

Muscarine M₂ Receptor-mediated Presynaptic Inhibition of GABAergic Transmission in Rat Meynert Neurons

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Cholinergic modulation of GABAergic spontaneous miniature inhibitory postsynaptic currents (mIPSCs) by the activation of muscarine receptors was investigated in mechanically dissociated rat nucleus basalis of the Meynert neurons using the conventional whole-cell patch recording configuration. Muscarine (10 μ M) reversibly and concentration-dependently decreased mIPSC frequency without affecting the current amplitude distribution. Muscarine action on GABAergic mIPSCs was completely blocked by 1 μ M methoctramine, a selective M₂ receptor antagonist, but not by 1 μ M pirenzepine, a selective M₁ receptor antagonist. NEM (10 μ M), a G-protein uncoupler, attenuated the inhibitory action of muscarine on GABAergic mIPSC frequency. Muscarine still could decrease GABAergic mIPSC frequency even in the Ca²⁺-free external solution. However, the inhibitory action of muscarine on GABAergic mIPSCs was completely occluded in the presence of forskolin. The results suggest that muscarine acts presynaptically and reduces the probability of spontaneous GABA release, and that such muscarine-induced inhibitory action seems to be mediated by G-protein-coupled M₂ receptors, via the reduction of cAMP production. Accordingly, M₂ receptor-mediated disinhibition of nBM neurons might play one of important roles in the regulation of cholinergic outputs from nBM neurons as well as the excitability of nBM neurons themselves.

Key Words: Meynert, Cholinergic neurons, M₂ receptor, GABAergic presynaptic nerve terminal, GABAergic mIPSCs, Mechanical dissociation, Presynaptic inhibition

INTRODUCTION

The nucleus basalis of the Meynert (nBM) contains magnocellular cholinergic neurons, which innervate their cholinergic axons to the cerebral cortex, the amygdala and the thalamus (Divac, 1975; Mesulam & Van Hoesen, 1976), and non-cholinergic neurons, which include GABAergic and peptidergic ones (Walker et al, 1989; Gritti et al, 1993). The cholinergic innervation to the cerebral cortex plays a pivotal role in the higher brain function including attention, learning and memory (Drachman & Leavitt, 1974; Muir et al, 1992; Mesulam, 1995). On the other hand, the nBM receives extensive glutamatergic, GABAergic, dopaminergic and histaminergic inputs from the amygdala, cortex, thalamus, hypothalamus and septal area (Inagaki et al, 1988; Carnes et al, 1990; Pare & Smith, 1994; Zaborszky et al, 1997). These synaptic inputs into the nBM and their modulation might play important roles in shaping its cholinergic outputs.

Muscarinic acetylcholine receptors (mAChRs) are G-protein-coupled receptors and five subtypes (M₁–M₅) have been cloned and pharmacologically divided (for review; Caufield & Birdsall, 1998). mAChRs are widely expressed in the CNS (Levey, 1993) and play important roles in motor

control, temperature regulation, and higher brain function including attention, learning and memory, and sleep/waking cycle (Drachman & Leavitt, 1974; Muir et al, 1992; Mesulam, 1995; Caufield & Birdsall, 1998). It is well known that M₁, M₃ and M₅ receptors couple via the α subunits of G_{q/11} family and stimulate neurotransmitter release, whereas M₂ and M₄ receptors couple via G_i and/or G_o subunits and inhibit a variety of neurotransmitter release at presynaptic sites (Caufield & Birdsall, 1998). Especially, M₂ receptors are known to inhibit the release of a variety of neurotransmitters as a heteroreceptors as well as to inhibit the acetylcholine release as autoreceptors (Onge et al, 1986; Slutsky et al, 1999).

In the previous study, we have reported that group II metabotropic glutamate receptors on the GABAergic presynaptic nerve terminals regulate presynaptically the probability of spontaneous GABA release (Doi et al, 2002). However, much less is known whether mAChRs modulates spontaneous GABAergic transmission at these synapses. In the present study, therefore, we investigated the mechanism of the cholinergic modulation of spontaneous GABAergic transmission and its signal transduction pathway using mechanically dissociated nBM neurons, which are retaining functional GABAergic presynaptic nerve terminals (namely, 'synaptic bouton' preparation, Rhee et al, 1999).

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ABBREVIATIONS: nBM, nucleus basalis of the Meynert; mIPSCs, spontaneous miniature inhibitory postsynaptic currents; mAChRs, Muscarinic acetylcholine receptors.

METHODS

Preparation

Wistar rats (12~15 day old) were decapitated under pentobarbital anesthesia (50 mg/kg, i. p.). The brain was quickly removed and transversely sliced at a thickness of 350 μ M by using microslicer (VT1000S; Leica, Nussloch, Germany). Slices were kept in the control incubation medium (see below) saturated with 95% O₂ and 5% CO₂ at room temperature (21~24°C) for at least 1 hr before the mechanical dissociation. For dissociation, slices were transferred into 35 mm culture dish (Primaria 3801; Becton Dickinson, Rutherford, NJ), and the Meynert 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 50~60 Hz (0.5~1.0 mm). The tip of the fire-polished glass pipette was lightly placed on the surface the Meynert region with a micromanipulator. The tip of glass pipette was vibrated horizontally for about 2 min. Slices were removed, and the mechanically dissociated neurons were allowed to settle for 15 min and adhere to the bottom of the dish. Such neurons underwent dissociation retained short portions of their proximal dendrites.

All experiments conformed to the guiding principles for the care and use of animals approved by the Council of the Physiological Society of Japan, and all efforts were made to minimize the number of animals and any suffering.

Electrical measurements

All voltage-clamp recordings were made using the conventional whole-cell patch recording mode at a holding potential (V_H) of -60 mV (CEZ-2300; Nihon Kohden, Tokyo, Japan). Patch pipettes were made from borosilicate capillary glass (1.5 mm outer diameter, 0.9 mm inner diameter; G-1.5; Narishige, Tokyo, Japan) in two stages on a vertical pipette puller (PB-7; Narishige). The resistance of the recording pipettes filled with internal solution was 5~6 M Ω . Electrode capacitance and liquid junction potential were compensated, but the series resistance was not compensated. Neurons were viewed under phase contrast on an inverted microscope (Diaport; Nikon). Current and voltage were continuously monitored on an oscilloscope (VC-6023; Hitachi) and a pen recorder (RECTI-HORIT-8K; Sanei, Tokyo, Japan), and recorded on a digital-audio tape recorder (RD-120TE; TEAC). The membrane currents were filtered at 1 kHz (E-3201A Decade Filter; NF Electronic Instruments, Tokyo, Japan), digitized at 4 kHz, and stored on a computer equipped with pCLAMP 8.0 (Axon Instruments). During recording, 10 mV hyperpolarizing step pulses (30 msec in duration) were periodically delivered to monitor the access resistance. All experiments were performed at room temperature (21~24°C).

Data analysis

Spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were counted and analyzed in pre-set epochs before, during and after each experimental condition using the MiniAnalysis Program (Synaptosoft, NJ). Briefly, spontaneous events were automatically screened using an am-

plitude threshold of 10 pA and then were visually accepted or rejected based upon the rise and decay times. In complex waveforms where the event started to rise before the previous event went back to the baseline, the baseline for the second event was estimated by extrapolating the decay of the first peak at the location of the double peak. Then the peak amplitude of the second event was determined from this calculated baseline but not from the onset point of event. The average values of mIPSC frequency and amplitude during the control period (10~15 min) were calculated, and the frequency and amplitude of all the events during agonist application (5 min) were normalized to these values. The effect of the agonist was quantified as a percent decrease in mIPSC frequency compared to the control value. Numerical values were provided as means \pm standard error of the mean (S.E.M.) using values normalized to the control levels. Any differences in the amplitude and frequency distribution were tested by Student's paired two-tailed *t*-test using their absolute values but not normalized ones. Values of $p < 0.05$ were considered to be significant. On the other hand, the inter-event intervals and amplitudes of a large number of mIPSCs obtained from the same neuron were examined by constructing cumulative probability distributions and compared using Kolmogorov-Smirnov (K-S) test with Stat View software (SAS Institute, Inc.). The continuous curve for concentration-response relationship was constructed according to a modified Michaelis-Menten equation, using a least-squares fitting routine:

$$I = 1 - I_{max} \times C^{nH} / (C^{nH} + EC_{50}^{nH}),$$

where *I* is the muscarine-induced inhibition ratio of mIPSC frequency and *C* is the corresponding agonist concentration. EC₅₀ and *nH* denote the half-effective concentration and the Hill coefficient, respectively.

Solutions

The incubation medium consisted of (in mM) 124 NaCl, 5 KCl, 1.2 KH₂PO₄, 24 NaHCO₃, 2.4 CaCl₂, 1.3 MgSO₄, and 10 glucose saturated with 95% CO₂ and 5% O₂. The pH was about 7.45. The standard external solution consisted of (in mM) 150 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES. The Ca²⁺-free external solution consisted of (in mM) 150 NaCl, 5 KCl, 5 MgCl₂, 2 EGTA, 10 glucose, and 10 HEPES. These external solutions were adjusted to pH 7.4 with Tris-base. For recording mIPSCs, these external solutions routinely contained 300 nM tetrodotoxin (TTX) to block voltage-dependent Na⁺ channels, and 10 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 20 μ M DL-2-amino-5-phosphonovaleric acid (AP5) to block ionotropic glutamatergic currents. The ionic composition of the internal (patch-pipette) solution for the whole-cell patch recording was (in mM) 40 Cs-methanesulfonate, 105 CsCl, 5 TEA-Cl, 10 HEPES and 4 Mg ATP with pH adjusted to 7.2 with Tris-base.

Drugs

Drugs used in the present study were TTX, CNQX, AP5, bicuculline, muscarine, methocramine, pirenzepine, *N*-ethylmaleimide (NEM), EGTA, Mg-ATP and forskolin (all from Sigma, St. Louis, MO). CNQX, forskolin and bicuculline were dissolved in dimethyl sulfoxide at 10 mM as a stock solution. All solutions containing drugs were ap-

plied by the Y-tube system for complete solution exchange within 20 ms (Akaike & Harata, 1994).

RESULTS

GABAergic miniature inhibitory postsynaptic currents

The nBM consists of two populations of neurons, choline acetyltransferase-immunoreactive (ChAT-ir) cholinergic ones and calbindin-D28k-immunoreactive (CB-ir) noncholinergic ones (Smith et al, 1994; Bengtson & Osborne, 2000). After the mechanical dissociation of the nBM, the individual neurons were divided into two groups based on their soma diameters. Based on the previous our morphological results showing that ChAT-ir neuron has large-sized (>20 μM) and oval-shaped somata whereas CB-ir neuron has relatively small-sized (<15 μM) somata (Doi et al, 2002), present study was performed with large-sized cholinergic neurons.

As shown in Fig. 1A, the spontaneous inhibitory currents were completely and reversibly blocked by adding 10 μM bicuculline in the presence of 300 nM TTX, 10 μM CNQX and 20 μM AP-5, indicating that the spontaneous miniature inhibitory postsynaptic currents (mIPSCs) are GABAergic. Fig. 1B shows typical spontaneous GABAergic mIPSCs at various V_H values. The equilibrium potential (E_{GABA}) of

these mIPSCs, estimated from the $I\sim V$ relationship, was -12.7 mV ($n=4$). The measured value was almost identical to the theoretical Cl^- equilibrium potential (E_{Cl^-} , -10.4 mV) calculated from the Nernst equation using extra- and intracellular Cl^- concentrations (161 and 110 mM, respectively). Thus the spontaneous events were identified as GABAergic mIPSCs mediated by GABA_A receptors.

Modulation of GABAergic mIPSCs by muscarine

Application of muscarine (10 μM) decreased the mIPSC frequency in the majority (69 of 91; 76%) of nBM neurons tested. Upon washing out muscarine, the mIPSC frequency transiently rebounded before returning to control levels (Fig. 2Aa, b). Mean responses show a rapid and sustained decrease in mIPSC frequency (Fig. 2Ab). Fig. 2B shows cumulative probability plots for inter-event interval and current amplitude of mIPSCs. Muscarine shifted the distribution of mIPSC frequency to the right, indicating the reduction of mIPSC frequency, without affecting the amplitude distribution. The pooled data ($n=8$) showed that muscarine decreased the mean mIPSCs frequency to $64.5 \pm 5.9\%$ of the control ($p < 0.05$), but the mean amplitude was not affected ($103.4 \pm 5.2\%$ of the control, $p=0.41$; Fig. 2Ba, b insets). Muscarine reduced mIPSC frequency in a concentration-dependent manner (Fig. 2C).

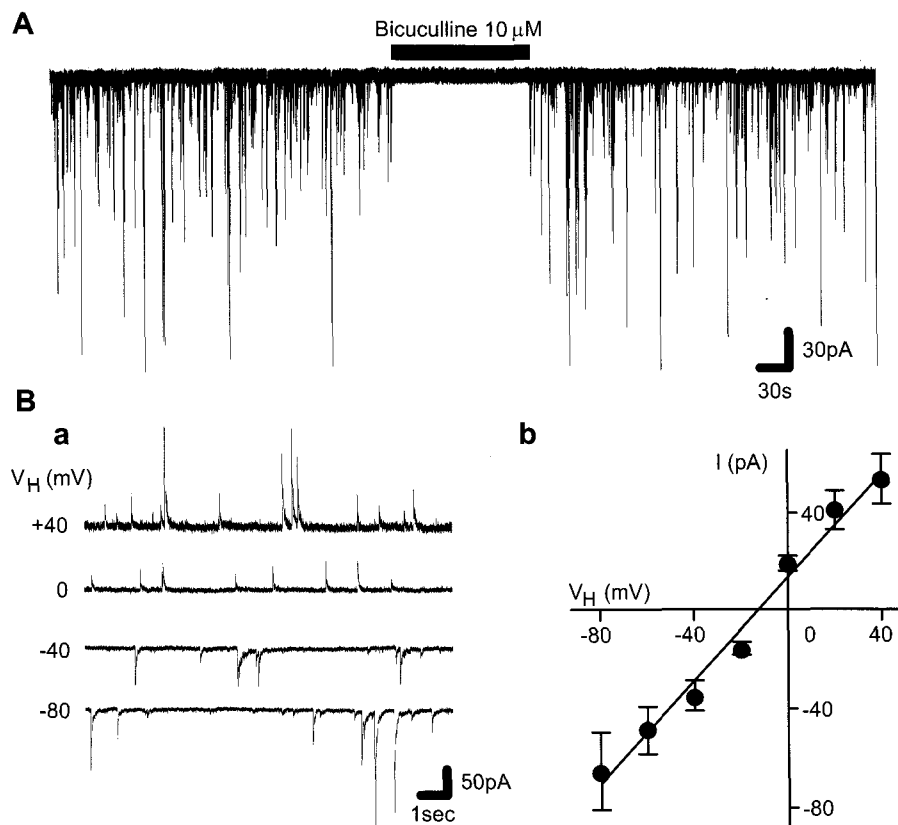


Fig. 1. GABAergic mIPSCs recorded from mechanically dissociated Meynert neurons. **A**, Typical traces of mIPSCs observed before, during and after the application of 10 μM bicuculline in the presence of 300 nM TTX, 10 μM CNQX, and 20 μM AP5. **B**, Traces of mIPSCs recorded at each V_H (**a**) and their mean amplitude $I\sim V$ curve (**b**). In **b**, each point is the mean from 4 neurons. The continuous line is the least-squares linear fit to the mean mIPSC amplitude values at respective V_H . Each point and error bar represent the mean \pm S.E.M.

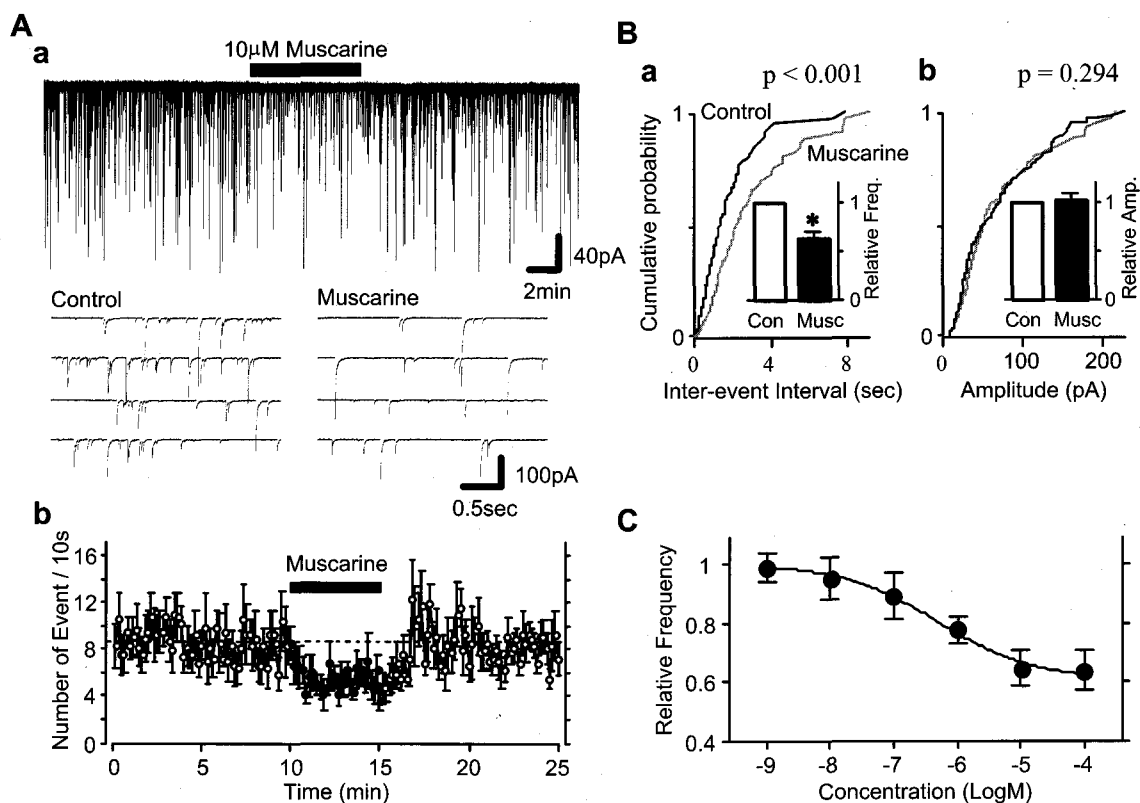


Fig. 2. Muscarine-induced inhibition of GABAergic mIPSCs. *Aa*, Typical traces of mIPSCs observed before, during and after the application of 10 μ M muscarine. *Insets* represent typical traces with an expanded time scale. *b*, The time course of mIPSC frequency before, during and after the application of muscarine. The number of events in every 10 sec period (open circle, absence of muscarine; closed circle, presence of muscarine) was summed and plotted. Each point is the mean \pm S.E.M. from 8 neurons. *B*, Cumulative distributions for inter-event interval (*a*) and current amplitude (*b*) of mIPSCs recorded from the same neuron. *p* values indicate the results of K-S test for frequency and amplitude (339 events for the control, and 104 events for muscarine). *Insets*, each column is the mean from 8 neurons. All frequencies (*a*) and amplitudes (*b*) are normalized to those of control mIPSCs. *, $p < 0.05$. These definitions are applied to all subsequent figures. *C*, Concentration-response relationship for the normalized mIPSC frequency in the presence of muscarine at various concentrations. EC_{50} value of muscarine was about 480 nM. A continuous line represents the least-squares fit. Each point is the mean from 4–12 neurons.

Effects of muscarine receptor antagonists

To identify the subtypes of muscarine receptors participating in the decrease of mIPSC frequency, the effect of muscarine receptor antagonists were examined. Methoctramine (1 μ M), a selective M_2 receptor antagonist, completely blocked the inhibitory action of muscarine on GABAergic mIPSC frequency ($97.6 \pm 9.2\%$ of the methoctramine condition, $p = 0.34$, $n = 5$, Fig. 3Ab, B). However, muscarine still could reduce mIPSC frequency even in the presence of pirenzepine (1 μ M, $n = 5$), a selective M_1 receptor antagonist ($64.1 \pm 7.1\%$ of the pirenzepine condition, $p < 0.05$, $n = 5$, Fig. 3Ac, C). Such results suggest that the modulation of GABAergic synaptic transmission by muscarine is mediated by presynaptic muscarine M_2 receptors.

Effect of NEM on M_2 receptor-mediated presynaptic inhibition

Muscarine M_2 receptors are known to couple via G_i and/or G_o subunits (for review, Caulfield & Birdsall, 1998). To examine whether the muscarine-induced inhibition of

mIPSCs frequency is coupled to a pertussis toxin-sensitive G-protein pathway, we utilized NEM, a sulphydryl alkylating agent (Asano & Ogasawara, 1986). Pretreatment of 10 μ M NEM for 15 min increased mIPSC frequency to $213.4 \pm 19.6\%$ of the control ($p < 0.05$, $n = 5$) without affecting the mean amplitude (Fig. 4A, B). In the presence of NEM, however, the inhibitory effect of muscarine on GABAergic mIPSCs was completely occluded to $108.2 \pm 10.4\%$ of the NEM condition ($p = 0.29$, $n = 5$), without affecting the amplitude distribution (Fig. 4A, B). The results suggest that M_2 receptors on the GABAergic presynaptic nerve terminals projecting to nBM neurons may be coupled to G_i/G_o proteins.

Mechanisms of M_2 receptor-mediated presynaptic inhibition

M_2 receptors are known to inhibit voltage-dependent Ca^{2+} channels (VDCCs) (Wanke et al, 1987; Wessler et al, 1987). Since Ca^{2+} influx through the VDCCs plays an important role in the release of neurotransmitter from the presynaptic nerve terminals (Wu & Saggau, 1997), we tested whether

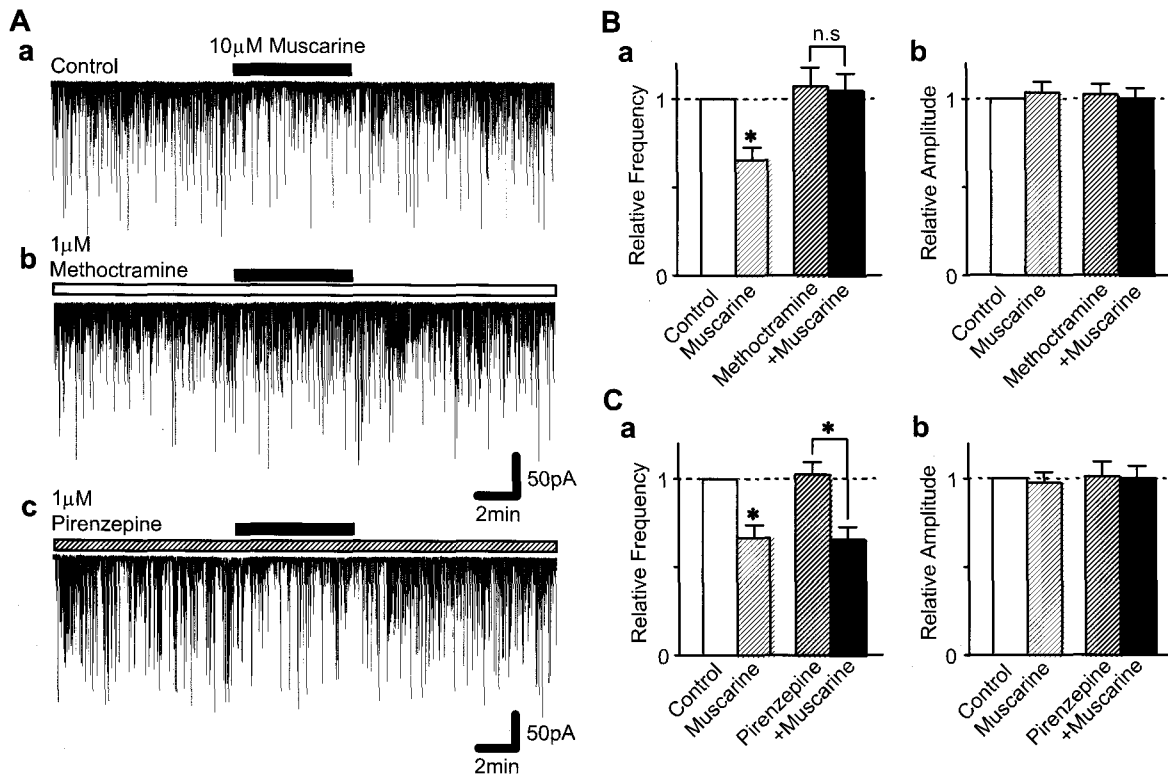


Fig. 3. M₂ receptor-mediated presynaptic inhibition of GABAergic mIPSCs. *A*, Typical traces of mIPSCs observed before, during and after the application of 10 μM muscarine in the absence (*a*) or presence (*b*) of 1 μM methoctramine, or the application of 10 μM muscarine in the presence of 1 μM pirenzepine (*c*). *a* and *b* were obtained from the same neuron. *B* and *C*, Each column is the mean from 5 neurons. All frequencies and amplitudes of mIPSCs are normalized to the control.

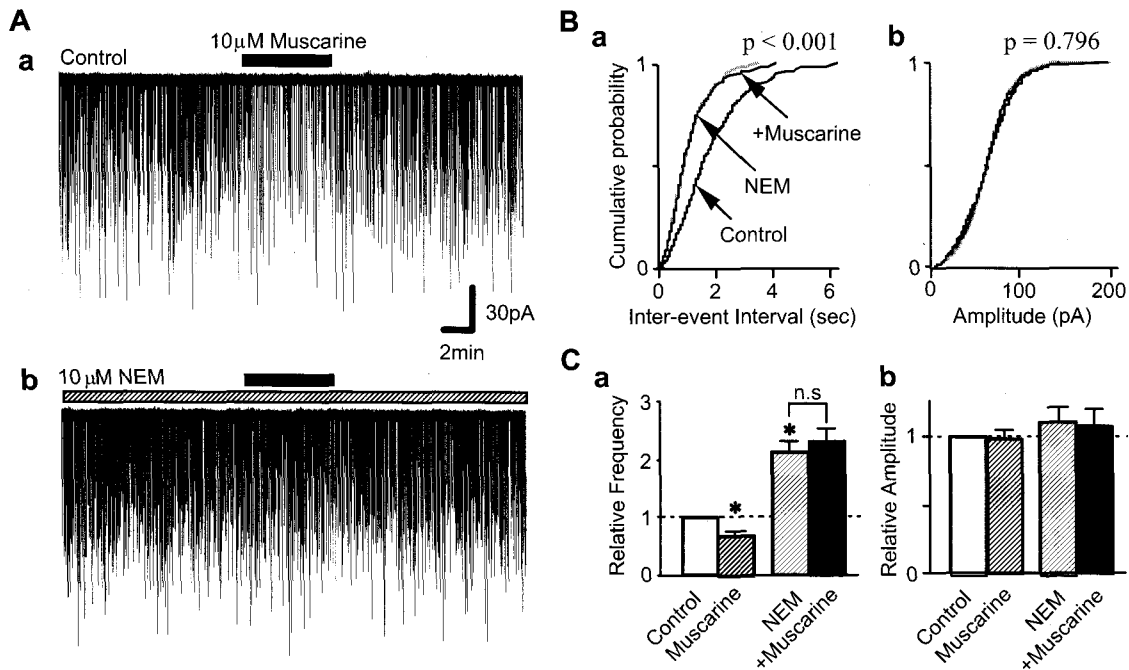


Fig. 4. Effect of NEM on muscarine-induced mIPSCs inhibition. *A*, Typical traces of mIPSCs observed before, during and after the application of 10 μM muscarine in the absence (*a*) and presence (*b*) of 10 μM NEM. *B*, Cumulative distributions for inter-event interval (*a*) and current amplitude (*b*) of mIPSCs recorded from the same neuron. *p* values indicate the results of K-S test for frequency and amplitude (712 events for the control, 1,256 events for the NEM condition, and 411 events for muscarine). *C*, Each column is the mean from 5 neurons. All amplitude and frequencies are normalized to the control.

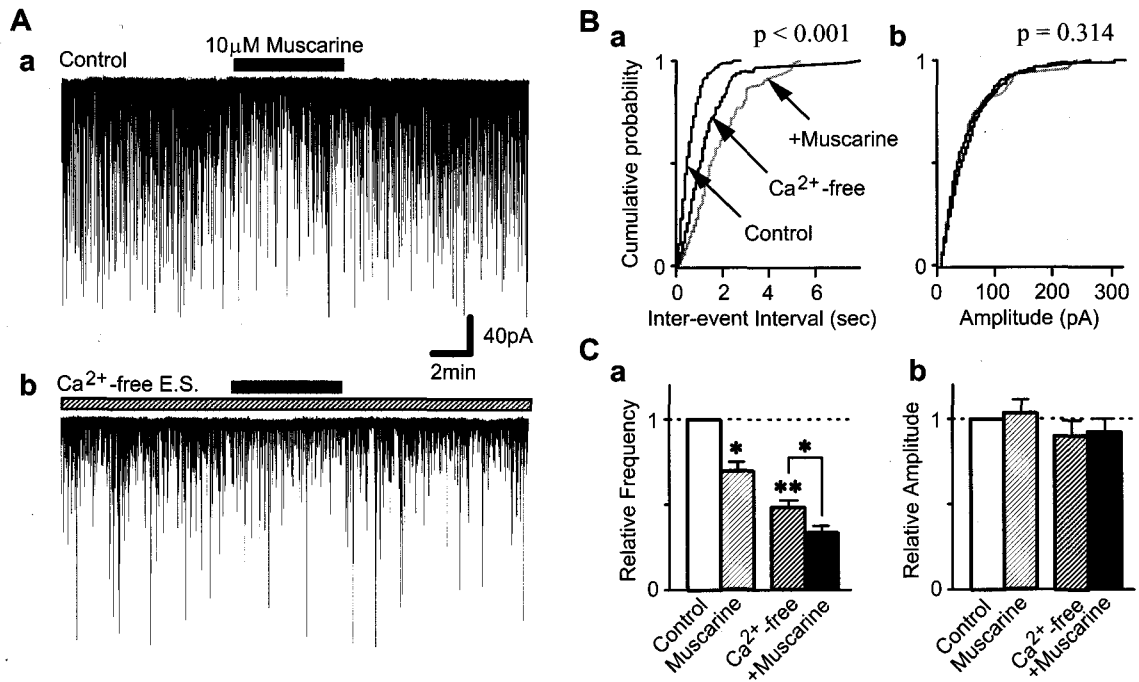


Fig. 5. Effect of the Ca²⁺-free external solution on muscarine-induced mIPSCs inhibition. *A*, Typical traces of mIPSCs observed before, during and after the application 10 μM muscarine in the standard solution (*a*) and in the Ca²⁺-free external solution (*b*). *B*, Cumulative distributions for inter-event interval (*a*) and current amplitude (*b*) of mIPSCs recorded from the same neuron. *p* values indicate the results of K-S test for frequency and amplitude (1,755 events for the control, 615 events for Ca²⁺-free condition, and 106 events for muscarine). *C*, Each column is the mean from 6 neurons. All amplitudes and frequencies are normalized to the control. **, *p* < 0.01.

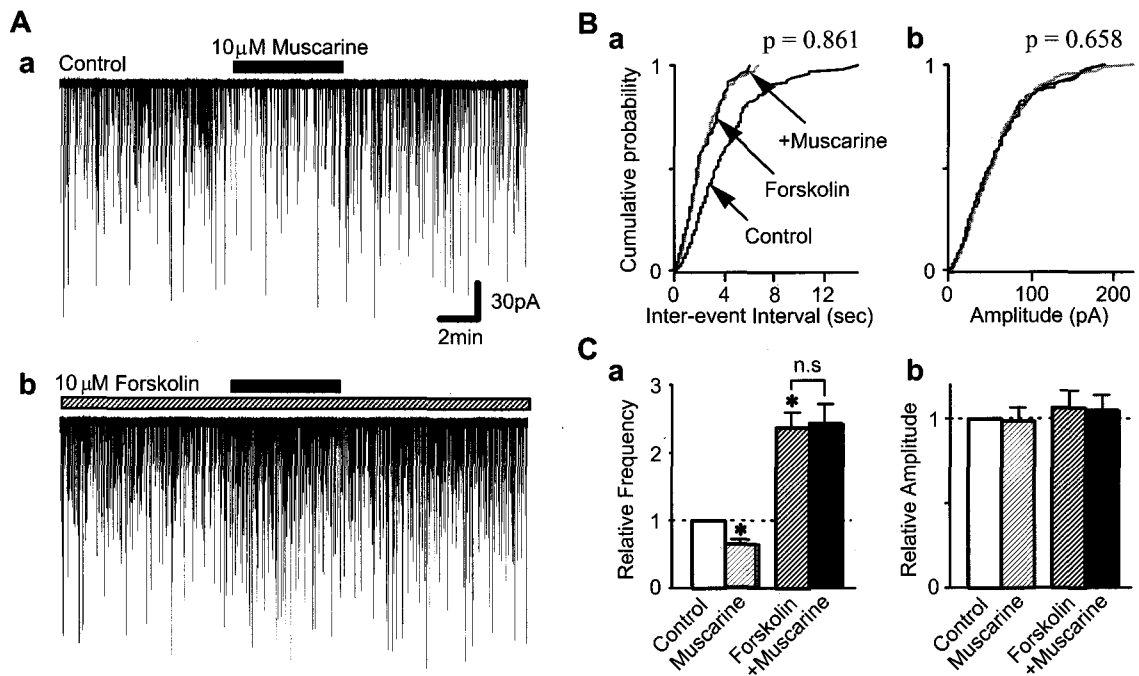


Fig. 6. Effect of forskolin on muscarine-induced mIPSCs inhibition. *A*, Typical traces of mIPSCs observed before, during and after the application of 10 μM muscarine in the absence (*a*) or presence (*b*) of 10 μM forskolin. *B*, Cumulative distributions for inter-event interval (*a*) and current amplitude (*b*) of mIPSCs recorded from the same neuron. *p* values indicate the results of K-S test for frequency and amplitude (427 events for the control, 783 events for the forskolin condition, and 359 events for muscarine). *C*, Each column is the mean from 6 neurons. All amplitudes and frequencies are normalized to the control.

the M₂ receptor-mediated inhibition of GABAergic mIPSCs requires Ca²⁺ influx from the extracellular site. Exposure of nBM neurons to the Ca²⁺-free external solution significantly reduced GABAergic mIPSC frequency to 48.1 ± 4.6% of the control ($p < 0.05$, $n = 5$, Fig. 5A, Ba, Ca), without affecting the mean mIPSC amplitude (91.3 ± 8.1% of the control, $p = 0.19$, Fig. 5Bb, Cb). This indicates that Ca²⁺ influx from the extracellular site may contribute to the generation of GABAergic mIPSCs. In the Ca²⁺-free external solution, however, muscarine still reduced mIPSC frequency (71.5 ± 5.9% of the Ca²⁺-free condition, Fig. 5B, C).

Since M₂ receptor activation is also negatively coupled to cAMP formation by inhibiting adenylyl cyclase (AC) in brain (Caulfield & Birdsall, 1998; Krsmanovic et al, 1998), we tested the effect of forskolin, an AC activator, on M₂ receptor-mediated inhibition of GABAergic mIPSCs. Activation of AC with forskolin (10 μM) significantly increased mIPSC frequency to 238.9 ± 21.1% of the control ($p < 0.05$, $n = 6$, Fig. 6A, Ba, Ca) without affecting the mean mIPSC amplitude (Fig. 6Bb, Cb). In the presence of forskolin, however, muscarine action on GABAergic mIPSC frequency was completely occluded to 102.9 ± 8.3% of the forskolin condition ($p < 0.05$, $n = 6$, Fig. 6B, C). The results suggest that M₂ receptors on the GABAergic presynaptic nerve terminals projecting to nBM neurons are coupled to AC-cAMP pathway.

DISCUSSION

In the present study, we have investigated the effect of muscarine on spontaneous GABAergic transmission in mechanically dissociated rat nBM neurons. The results suggest that muscarine-mediated inhibition of GABAergic transmission might be primarily mediated by the reduction of cAMP production via G_i/G_o-protein coupled M₂ receptors.

Muscarine reversibly decreased GABAergic mIPSC frequency without affecting the amplitude distribution, indicating that muscarine acts presynaptically to inhibit spontaneous GABA release from the presynaptic nerve terminals. This effect was completely blocked by methoctramine, a selective M₂ receptor antagonist, but not affected by pirenzepine, a selective M₁ receptor antagonist. Thus muscarine primarily seems to act on presynaptic M₂ receptors. Since muscarine did not change GABAergic mIPSC frequency after the blockade of M₂ receptors with methoctramine, GABAergic presynaptic nerve terminals projecting to nBM neurons might express only M₂ receptors.

M₂ receptors are generally coupled to the G_i/G_o protein (Caulfield & Birdsall, 1998). In the present study, M₂ receptor-mediated inhibition of mIPSC frequency was completely occluded in the presence of NEM. The results are consistent with the previous findings showing that M₂ receptor-mediated presynaptic inhibition is coupled to NEM-sensitive G_i/G_o proteins in a number of tissues (Felder 1995; Caulfield & Birdsall, 1998). G protein-coupled receptors have three possible modes of action in causing presynaptic inhibition for neurotransmitter release: inhibition of VDCCs, an increase in K⁺ conductance or direct modulation of synaptic release machinery in downstream of Ca²⁺ influx (for reviews; Wu & Saggau, 1997). Similarly, the inhibitory action of G-protein-coupled M₂ receptors on neurotransmitter release is mediated by inhibition of AC,

inhibition of Ca²⁺ influx, or activation of K⁺ channels (Caulfield & Birdsall, 1998).

Exposure of nBM neurons to the Ca²⁺-free external solution significantly reduced the occurrence of GABAergic mIPSCs, indicating that the mIPSCs at these synapses are closely related to Ca²⁺ influx from the extracellular sites. The results suggest that these GABAergic presynaptic terminals might have somewhat depolarized membrane potential, and thus it might lead to spontaneous opening of VDCCs even in the presence of TTX. Thus the events that remain in the Ca²⁺-free external solution should be classical miniature events, which are not sensitive to extracellular Ca²⁺. In the present study, muscarine action on GABAergic mIPSC frequency was still developed in the Ca²⁺-free external solution. The results indicate that muscarine action on GABAergic mIPSCs might not be related to Ca²⁺ influx from the extracellular sites, suggesting no involvement of VDCCs inhibition. More reasonably, muscarine-mediated presynaptic inhibition of GABAergic transmission might be closely related to inhibition of synaptic release machinery in downstream of Ca²⁺ influx.

The cAMP-dependent modulation of neurotransmitter release at central synapses is recently reported (Capogna et al, 1995; Katsurabayashi et al, 2001; Doi et al, 2002). In the present study, muscarine action on GABAergic mIPSC frequency was completely occluded in the presence of forskolin, which activates AC to increase intracellular cAMP concentration. Accordingly, most plausible mechanism for M₂ receptor-mediated presynaptic inhibition of GABAergic transmission seems to be a reduction of cAMP formation within the GABAergic presynaptic nerve terminals. This decrease in cAMP concentration might reduce the probability of spontaneous GABA release by modulating synaptic release machinery. Similarly, metabotropic glutamate receptor-mediated presynaptic inhibition has been reported to inhibit cAMP production in rat spinal cord (Katsurabayashi et al, 2001) and nBM neurons (Doi et al, 2002).

The cholinergic innervation from the nBM to the cerebral cortex plays a pivotal role in the higher brain function including attention, learning and memory, and sleep/waking cycle (Drachman & Leavitt, 1974; Muir et al, 1992; Mesulam, 1995). The deficit of this pathway is one of representative neuropathological features of Alzheimer's disease, and provides one of the several anatomical substrates for the dementia, especially the memory loss (Arendt et al, 1985; Geula & Mesulam, 1994). Accordingly, M₂ receptor-mediated disinhibition of nBM neurons might play important roles in not only the regulation of neuronal excitability and cholinergic outputs but also the modulation of higher brain functions.

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