Pulmonary, Gastrointestinal and Urogenital Pharmacology

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

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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 Ca2+ 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 cannabinoids (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
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 Psychological 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% O2 and 5% CO2 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 (Vh) 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 ºC).

2.3. Data analysis

Spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were counted and analyzed using the MiniAnalysis program (Synapsoft, 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 comparing 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 KH2PO4, 24 NaHCO3, 2 CaCl2, 1.3 MgSO4 and 10 glucose saturated with 95% O2 and 5% CO2. The pH was about 7.4–7.5. The standard external solution was (in mM) 150 NaCl, 3 KCl, 2 CaCl2, 1 MgCl2, 10 glucose and 10 Hepes. The Ca2+–free external solution was (in mM) 150 NaCl, 3 KCl, 2 EGTA, 3 MgCl2, 10 glucose and 10 Hepes. The Na+–free external solution was (in mM) 150 N-methyl-D-glucamine-Cl, 3 KCl, 2 CaCl2, 1 MgCl2, 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-phosphonovonic 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 CsMeHSO3, 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
dissociation of the PAG region, we found several kinds of neurons in soma diameter (≤15 μm) and shape (multipolar, bipolar and pyramidal-shaped). In the case of smaller bipolar or multipolar neurons (<10 μm in soma diameter), GABAergic synaptic events were hardly detected during electrophysiological recordings, so that it was hard to investigate the effect of acetylcholine on GABAergic synaptic transmission. In contrast, we could record abundant spontaneous synaptic events in bipolar and pyramidal-shaped neurons (10–15 μm in soma diameter), and there was no difference in the presynaptic response to acetylcholine or nicotine between bipolar and pyramidal-shaped neurons. Therefore, we performed all electrophysiological recordings with these bipolar and pyramidal-shaped neurons.

In the presence of 300 nM TTX, 10 μM CNQX and 20 μM APV, the spontaneous miniature currents were recorded from the mechanically dissociated PAG neurons at a V_H of 0 mV. The observed spontaneous currents were completely and reversibly blocked by 10 μM SR95531, a selective GABA_A receptor antagonist (Fig. 1A). The amplitude of spontaneous currents varied with the holding potentials (V_H; Fig. 1B) and their reversal potential was −72.3 mV. This value was very similar to the theoretical Cl− equilibrium potential (E_{Cl}) of −78.7 mV, which was calculated from the Nernst equation using extracellular and intracellular Cl− concentrations ([Cl−]_o: 159 mM and [Cl−]_i: 7 mM, respectively). These results indicate that the spontaneous miniature currents are GABAergic mIPSCs mediated by GABA_A receptors.

### 3.2. Effect of acetylcholine on GABAergic mIPSCs

To investigate whether functional nicotinic receptors exist on GABAergic presynaptic nerve terminals projecting to PAG neurons and their activation can modulate GABAergic synaptic transmission, we first tested the effect of exogenously applied acetylcholine, an endogenous ligand of cholinergic receptors, on GABAergic mIPSCs. The application of acetylcholine (30 μM) during 3 min elicited a brief increase in the frequency of GABAergic mIPSCs and this increase was rapidly subsided to the control level within 40 s (Fig. 2A and B). In 11 neurons tested, acetylcholine increased both the mean mIPSC frequency to 351±69% of the control (n = 11, P < 0.01) and the mean mIPSC amplitude (152±21% of the control, n = 11, P < 0.01) (Fig. 2C insets). In addition, as shown in Fig. 2C, acetylcholine significantly shifted the distributions of inter-event interval and current amplitude of GABAergic mIPSCs to the left and right (P < 0.05, K-S test), respectively, indicating increases in the frequency and amplitude of GABAergic mIPSCs. An increase in mIPSC amplitude might result from the acetylcholine-mediated postsynaptic effects, such as an increase in the GABA sensitivity. However, this was not the case because acetylcholine (30 μM) did not affect 30 μM GABA-induced currents (101±2% of the control, n = 6, P = 0.57, Fig. 2D). Taken together, these results suggest that acetylcholine acts presynaptically to increase spontaneous GABA release onto PAG neurons.

### 3.3. Facilitation of GABAergic mIPSCs mediated by presynaptic nicotinic receptors

A previous study has shown that carbachol, a nonselective cholinergic agonist which can activate nicotinic and muscarinic receptors, decreases GABAergic transmission onto PAG neurons by activating presynaptic muscarinic receptors (Lau and Vaughan, 2008). Therefore, we further examined which muscarinic receptors also contribute to the acetylcholine-induced modulation of mIPSC frequency. To test this, we observed the effect of MCA, a nonselective nicotinic receptor antagonist, on the acetylcholine-induced increase in spontaneous GABA release. In 8 neurons, in which MCA effect was fully analyzed, the acetylcholine (30 μM)-induced initial increase in mIPSC

![Fig. 1. GABAergic mIPSCs recorded from acutely isolated PAG neurons. A, A typical trace of GABAergic mIPSCs observed before, during and after the application of 10 μM SR95531 at a V_H of 0 mV in the presence of 300 nM TTX, 10 μM CNQX and 20 μM APV. Insets represent GABAergic mIPSCs with an expanded time scale in each condition. B, Typical traces of GABAergic mIPSCs at various holding potentials (V_H). A, A plot of the mean amplitude of mIPSCs at various V_H values. The reversal potential was estimated to be −72.3 mV using the Nernst equation, which was very similar to the theoretical E_{Cl} (−78.8 mV). Each point was the mean and S.E.M. from 5 experiments.

![Fig. 2. Effects of acetylcholine on GABAergic mIPSCs. A, A typical trace of GABAergic mIPSCs observed before, during and after application of 30 μM acetylcholine (Ach). Insets represent GABAergic mIPSCs with an expanded time scale in each condition. B, A time course of the acetylcholine-induced change in mIPSC frequency. Each point was the mean and S.E.M. from 11 experiments. C, Cumulative probability distributions for inter-event interval (a) and current amplitude (b) of GABAergic mIPSCs. 222 for control and 275 events for acetylcholine were plotted. Inset columns were the mean and S.E.M. from 11 experiments, respectively. **, P < 0.05; ***, P < 0.01. D, Typical traces of GABA (30 μM)-induced membrane currents (i_{GABA}) observed before, during and after the application of 30 μM acetylcholine.](image-url)
frequency (during the first 30 s, 565 ± 135% of the control, n = 8, P < 0.01) was completely blocked by 10 μM MCA (during the first 30 s, 110 ± 21% to the MCA condition, n = 8, P = 0.94, Fig. 3A and B), suggesting that nicotinic receptors are involved in the acetylcholine-induced initial increase in mIPSC frequency. However, acetylcholine did not decrease GABAergic mIPSC frequency in the presence of MCA (Fig. 3A and B). In addition, muscarine (10 μM), a muscarinic receptor agonist, did not affect GABAergic mIPSC frequency (103 ± 10% to the condition, n = 6, P = 0.94, data not shown), indicating the involvement of muscarinic receptors in the acetylcholine-induced modulation of spontaneous GABA release might be negligible. Next, we observed the effect of nicotine, a nicotinic receptor agonist, on GABAergic mIPSCs. Nicotine (3 μM, 30 s application) also increased both the mean mIPSC frequency to 620 ± 110% of the control (n = 10, P < 0.01) and the mean mIPSC amplitude (120 ± 7% to the control, n = 10, P < 0.05) (Fig. 3C and D insets). In addition, as shown in Fig. 3D, nicotine significantly shifted the distributions of inter-event interval and current amplitude to the left and right (P < 0.05, K-S test), respectively, indicating increases in the frequency and amplitude of GABAergic mIPSCs. The results suggest that function nicotinic receptors are expressed on GABAergic nerve terminals projecting to PAG neurons and that nicotine but not muscarinic receptors are responsible for the cholinergic modulation of spontaneous GABAergic transmission in acutely isolated PAG neurons. In all subsequent pharmacological experiments, nicotine was used to activate presynaptic nicotinic receptors based on its selectivity for nicotinic receptors.

3.4. Subunit composition of presynaptic nicotinic receptors

In the mammalian brain, both heteromeric α4β2 and homomeric α7 nicotinic receptors are abundantly expressed (Gotti et al., 2006). Therefore, we examined the subunit composition of presynaptic nicotinic receptors expressed on GABAergic nerve terminals by use of pharmacological tools. We first observed the effect of DHβE on the nicotine-induced increase in spontaneous GABA release. DHβE at a concentration of 1–10 μM is known to block β2-containing nicotinic receptors including α4β2 nicotinic receptors (Dickinson et al., 2008; Livingstone et al., 2009). In the presence of 10 μM DHβE, the nicotine-induced increase in mIPSC frequency was almost suppressed (133 ± 36% of the DHβE condition, n = 6, P = 0.99; Fig. 4A and B). We also observed the effect of MLA, a selective α7 nicotinic receptor antagonist, on the nicotine-induced increase in spontaneous GABA release. In the presence of 300 nM MLA, 3 μM nicotine still increased GABAergic mIPSC frequency (524 ± 154% to the MLA condition, n = 6, P < 0.01; Fig. 4A and B). Furthermore, choline (1 mM), which has a relatively higher sensitivity to α7 nicotinic receptors (Alkondon et al., 1997), had no facilitatory effect on GABAergic mIPSC frequency (Fig. 4B). Taken together, the results suggest that presynaptic nicotinic receptors at least contain β2 subunits rather than α7 subunits.

3.5. Ion permeability of presynaptic nicotinic receptors

To elucidate the mechanisms underlying the nicotinic receptor-mediated facilitation of mIPSC frequency, we examined the effect of Ca2+-free (plus 2 mM EGTA) external solution on the nicotine-induced facilitation of mIPSC frequency. In the Ca2+-free external solution, both the frequency and amplitude of GABAergic mIPSCs were significantly reduced (30 ± 11% and 79 ± 8% of the control, n = 6, P < 0.05, respectively). This suggests indicate that GABAergic mIPSCs...
4. Discussion

4.1. Presynaptic nicotinic receptors facilitate spontaneous GABA release onto PAG neurons

Previous studies have shown that nicotinic receptors are widely expressed on presynaptic terminals and their activation modulates neurotransmitter release at a variety of central synapses (Clarke and Reuben, 1996; Fu et al., 1998; Genzen and McGehee, 2003; Guo et al., 1998; Kiyosawa et al., 2001). In the present study, we initially examined the effect of acetylcholine, an endogenous ligand of cholinergic receptors, on spontaneous GABA release onto acutely isolated PAG neurons. As acetylcholine can bind to both ionotropic nicotinic and metabotropic muscarinic receptors, these two receptor subtypes might be involved in the acetylcholine-induced modulation of spontaneous GABA release. However, several lines of evidence suggest that acetylcholine acts presynaptic nicotinic but not muscarinic receptors to increase spontaneous GABAergic transmission onto PAG neurons. First, acetylcholine significantly increased the frequency of GABAergic mIPSCs, indicating that acetylcholine acts presynaptically to change the probability of spontaneous GABA release. Although acetylcholine also increased the mean amplitude of mIPSCs, acetylcholine is likely to act presynaptically because acetylcholine had no effect on the sensitivity of postsynaptic GABA$_A$ receptors. An increase in mIPSC amplitude by acetylcholine might result from multivesicular release due to an increase in the intraterminal Ca$^{2+}$ concentration ([Ca$^{2+}$]$_{terminal}$) (see also Sharma et al., 2008). Second, the acetylcholine-induced transient facilitation of GABAergic mIPSC frequency was completely blocked by MCA, a nonselective nicotinic receptor antagonist, and such an effect was closely mimicked by nicotine, a selective nicotinic receptor agonist. In contrast, hippocampal mossy fibers had no effect on GABAergic mIPSC frequency after the blockade of nicotinic receptors with MCA, muscarinic receptors might not be involved in the acetylcholine-induced modulation of spontaneous GABA release. Third, the preparation used in this study should exclude any non-presynaptic actions, such as changes in the excitability of soma, because mechanically dissociated neurons retain functional cell-free presynaptic nerve terminals (for review, Akaike and Morshouse, 2003).

A recent study has shown that carbachol, a nonselective acetylcholine receptor agonist, suppresses GABAergic transmission in PAG neurons (Lau and Vaughan, 2008). However, the carbachol-induced presynaptic inhibition of GABAergic transmission would be largely mediated by the indirect M1/M3 muscarinic receptor-induced endocannabinoid signaling (Lau and Vaughan, 2008). In fact, the activation of M1 muscarinic receptors is known to produce endocannabinoids to inhibit neurotransmitter release (Ohno-Shosaku et al., 2003; Fukudome et al., 2004; Narushima et al., 2007). In contrast to these studies, our present results indicate that muscarinic receptors might be not involved in the acetylcholine-induced modulation of spontaneous GABA release. As acetylcholine can bind to both ionotropic cholinergic receptors, on spontaneous GABA release onto acutely isolated PAG neurons. As acetylcholine can bind to both ionotropic nicotinic and metabotropic muscarinic receptors, these two receptor subtypes might be involved in the acetylcholine-induced modulation of spontaneous GABA release. However, several lines of evidence suggest that acetylcholine acts presynaptically on the nicotine-induced increase in mIPSC frequency. In the absence of extracellular Na$^+$, nicotine again failed to increase the frequency of GABAergic mIPSCs, indicating that acetylcholine acts presynaptically to change the probability of spontaneous GABA release. Although acetylcholine also increased the mean amplitude of mIPSCs, acetylcholine is likely to act presynaptically because acetylcholine had no effect on the sensitivity of postsynaptic GABA$_A$ receptors. An increase in mIPSC amplitude by acetylcholine might result from multivesicular release due to an increase in the intraterminal Ca$^{2+}$ concentration ([Ca$^{2+}$]$_{terminal}$) (see also Sharma et al., 2008). Second, the acetylcholine-induced transient facilitation of GABAergic mIPSC frequency was completely blocked by MCA, a nonselective nicotinic receptor antagonist, and such an effect was closely mimicked by nicotine, a selective nicotinic receptor agonist. In addition, since acetylcholine had no effect on GABAergic mIPSC frequency after the blockade of nicotinic receptors with MCA, muscarinic receptors might not be involved in the acetylcholine-induced modulation of spontaneous GABA release. Third, the preparation used in this study should exclude any non-presynaptic actions, such as changes in the excitability of soma, because mechanically dissociated neurons retain functional cell-free presynaptic nerve terminals (for review, Akaike and Morshouse, 2003).

4.2. Presynaptic nicotinic receptors are less permeable to Ca$^{2+}$

Despite of the large number of nicotinic receptor subunits, most of neuronal nicotinic receptors expressed in the CNS are heteromeric α4β2 or homomeric α7 nicotinic ones (for review, Gotti et al., 2006), and there are distinct differences in pharmacological and physiological properties between these two types of nicotinic receptors. For example, while α4β2 nicotinic receptors exhibit high affinity of...
nicotine (EC50 ~ 15 μM) and low Ca2+ permeability (PNa/PCa ~ 1.5), α7 nicotinic receptors do low affinity of nicotine (EC50 ~ 90 μM) and high Ca2+ permeability (PNa/PCa > 10) (Role and Berg, 1996; Giniatullin et al., 2005). In the present study, while DH4/SE, a selective antagonist of β2-containing nicotinic receptors (Dickinson et al., 1987; Livingstone et al., 2009), completely suppressed the nicotine-induced facilitated spontaneous GABA release, MLA or α-bungarotoxin, selective antagonists of α7-containing nicotinic receptors, did not block the nicotine action. In addition, choline, which is more sensitive to α7 than α4/2 nicotinic receptors (Alkondon et al., 1997), had no effect on GABAergic mIPSC frequency. These pharmacological properties indicate that presynaptic nicotinic receptors responsible for the nicotine-induced increase in spontaneous GABA release might be β2-containing, possibly α4/2 nicotinic receptors. However, further studies should be needed to elucidate the exact subunit composition of presynaptic nicotinic receptors as DH4/SE affects other subtypes such as α3/2 nicotinic receptors (Harvey and Luetje, 1996).

Since nicotinic receptors are nonselective cation channels permeable to Na+ and Ca2+ (Role and Berg, 1996; Giniatullin et al., 2005), the activation of presynaptic nicotinic receptors is expected to enhance the probability of neurotransmitter release either by permitting Ca2+ influx passing through nicotinic receptors themselves or by eliciting a presynaptic depolarization, which subsequently activates presynaptic VDCCs. In the present study, the nicotine action on GABAergic mIPSCs was completely occluded by deleting extracellular Ca2+, indicating that the nicotine-induced increase in spontaneous GABA release might be β2-containing, possibly α4/2 nicotinic receptors. However, further studies should be needed to elucidate the exact subunit composition of presynaptic nicotinic receptors as DH4/SE affects other subtypes such as α3/2 nicotinic receptors (Harvey and Luetje, 1996).

4.3. Physiological implications

The PAG is closely involved in the descending pain inhibitory pathway, which is related to central analgesia (Yaksh, 1997; Lichtman et al., 1996; Finn et al., 2003), as the electrical stimulation of the PAG region is known to activate the descending inhibitory pathway to reduce pain (Reynolds, 1969; Monhemius et al., 2001). Considering that the major intrinsic neural circuit is a tonically active spontaneous GABAergic network within the PAG (Behbehani et al., 1990; Ogawa et al., 1994), GABAergic transmission would contribute to maintain the excitability of PAG neurons. For example, a behavioral study has revealed that the focal microinjection of bicuculline, a GABA(A) receptor antagonist, into the PAG abolishes the heat-evoked nociception (Sandkühler et al., 1989). In addition, the antinoceptive action of opioids is mainly mediated by decreasing GABAergic inhibition within the PAG (Moreau and Fields, 1986; Depaulis et al., 1987; Kalyuzhny et al., 1996). On the other hand, as the PAG receives innervations of cholinergic inputs from the pontine tegmentum (Woolf et al., 1990), the cholinergic system might be involved in the pain modulation by changing the excitability of PAG neurons. For example, Guimarães and Prado (1994), Guimarães et al. (2000) have reported that the microinjection of carbachol into the PAG produces analgesic actions, suggesting that cholinergic receptor-mediated mechanisms contribute to the modulation of nociceptive suppression in the PAG. Similarly, a recent study has shown that muscarinic receptors inhibit GABAergic transmission onto PAG neurons (Lau and Vaughan, 2008). In the present study, we have shown that functional nicotinic receptors, presumably α4/2 nicotinic receptors, are expressed on GABAergic nerve terminals projecting to PAG neurons, and that their activation transiently increases spontaneous GABA release. However, it is poorly known whether bipolar and pyramidal-shaped neurons used in this study are projection neurons innervating serotoninergic neurons or local interneurons, because there is little information available regarding the relationship between morphological cell types and their projection (see also Beitz, 1990). Although further study should be needed to elucidate the functional roles of presynaptic nicotinic receptors in the modulation of the descending pain inhibitory pathway, the present results suggest that presynaptic nicotinic receptors might temporally regulate the excitability of PAG neurons being not overexcited and eventually contribute to the cholinergic modulation of output from the PAG.

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References


