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European Journal of Pharmacology 586 (2008) 114–122
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5-HT_{1A} receptor-mediated activation of a G-protein-coupled inwardly rectifying K^+ current in rat medial preoptic area neurons

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Received 2 September 2007; received in revised form 12 February 2008; accepted 20 February 2008
Available online 4 March 2008

Abstract

The medial preoptic area plays an important role in the regulation of sexual behavior, and serotonin (5-hydroxytryptamine, 5-HT) exerts an inhibitory effect on sexual behavior by acting on the medial preoptic area region. This study was designed to clarify the inhibitory effect of 5-HT on the medial preoptic area neurons and to elucidate the electrophysiological mechanisms involved in the action of 5-HT. Superfusion of 100 nM 5-HT hyperpolarized the membrane potential and inhibited the action potential firing. When the membrane potential was stepped to various potentials, the inward K^+ currents were potentiated in the presence of 100 nM 5-HT. When the concentration of K^+ in the external solution was increased from 5 mM to 30 mM, 5-HT markedly potentiated the inward K^+ currents. In the steady-state current–voltage relationship, the 5-HT-activated inward current was carried by K^+ ions and showed characteristics typical of an inwardly rectifying K^+ current. The 5-HT-activated K^+ current was mimicked by a 5-HT_{1A} receptor agonist, (±)-8-hydroxy-2-dipropylaminotetralin hydrobromide, and was reversibly blocked by a 5-HT_{1A} receptor antagonist, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide, but not by a 5-HT_2 receptor antagonist, ketanserin. The 5-HT-activated K^+ current was sensitively blocked by Ba^{2+}, but not by 4-aminopyridine, and was completely suppressed by *N*-ethylmaleimide. These results indicate that 5-HT-induced hyperpolarization of the medial preoptic area neurons occurs as a result of activation of the G-protein-coupled inwardly rectifying K^+ currents by 5-HT_{1A} receptors.
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Keywords: Medial preoptic area; Serotonin; 5-Hydroxytryptamine; K^+ channel

1. Introduction

The medial preoptic area is a critical integrative site for male sexual behavior in most vertebrate species. Damage to the medial preoptic area impairs sexual behavior (Liu et al., 1997; Paredes et al., 1998), whereas stimulation of the medial preoptic area enhances sexual behavior (Rodriguez-Manzo et al., 2000).

Serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter as well as a neuromodulator, is widely distributed in the brain and affects many functions controlled by the central nervous system, including sleep, cognition, sensory perception, motor activity, temperature regulation, appetite, hormone secretion, nociception, and sexual behavior. Regulation of male sexual behavior by serotonin has been studied in humans, primates, and rodents, and most pharmacological manipulations of 5-HT suggest that it has an inhibitory effect on sexual motivation and performance (Bitran and Hull, 1987; Gorzalka et al., 1990; Zajecka et al., 1991). Several regions of the brain, including preoptic and hypothalamic structures, are believed to be involved in mediating these serotonergic effects, and microinjection of 5-HT into the medial preoptic area of male rats has been shown to impair sexual activity (Verma et al., 1989; Fernandez-Guasti et al., 1992). In addition, antidepressants of the selective serotonin reuptake inhibitor (SSRI) have been shown to impair ejaculatory/orgasmic function and frequently interfere with erectile function as well (Gitlin, 1994). Conversely, decreasing the serotonergic activity, through the use of lesions or by inhibition of synthesis, has been shown to facilitate sexual behavior (Bitran and Hull, 1987; Gorzalka et al., 1990).

Some agonists or neurotransmitters inhibit neuronal activities by hyperpolarizing the membrane potential. In many central

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doi:10.1016/j.ejphar.2008.02.051

neurons, 5-HT activates an inwardly rectifying K^+ current, which leads to hyperpolarization (Andrade et al., 1986; Penington et al., 1993; Oh et al., 1995; Bayliss et al., 1997; Katayama et al., 1997; Larkman and Kelly, 1998; Sodickson and Bean, 1998; Takigawa and Alzheimer, 1999). Furthermore, it has been reported that 5-HT-activated inwardly rectifying K^+ channels are coupled to pertussis toxin-sensitive G proteins (Andrade et al., 1986; Penington et al., 1993; Oh et al., 1995; Bayliss et al., 1997; Katayama et al., 1997). However, electrophysiological characteristics and mechanisms of the modulatory action of 5-HT on the neuronal excitability of the medial preoptic area have not yet been elucidated. Thus, this study was designed to examine the electrophysiological mechanism for serotonergic inhibition of neuronal activity by performing nystatin-perforated patch-clamp recordings in acutely dissociated rat medial preoptic area neurons under current- and voltage-clamp conditions.

2. Materials and methods

2.1. Preparation of the medial preoptic area neurons

All experiments conformed to the guiding principles for the care and use of animals approved by the Council of the Korean Physiological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals as well as suffering. The medial preoptic area neurons were dissociated using techniques described in detail elsewhere (Cho et al., 2001; Lee et al., 2003). Briefly, 2 weeks-old Sprague–Dawley rats of both sexes were decapitated under Zoletil® anesthesia (50 mg/kg, intraperitoneal injection). The brain was removed and transverse slices (400 μm thickness) were made using a vibratome (Series 1500, Vibratome, St. Louis, Missouri). The slices were pre-incubated in the incubation solution that had been well saturated with 95% O_2 and 5% CO_2 at room temperature for 30 min. Next, the slices were treated with pronase (1 mg/7 ml of the oxygenated incubation solution) for 40–80 min at 32 °C and then subsequently treated with thermolysin (1 mg/7 ml) for 10–15 min at 32 °C. Following the enzyme treatments the slices were kept in the incubation solution for 1 h. The medial preoptic area region was identified in a 60 mm culture dish coated with silicon using a binocular microscope (SZ-ST, Olympus, Tokyo, Japan), and then was micropunched out from the slices using an electrolytically polished injection needle. The pieces that had been micropunched out were then mechanically dissociated in a different dish using fire-polished fine glass Pasteur pipettes in 35 mm plastic culture dishes (Falcon Primaria 3801, Becton Dickinson, Rutherford, New Jersey) filled with the standard external solution under an inverted phase-contrast microscope (CK-2, Olympus, Tokyo, Japan). The dissociated neurons usually adhered to the bottom of the dish within 20 min and remained viable for electrophysiological studies for up to 6 h after dissociation.

2.2. Electrical measurements

Electrical recordings were performed in the nystatin-perforated whole-cell patch-recording mode (Akaike and Harata, 1994) under current- and voltage-clamp conditions. Patch pipettes were prepared from glass capillaries (1B150F-4, World Precision Instruments Inc., Sarasota, Florida) on a vertical pipette puller (PP-830, Narishige, Tokyo, Japan) in two stages. The patch pipette was positioned on the neuron using a water-driven micromanipulator (WR-60, Narishige, Tokyo, Japan). The resistance between the recording electrode filled with the internal pipette solution and the reference electrode was 5–7 MΩ. The neurons were visualized using phase-contrast equipment on an inverted microscope (IX-70, Olympus, Tokyo, Japan). Electrical stimulation, current recordings, and filtration of currents (at 1 kHz) were obtained using an EPC-10 patch-clamp amplifier (HEKA Electronik, Lambrecht, Germany) linked to an IBM-compatible Pentium PC controlled by HEKA software. The current and voltage were also monitored on a pen recorder (Recti-Horiz-8K, NEC San-ei, Tokyo, Japan) and stored on videotapes with a data recorder (RD-120TE, TEAC, Tokyo, Japan). All experiments were performed at room temperature (22–25 °C).

2.3. Solutions and drugs

The ionic composition of the incubation solution was (in mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, glucose 10, and $NaHCO_3$ 24, and the pH was adjusted to 7.4 by continuous bubbling with 95% O_2 and 5% CO_2. The standard external solution was (in mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES) 10, and the pH was adjusted to 7.4 with tris-hydroxymethylaminomethane (Tris-base). The high K^+ external solution, which contained 30 mM K^+, was prepared by substituting the equimolar Na^+ in the standard external solution with K^+. When the current–voltage relationship of serotonin-activated current was recorded, both tetrodotoxin (0.3 μM) and $LaCl_3$ (10 μM) were included in the standard external solution to block voltage-activated Na^+ and Ca^{2+} channels, respectively. The composition of the internal pipette solution used for the nystatin-perforated recording was (in mM): *N*-methyl-D-glucamine (NMG)-methanesulfonate 20, potassium methanesulfonate 80, $MgCl_2$ 5, KCl 40, HEPES 10, and the pH was adjusted to 7.2 with Tris-base. The nystatin-stock solution, which contained 10 mg/ml of nystatin in methanol, was prepared and then diluted to a final concentration of 200 μg/ml using the internal pipette solution.

Zoletil 50® (tiletamine HCl 125 mg/5 ml+zolazepam HCl 125 mg/5 ml) was purchased from Virbac laboratory (06516 Carros, France). Pronase (protease XIV), thermolysin (protease X), nystatin, tetrodotoxin, lanthanum chloride ($LaCl_3$), methanesulfonic acid, dimethyl sulfoxide (DMSO), *N*-methyl-D-glucamine (NMG), $BaCl_2$, 4-aminopyridine (4-AP), *N*-ethylmaleimide (NEM), and 5-hydroxytryptamine HCl (5-HT, serotonin) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). (±)-8-Hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT), α-methyl-5-hydroxytryptamine maleate (α-methyl-5-HT), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN-190), and ketanserin tartrate were purchased from Tocris Cookson Ltd. (Bristol, UK).

Drugs were added to the external solutions at the final concentrations indicated in the text and the vehicle concentrations

never exceeded 0.01%. Drugs were applied using a rapid application system termed the "Y-tube method", as described elsewhere (Min et al., 1996).

2.4. Statistical analysis

Data were presented as the mean±S.E.M. and Student's paired two-tailed *t*-test was used for statistical analysis. *P* values less than 0.05 were considered significant.

3. Results

3.1. 5-HT reversibly hyperpolarizes the medial preoptic area neurons

We examined the effects of 5-HT on the membrane potentials and firing properties of the medial preoptic area neurons to determine whether the action of 5-HT on the medial preoptic area neurons was inhibitory or excitatory. Under current-clamp conditions, the resting membrane potentials of the medial preoptic area neurons ranged from −55 to −60 mV and the firing rate ranged from 2 to 3 Hz (Fig. 1). Application of 100 nM 5-HT hyperpolarized the membrane potential to −70 to −75 mV and inhibited the action potential firing (less than 1 Hz). After washing out 5-HT, the membrane potentials and firing properties gradually returned to the control level.

Among the medial preoptic area neurons tested, 30% were hyperpolarized by 5-HT, however the remaining 70% of the neurons did not respond to 5-HT. Most of the medial preoptic area neurons had an oval-shaped soma with two to three proximal dendrites, although some cells had a round-, triangular-, or rectangular-shaped soma with multiple dendrites. The size of the neuronal soma was usually 10 to 20 μM and there were no morphological differences observed between the 5-HT responsive and non-responsive cells. In addition, there was no difference observed between male and female response to 5-HT.

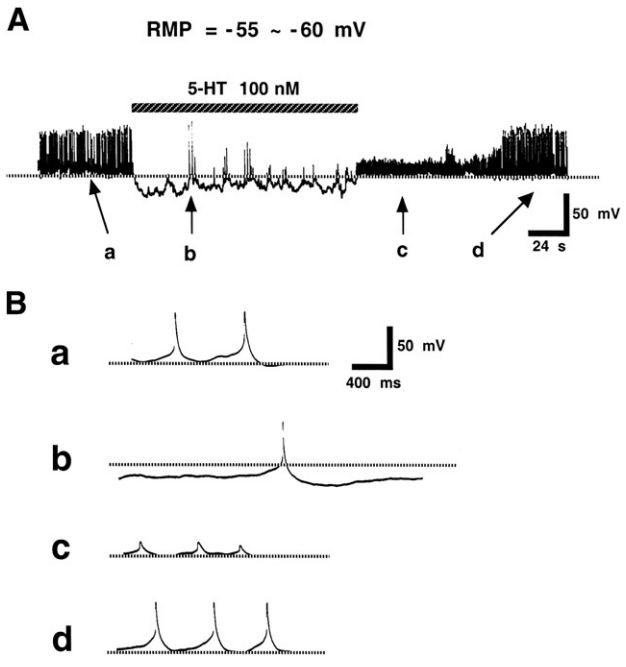

Fig. 1. 5-HT-induced hyperpolarization of the medial preoptic area neuron. (A) Application of 100 nM 5-HT hyperpolarizes the neuronal membrane potential and suppresses the frequency and amplitude of action potential firing. RMP indicates resting membrane potential. (B) Wave form of action potential in each point indicated as a, b, c, and d. Horizontal dashed lines indicate the control resting membrane potential.

3.2. 5-HT-induced hyperpolarization is caused by activation of inwardly rectifying potassium currents

In neuronal tissues, an agonist-induced hyperpolarization is usually caused by an increase in K^+ conductance via activation of inwardly rectifying K^+ currents. To investigate the electrophysiological mechanisms involved in the 5-HT-induced hyperpolarization, we examined the change of K^+ conductance in the medial preoptic area neuron. Under voltage-clamp conditions, the membrane potential was held at −60 mV, and then stepped to various potentials from −160 mV to +20 mV in 20 mV steps for 100 ms (Fig. 2). The amplitude of each current was measured as the mean current between 30 ms and 70 ms after application of each step pulse. K^+ currents evoked by step pulses primarily consisted of two components, a large, voltage-dependent, outward current, and a small, inward current. Although the voltage-dependent outward K^+ currents were not changed by 5-HT, the inward K^+ currents were potentiated at potentials less negative to as much as −80 mV in the presence of 100 nM 5-HT (Fig. 2Aa and Ba). At the step potential of −160 mV, the inward current was significantly potentiated by 5-HT (from −59.7±7.7 pA to −105.7±7.1 pA, $P<0.05$, $n=5$, Fig. 2Ba).

When the concentration of K^+ in the external solution was increased from 5 mM to 30 mM, the amplitudes of inward K^+ currents were potentiated, whereas those of the outward currents showed no change in amplitudes. When 100 nM 5-HT was applied to the high K^+ solution, the inward K^+ currents activated by the voltage steps were markedly potentiated at potentials less negative to −60 mV in the presence of 100 nM 5-HT (Fig. 2Ab and Bb). At the step potential of −160 mV, 5-HT significantly potentiated the inward current from −112.5±0.5 pA to −272.5±12.5 pA ($P<0.05$, $n=7$, Fig. 2). However, similar to the results observed when the 5 mM K^+ solution was tested, the outward K^+ currents were not changed by 5-HT in the high K^+ solution.

We examined the steady-state current–voltage (*I–V*) relationship of the 5-HT-activated inward current to elucidate the electrophysiological characteristics of the K^+ currents involved in the 5-HT-induced hyperpolarization of the medial preoptic area neurons. To accomplish this, we subtracted the current activated in the absence of 5-HT from the current activated in the presence of 5-HT at each holding potential to determine the currents activated by 100 nM 5-HT (Fig. 2Bc). When the medial preoptic area neurons were superfused with external solutions containing various K^+ concentrations (5 and 30 mM), the reversal potentials of the 5-HT-activated currents ($E_{5\text{-HT}}$), as estimated from the intersection of the potentials with the voltage axis in the *I–V* curve, were −80 and −40 mV, respectively, which were similar to the theoretical K^+ equilibrium potentials (E_K) that were calculated

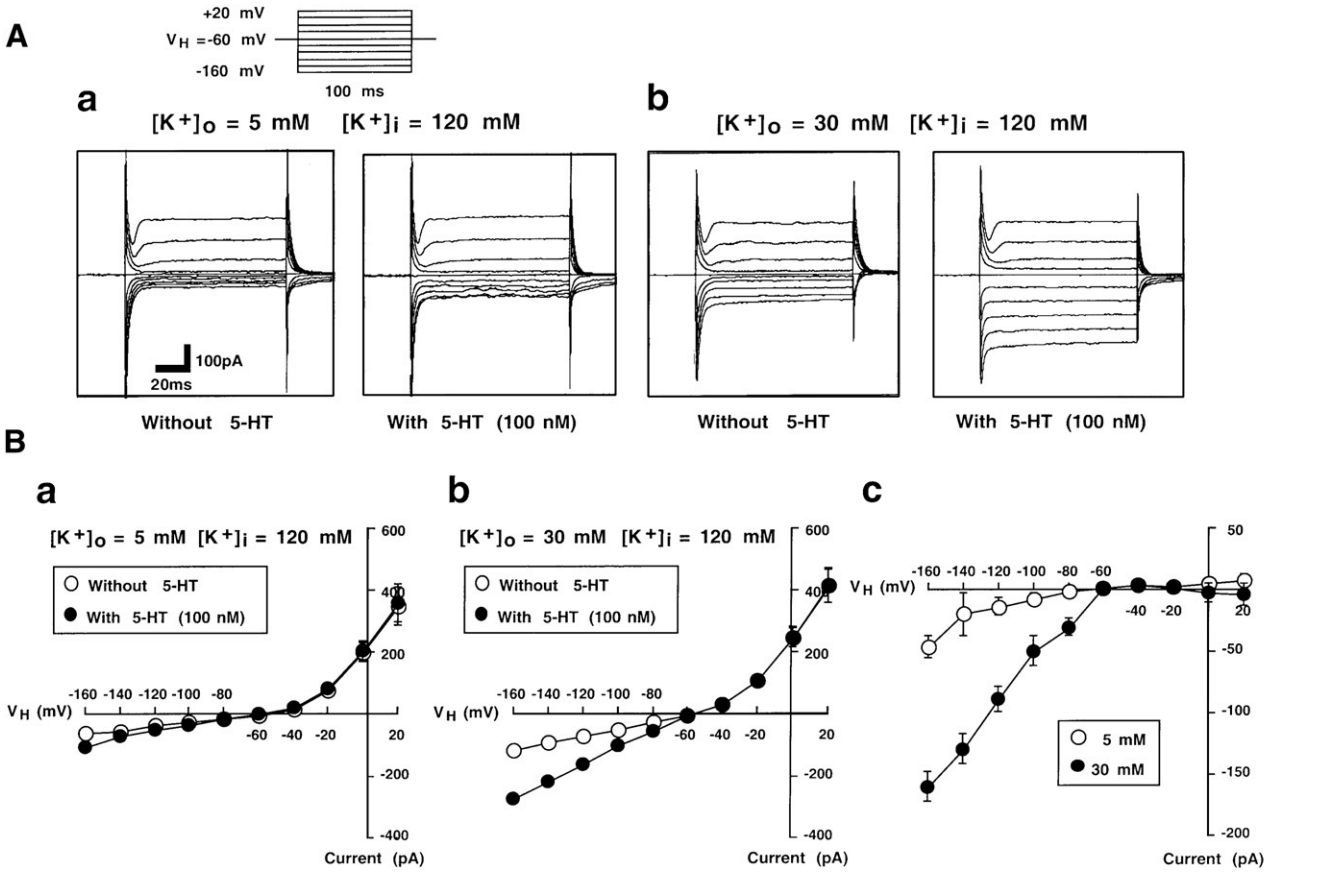

Fig. 2. 5-HT-induced activation of inwardly rectifying K^+ current. At the holding potential (V_H) of −60 mV, voltage steps were applied from −160 mV to +20 mV in 20 mV steps for 100 ms. (A) K^+ currents induced by voltage steps were cumulatively plotted in the presence and absence of 100 nM 5-HT. (a) In the control external solution containing 5 mM K^+. (b) In the high K^+ external solution containing 30 mM K^+. (B) Steady-state current–voltage curves were plotted to compare the change of K^+ conductance in the presence (filled circle) and absence (open circle) of 100 nM 5-HT. (a) In the control external solution (5 mM K^+). (b) In the high K^+ external solution (30 mM K^+). (c) Each point was plotted by subtracting the current activated in the absence of 5-HT from the current activated in the presence of 5-HT. As the extracellular K^+ concentration was increased from 5 mM (open circle) to 30 mM (filled circle), the *I–V* curve showed severe inward rectification.

using the Nernst equation, which were −89 and −42 mV, respectively. When the extracellular K^+ concentration was raised from 5 to 30 mM, the reversal potential shifted from −80 to −40 mV and the inward current was potentiated from −46.0±9.2 pA to −160.0±12.0 pA at the step potential of −160 mV ($P<0.01$, $n=7$, Fig. 2Bc).

3.3. 5-HT receptor subtypes involved in the 5-HT-activated K^+ currents ($I_{5\text{-}HT}$)

We examined the effects of 5-HT receptor agonists and antagonists to identify the 5-HT receptor subtypes involved in the 5-HT response. Fig. 3A shows the changes in K^+ currents induced by voltage steps in the presence of 5-HT, (±)-8-hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT, a 5-HT_{1A} receptor agonist), or α-methyl-5-hydroxytryptamine maleate (α-methyl-5-HT, a 5-HT_2 receptor agonist). The amplitude of the inward K^+ current augmented by 100 nM of 8-OH-DPAT was similar to the change in amplitude that occurred in response to 100 nM of 5-HT (81.7±16.7% of the 5-HT response, $P=0.353$, $n=4$, Fig. 3C). In contrast, the amplitude of the inward K^+ current was not augmented by 100 nM of α-methyl-5-HT (1.2±0.7% of the 5-HT response, $P<0.01$, $n=4$, Fig. 3C). However, similar to the 5-HT response, outward K^+ currents were not changed by 8-OH-DPAT (104.5±8.0% of the 5-HT response, $P=0.616$, $n=4$, Fig. 3D) or α-methyl-5-HT (95.8±2.7% of the 5-HT response, $P=0.225$, $n=4$, Fig. 3D).

The neurons were superfused with external solution containing each 5-HT receptor antagonist for 1 min before simultaneous application of 100 nM 5-HT with one of the antagonists (Fig. 4A). 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN-190, 10 μM), a 5-HT_{1A} receptor antagonist, completely blocked the effect of 5-HT on the inward K^+ current (2.0±1.3% of the control, $P<0.01$, $n=5$, Fig. 4C), whereas, ketanserin tartrate (ketanserin, 10 μM), a 5-HT_2 receptor antagonist, had little effect on the 5-HT action (91.8±5.2% of the control, $P=0.208$, $n=4$, Fig. 4C). However, the effect of 5-HT on outward K^+ currents was not altered by NAN-190 (95.8±1.7% of the control, $P=0.063$, $n=5$, Fig. 4D) or ketanserin (103.4±1.8% of the control, $P=0.159$, $n=4$, Fig. 4D).

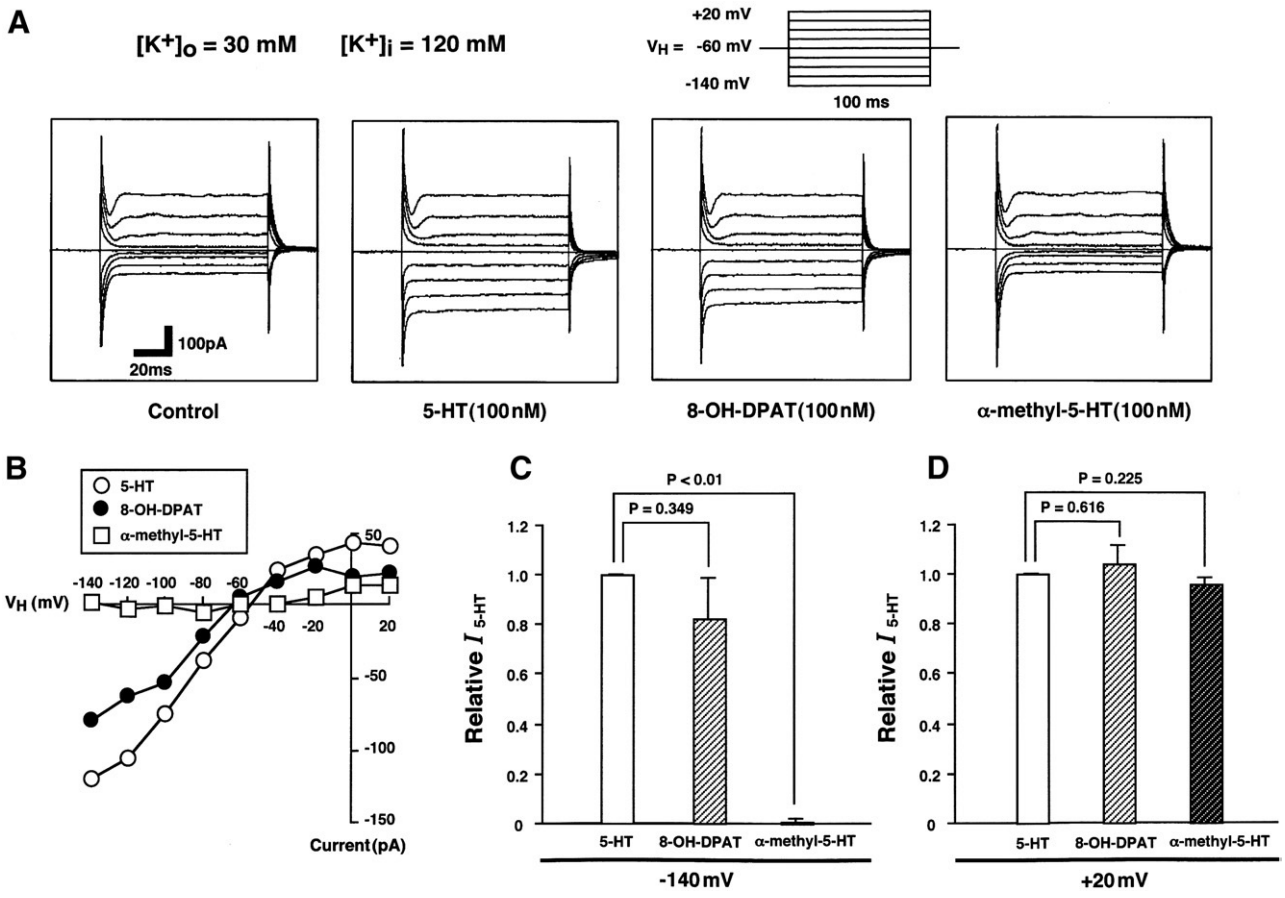

Fig. 3. Effect of 5-HT receptor subtype agonists on inwardly rectifying K^+ currents. All recordings were performed in an external solution containing 30 mM K^+. At a holding potential (V_H) of −60 mV, voltage steps were applied from −140 mV to +20 mV in 20 mV steps for 100 ms. (A) Representative current traces induced by voltage steps after treatment with 100 nM 5-HT, 8-OH-DPAT, or α-methyl-5-HT. (B) Steady-state currents plotted against voltage obtained by subtracting the current under control conditions from the current observed in response to treatment with 5-HT, 8-OH-DPAT, or α-methyl-5-HT. (C) Histograms showing the effects of 8-OH-DPAT and α-methyl-5-HT on the inward K^+ current evoked at a V_H of −140 mV. Vertical bars indicate the S.E.M. Note that the peak amplitude of the inward K^+ current induced by 8-OH-DPAT was similar to that induced by 5-HT ($P>0.05$, $n=4$). $I_{5\text{-HT}}$ indicates the 5-HT-activated K^+ current. (D) Histograms showing the effects of 8-OH-DPAT and α-methyl-5-HT on the outward K^+ current evoked at a V_H of +20 mV. The effect of 8-OH-DPAT ($P>0.05$, $n=4$) or α-methyl-5-HT ($P>0.05$, $n=4$) on the outward K^+ current was not different from that of 5-HT.

3.4. Effects of K^+ channel blockers on the 5-HT-activated K^+ currents

The effects of K^+ channel blockers on the 5-HT-activated K^+ current were examined to elucidate the pharmacological properties of the K^+ channel activated by 5-HT. The neurons were superfused with external solution containing each K^+ channel blocker for 1 min before simultaneous application of 100 nM 5-HT with one of the K^+ channel blockers (Fig. 5A). The 5-HT-induced inward K^+ current was abolished by the application of external solution containing 1 mM Ba^{2+} (52.4±14.0% of the control, $P<0.05$, $n=6$, Fig. 5C). Moreover, the inward current amplitude in the presence of both 5-HT and Ba^{2+} was less than that obtained under the control conditions, possibly indicating slight basal activation of the inwardly rectifying K^+ channels in the absence of an agonist. In contrast, 4-AP (1 mM) had little effect on the 5-HT-activated K^+ current (87.8±16.3% of the control, $P=0.294$, $n=6$, Fig. 5C).

Outward K^+ currents showed no change in amplitude in the presence of either Ba^{2+} (98.7±5.1% of the control, $P=0.813$, $n=6$, Fig. 5D) or 4-AP (79.2±11.9% of the control, $P=0.141$, $n=6$, Fig. 5D).

3.5. Effect of N-ethylmaleimide on the 5-HT-activated K^+ currents

N-ethylmaleimide (a sulfhydryl alkylating agent, NEM) is known to uncouple G-protein-coupled receptors from pertussis toxin-sensitive G proteins (G_i and/or G_o) in many tissues (Nakajima et al., 1990; Shapiro et al., 1994), and NEM at a concentration of 50 μM can uncouple the receptors from G_i and/or G_o without disrupting agonist-receptor binding or blocking the pertussis toxin-insensitive signaling pathway (Shapiro et al., 1994). It has previously been reported that 5-HT activates 5-HT_{1A} receptors coupled to a G protein linked directly to the K^+ channel, which hyperpolarizes the dorsal raphe neurons (Penington et al., 1993; Katayama et al., 1997). To examine whether the 5-HT-activated K^+ current was affected by NEM, the medial preoptic area neurons were superfused with an external solution containing NEM for 3 min before simultaneous application of 100 nM 5-HT with NEM

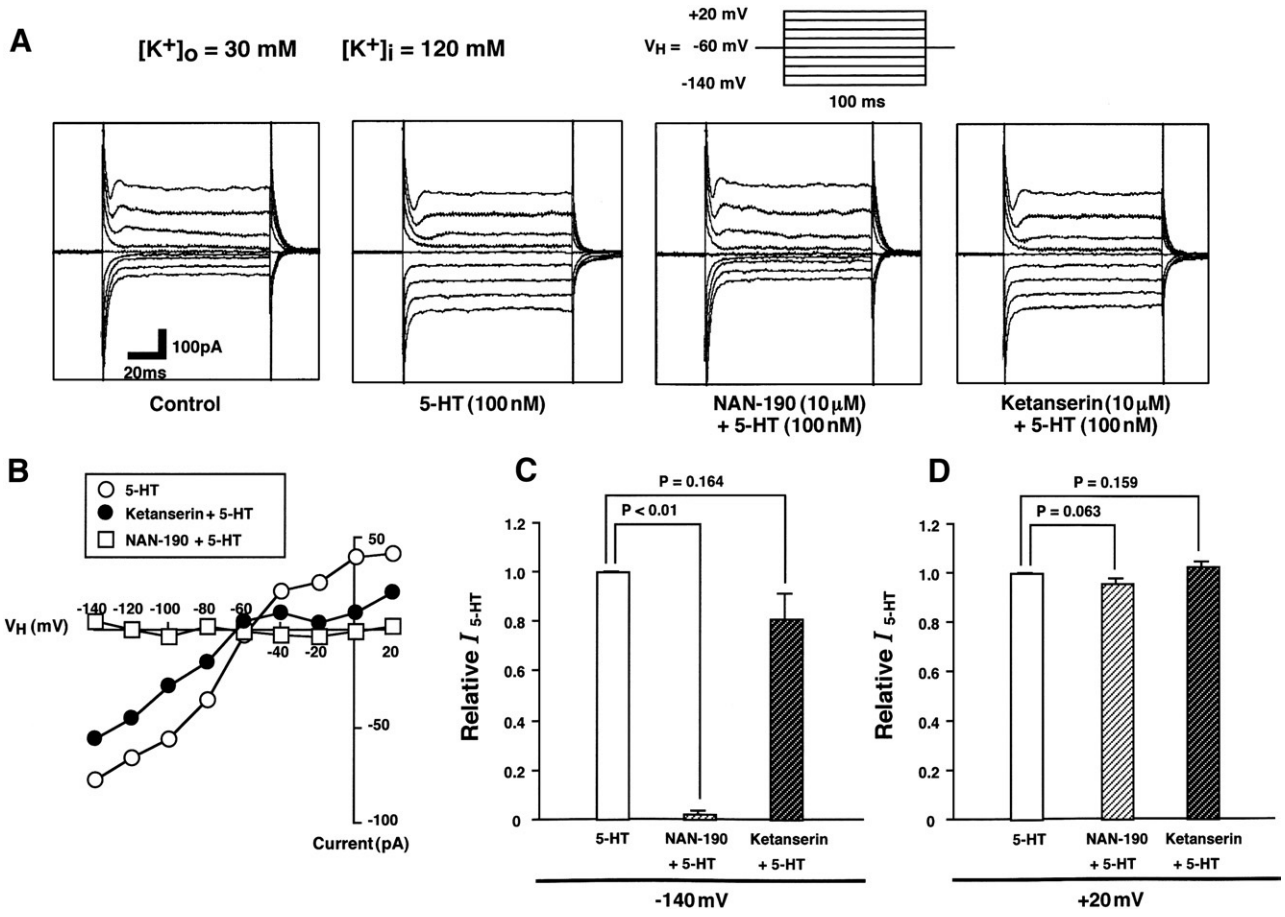

Fig. 4. Effect of 5-HT receptor antagonists on the 5-HT-activated K^+ current. All recordings were carried out in an external solution containing 30 mM K^+. At a V_H of −60 mV, voltage steps were applied from −140 mV to +20 mV in 20 mV steps for 100 ms. The neurons were pretreated for 1 min with each 5-HT antagonist before simultaneous application of 100 nM 5-HT. (A) Representative current traces showing the effects of 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN-190) or ketanserin on the 5-HT-activated K^+ current. (B) Steady-state currents plotted against voltage obtained by subtracting the current under control conditions from the current after treatment with 5-HT, ketanserin with 5-HT, and NAN-190 with 5-HT. (C) Histograms showing the effect of the 5-HT antagonists, NAN-190 or ketanserin, on the 5-HT potentiation of the inward K^+ current evoked at a V_H of −140 mV. Vertical bars indicate the S.E.M. Note that NAN-190 completely blocks the 5-HT response ($P<0.01$, $n=5$). (D) Histograms showing the effects of the 5-HT antagonists, NAN-190 and ketanserin, on the 5-HT modulation of the outward K^+ current evoked at a V_H of +20 mV. The outward K^+ current modulated by 5-HT did not show any alteration in the presence of NAN-190 ($P>0.05$, $n=5$) or ketanserin ($P>0.05$, $n=4$).

(Fig. 6A). NEM (50 μM) completely blocked the 5-HT-activated inward K^+ current (1.2±1.2% of the control, $P<0.01$, $n=5$, Fig. 6C), however, it had no effect on the outward K^+ currents (116.1±9.2% of the control, $P=0.156$, $n=5$, Fig. 6D).

4. Discussion

This study was conducted to investigate the effect of 5-HT on the medial preoptic area neuronal activity as well as the electrophysiological mechanism by which the 5-HT action occurs. 5-HT hyperpolarized the medial preoptic area neurons via activation of an inwardly rectifying K^+ channel that was barium sensitive and coupled to the 5-HT_{1A} receptors through a G-protein-mediated mechanism. Thirty percent of the medial preoptic area neurons tested were 5-HT responsive cells, whereas the remaining 70% did not respond to treatment with 5-HT. However, because the neurons were isolated by the enzyme treatments, it is possible that the negative response to 5-HT was due to the alterations of surface proteins, membrane receptors, the agonist binding affinity, and the receptor sensitivity.

Inward rectifying K^+ channels, which are expressed in a wide range of cells, play a major role in maintaining the resting membrane potential and regulating the duration of the action potential. In many central neurons, 5-HT activates an inwardly rectifying K^+ current, which leads to hyperpolarization and inhibition of the neuronal excitability (Andrade et al., 1986; Penington et al., 1993; Oh et al., 1995; Bayliss et al., 1997; Katayama et al., 1997; Larkman and Kelly, 1998; Sodickson and Bean, 1998; Takigawa and Alzheimer, 1999; Jeong et al., 2001). In this study, the medial preoptic area neurons exhibited a slow rate of spontaneous firing under current-clamp conditions, however the extracellular application of 5-HT produced a hyperpolarization of the membrane potential and reduced the firing frequencies (Fig. 1). Under voltage-clamp conditions, 5-HT reversed the potential of the I–V curve near the K^+ equilibrium potential and increased the slope of the I–V curve

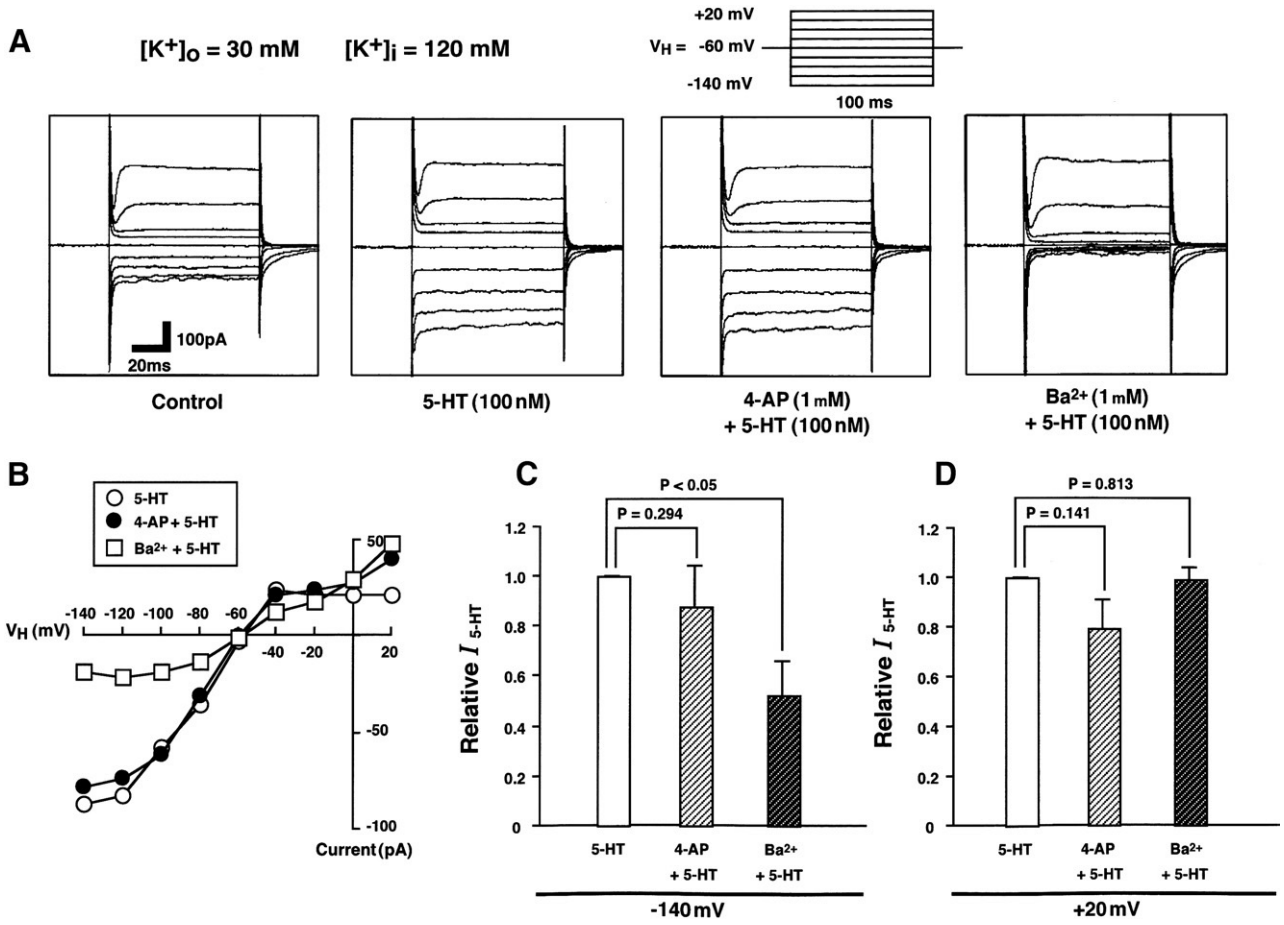

Fig. 5. Effects of K^+ channel blockers on the 5-HT-activated K^+ current. All recordings were conducted at a V_H of −60 mV in an external solution containing 30 mM of K^+. Voltage steps were applied from −140 mV to +20 mV in 20 mV steps for 100 ms. The neurons were pretreated for 1 min with each K^+ channel blocker before simultaneous application of 100 nM 5-HT. (A) Representative current traces showing the blocking effects of 1 mM 4-AP and Ba^{2+} on the 5-HT-activated K^+ current. (B) Steady-state currents plotted against voltage obtained by subtracting the current under control conditions from the current after treatment with 5-HT, 4-AP with 5-HT, and Ba^{2+} with 5-HT. (C) Histograms showing the blocking effects of 4-AP and Ba^{2+} on the 5-HT-activated inward K^+ current evoked at a V_H of −140 mV. Vertical bars indicate the S.E.M. Note that Ba^{2+} significantly blocks the effect of 5-HT on the inward K^+ current ($P<0.05$, $n=6$). (D) Histograms showing the effects of 4-AP and Ba^{2+} on the 5-HT modulation of the outward K^+ current evoked at a V_H of +20 mV. The outward K^+ currents did not change in the presence of 4-AP ($P>0.05$, $n=6$) or Ba^{2+} ($P>0.05$, $n=6$).

(Fig. 2). In addition, 5-HT activated inward currents, which were significantly blocked by Ba^{2+} (Fig. 5). These results indicate that 5-HT inhibits the medial preoptic area neuronal activity by activating inwardly rectifying K^+ channels.

In mammalian central neurons, 5-HT_{1A} receptors are usually involved in the 5-HT activation of inwardly rectifying K^+ currents (Andrade et al., 1986; Penington et al., 1993; Oh et al., 1995; Bayliss et al., 1997; Katayama et al., 1997; Larkman and Kelly, 1998; Sodickson and Bean, 1998; Takigawa and Alzheimer, 1999; Jeong et al., 2001). Furthermore, inwardly rectifying K^+ channels activated by agonists or neurotransmitters in central neurons are usually mediated by pertussis toxin-sensitive G proteins (Andrade et al., 1986; Trussell and Jackson, 1987; Inoue et al., 1988; Williams et al., 1988; Penington et al., 1993; Oh et al., 1995; Velimirovic et al., 1995; Bayliss et al., 1997; Katayama et al., 1997; Han et al., 1999; Jeong et al., 2001). A high density of both 5-HT_{1A} and 5-HT_{1B} receptor subtypes is found in the medial preoptic area (Pazos and Palacios, 1985). In this study, 5-HT-induced hyperpolarization was mimicked by 8-OH-DPAT, a 5-HT_{1A} receptor agonist and was antagonized by NAN-190, a 5-HT_{1A} receptor antagonist (Figs. 3 and 4). These results indicate that 5-HT-induced inhibition occurs as a result of action on the 5-HT_{1A} receptors. In addition, the application of NEM, an agent that uncouples the binding between pertussis toxin-sensitive G proteins and the receptors, completely suppressed the 5-HT-activated K^+ current (Fig. 6). Thus, the present study indicates that activation of the 5-HT_{1A} receptors in the medial preoptic area neurons inhibits the neuronal excitability via activation of GIRK channels.

Many *in vivo* studies have indicated that the medial preoptic area is closely associated with sexual functions. Sexual behavior was suppressed by lesions in the medial preoptic area (Liu et al., 1997; Paredes et al., 1998), but enhanced by stimulation of the medial preoptic area regions (Rodriguez-Manzo et al., 2000). The effect of 5-HT on the medial preoptic area is generally believed to be inhibitory to sexual behavior (Bitran and Hull, 1987; Verma et al., 1989; Gorzalka et al., 1990; Fernandez-Guasti et al., 1992; Gitlin, 1994; Hull et al., 2004), which is supported by the results of this *in vitro* study, which indicate that 5-HT inhibits the excitability of isolated medial preoptic

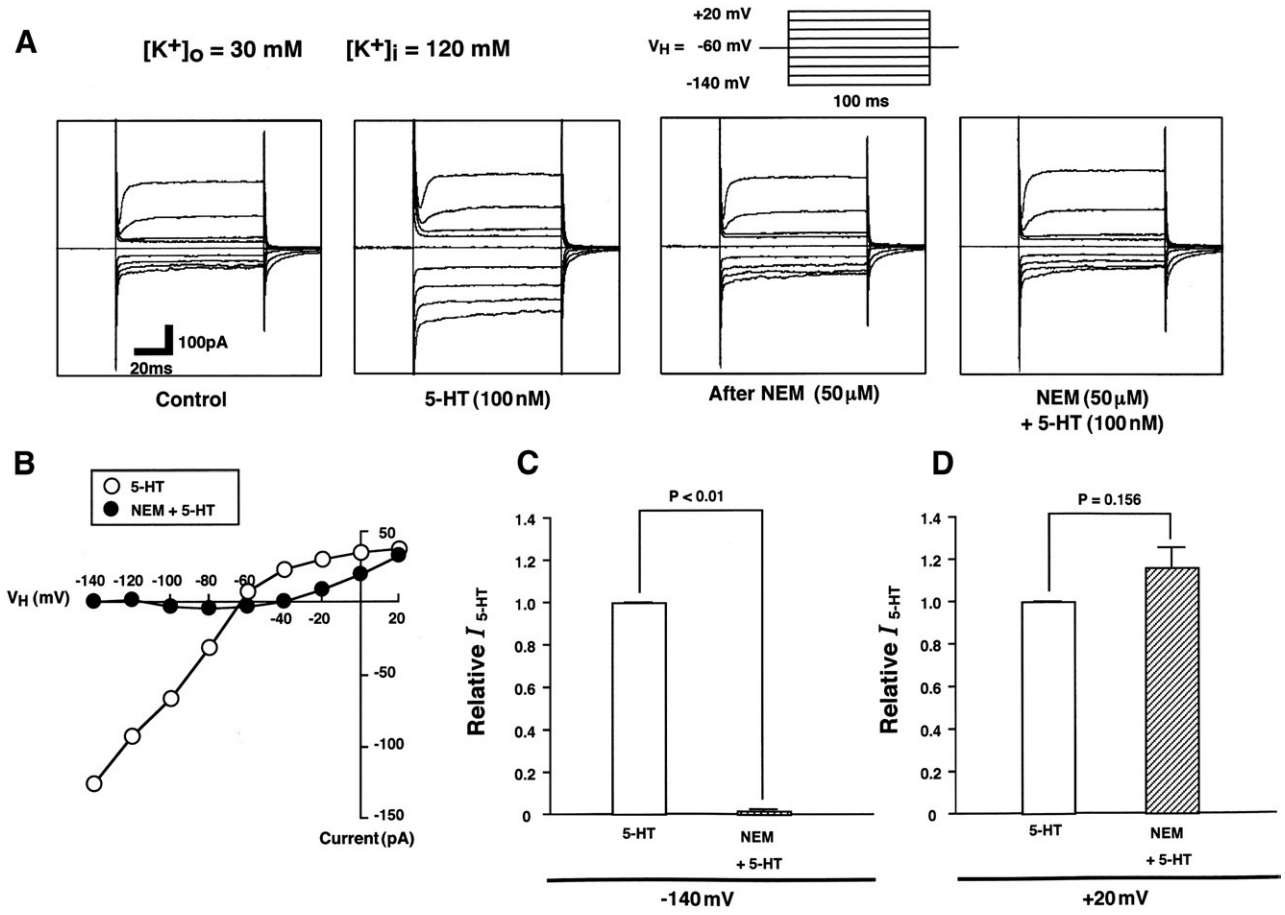

Fig. 6. The effect of *N*-ethylmaleimide on the 5-HT-activated K^+ current. All recordings were carried out at a V_H of −60 mV in an external solution containing 30 mM of K^+. Voltage steps were applied from −140 mV to +20 mV in 20 mV steps for 100 ms. The neurons were pretreated for 3 min with NEM before simultaneous application of 100 nM 5-HT. (A) Representative current traces showing the inhibitory effects of 50 μM NEM on the 5-HT-activated K^+ current. (B) Steady-state currents plotted against voltage obtained by subtracting the current under control conditions from the current after treatment with 5-HT and NEM with 5-HT. (C) Histograms showing the blocking effect of NEM on the 5-HT-activated inward K^+ current evoked at a V_H of −140 mV. Vertical bars indicate the S.E.M. Note that NEM completely blocks the effect of 5-HT on the inward K^+ current ($P<0.01$, $n=5$). (D) Histograms showing the effect NEM on the 5-HT modulation of the outward K^+ current evoked at a V_H of +20 mV. NEM did not alter the outward K^+ currents ($P>0.05$, $n=5$).

area neurons via hyperpolarization of the neuronal membrane potential. The inhibition of the medial preoptic area neuronal excitability by 5-HT might explain the mechanism of 5-HT-induced inhibition of sexual activity.

Some *in vivo* studies have shown that local application of 5-HT or selective 5-HT agonists to the medial preoptic area inhibits or facilitates male sexual behavior in rats (Verma et al., 1989; Fernandez-Guasti et al., 1992) and that 5-HT can facilitate and inhibit sexual behavior according to the receptor subtypes (Fernandez-Guasti et al., 1992). However, the relationship between sexual activity and 5-HT subtypes is not clear. Recently, we have investigated the effect of 5-HT on GABAergic and glutamatergic synaptic transmission in mechanically isolated single medial preoptic area neurons retaining their synaptic boutons, and we have demonstrated that 5-HT inhibits both GABAergic and glutamatergic synaptic input to the medial preoptic area via 5-HT_{1A} and 5-HT_{1B} receptors, respectively (Lee et al., 2008). Therefore, we have suggested that 5-HT can inhibit or facilitate neuronal excitability of the medial preoptic area and sexual behavior, which can be determined based on the subtypes that respond to 5-HT released from 5-HT input terminals. In the present study, however, we have found that 5-HT hyperpolarized the medial preoptic area neurons by the activation of the G-protein-coupled inwardly rectifying K^+ currents via 5-HT_{1A} receptors. As a result, we can suggest that serotonin activates 5-HT_{1A} receptors of the medial preoptic area neurons and inhibits the neuronal excitability, which may be a mechanism involving the inhibitory effect of 5-HT on sexual behavior.

Acknowledgement

This study was supported by the Kyung Hee University Research Fund in 2005 (KHU-20050425).

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