contrast on an inverted microscope (TE2000; Nikon). Membrane currents were filtered at 1 kHz, digitized at 4 kHz, and stored on a computer equipped with pCLAMP 10 (Molecular Devices). During the recordings, 10 mV hyperpolarizing step pulses (30 ms in duration) were periodically applied to monitor the access resistance. All experiments were performed at room temperature (22–25 °C).

2.3. Data analysis

Spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were counted and analyzed using the MiniAnalysis program (Synaptosoft, Inc., Decatur, GA) as described previously (Jang et al., 2002). Briefly, mIPSCs were screened automatically using an amplitude threshold of 10 pA, and then visually accepted or rejected based upon the rise and decay times. Basal noise levels during voltage-clamp recordings were typically less than 8 pA. The average values of both the frequency and amplitude of mIPSCs during the control period (5–10 min) or each drug condition (5–10 min) were calculated for each recording, and the frequency and amplitude of all the events during the agonist application (30 s or 3 min) were normalized to these values. The effects of these different conditions were quantified as a percentage increase in mIPSC frequency compared to the control 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 compared using the Kolmogorov–Smirnov (K–S) test with Stat View software (SAS Institute, Inc., Cary, NC, USA). 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 Student's paired two-tailed *t*-test, using absolute values rather than normalized ones. Values of $P<0.05$ were considered significant.

2.4. Solutions

The ionic composition of the incubation medium 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 7.4–7.5. The standard external solution was (in mM) 150 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 Hepes. The Ca^{2+}-free external solution was (in mM) 150 NaCl, 3 KCl, 2 EGTA, 3 $MgCl_2$, 10 glucose and 10 Hepes. 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. All these external solutions were adjusted to a pH of 7.4 with Tris-base. For recording mIPSCs, these standard external solutions routinely contained 300 nM tetrodotoxin (TTX), 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 20 μM DL-2-amino-5-phosphonovaleric acid (APV) to block voltage-dependent Na^+ channels and ionotropic glutamate receptors, respectively. The ionic composition of the internal (pipette) solution was consisted of (in mM) 135 $CsMeHSO_3$, 7 CsCl, 2 EGTA and 10 Hepes with a pH adjusted to 7.2 with Tris-base.

2.5. Drugs

The drugs used in the present study were APV, TTX, CNQX, acetylcholine, nicotine, choline-Cl, 6-imino-3-(4-methoxyphenyl)-1(6H)-pyridazinebutanoic acid HBr (SR95531), muscarine (from Sigma, St. Louis, MO, USA), dihydro-β-erythroidine (DHβE), methyllycaconitine (MLA), mecamylamine hydrochloride (MCA), α-bungarotoxin (from Tocris, Bristol, UK). All solutions containing drugs were applied using the 'Y-tube system' for rapid solution exchange (Murase et al., 1989).

3. Results

3.1. GABAergic mIPSCs in mechanically dissociated PAG neurons

Previous comparative studies of the morphology of the PAG of rat, cat and monkey have shown considerable similarities in the types of neurons and their distributions within the PAG (Mantyh, 1982; Beitz and Shepard, 1985). Four major types of rat PAG neurons ranging between 10 and 35 μm in soma diameter have been identified based on their morphological properties; fusiform or bipolar neurons, multipolar neurons that have a very large number of dendrites, stellate cells that have 3–6 dendrites, and pyramidal-shaped neurons (Mantyh, 1982; Beitz and Shepard, 1985). After the mechanical