Characterization of Outward Currents Induced by 5-HT in Neurons of Rat Dorsolateral Septal Nucleus

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Yamada, Kei, Hiroshi Hasuo, Masaru Ishimatsu, and Takashi Akasu. Characterization of outward currents induced by 5-HT in neurons of rat dorsolateral septal nucleus. J Neurophysiol 85: 1453–1460, 2001. Properties of the 5-hydroxytryptamine (5-HT)-induced current (I_5,HT) were examined in neurons of rat dorsolateral septal nucleus (DLSN) by using whole cell patch-clamp techniques. I_5,HT was associated with an increase in the membrane conductance of DLSN neurons. The reversal potential of I_5,HT was $-93 \pm 6$ (SE) mV ($n = 7$) in the artificial cerebrospinal fluid (ACSF) and was changed by 54 mV per decade change in the external K$^+$ concentrations, indicating that I_5,HT is carried exclusively by K$^+$. Voltage dependency of the K$^+$ conductance underlying I_5,HT was investigated by using current-voltage relationship. I_5,HT showed a linear I-V relation in 63%, inward rectification in 21%, and outward rectification in 16% of DLSN neurons. (±)-8-Hydroxy-dipropylaminotetralin hydrobromide (30 µM), a selective 5-HT_1A receptor agonist, also produced outward currents with three types of voltage dependency. Ba$^{2+}$ (100 µM) blocked the inward rectifier I_5,HT but not the outward rectifier I_5,HT. In I_5,HT with linear I-V relation, blockade of the inward rectifier K$^+$ current by Ba$^{2+}$ (100 µM) unmasked the outward rectifier current in DLSN neurons. These results suggest that I_5,HT with linear I-V relation is the sum of inward rectifier and outward rectifier K$^+$ currents in DLSN neurons. Intracellular application of guanosine-5’-O-(3-thiotriphosphate) (300 µM) and guanosine-5’-O-(2-thiodiphosphate) (5 mM), blockers of G protein, irreversibly depressed I_5,HT. Protein kinase C (PKC) $\alpha$ and $\beta$ isomers (300 µM), a selective PKC inhibitor, depressed the outward current I_5,HT, but not the inward rectifier I_5,HT. I_5,HT was depressed by N-ethylmaleimide, which uncouples the G-protein-coupled receptor from pertussis-toxin-sensitive G proteins. H-89 (10 µM) and adenosine 3’5’-cyclic monophosphothioate Rp-isomer (300 µM), protein kinase A inhibitors, did not depress I_5,HT. Phorbol 12-myristate 13-acetate (10 µM), an activator of PKC, produced an outward rectifying K$^+$ current. These results suggest that both 5-HT-induced inward and outward rectifying currents are mediated by different G proteins and that PKC is probably involved in the transduction pathway of the outward rectifying I_5,HT in DLSN neurons.

INTRODUCTION

Lateral septal neurons have been known to receive afferents containing 5-hydroxytryptamine (5-HT: serotonin) from the dorsal and medial raphe nuclei (Gall and Moore 1984; Gallagher et al. 1995; Jakab and Leranth 1995; Köhler et al. 1982). 5-HT receptors, especially those of the 5-HT_1A subtype, have been demonstrated by in vitro autoradiography with the highest levels in the lateral septum (Biegon et al. 1982; Hensler et al. 1991; Marcinkiewicz et al. 1984; Pazos and Palacios 1985; Vergé et al. 1986). Molecular cloning has established that 5-HT_1A receptors belong to the superfamily of the G-protein-coupled receptor (Albert et al. 1990; Fargin et al. 1989). In the lateral septum, Sim et al. (1997) have demonstrated that stimulation of 5-HT_1A receptors increases guanosine-5’-O-(3-thiotriphosphate) (GTPγS) binding. In situ hybridization revealed a substantial distribution of messenger RNA for the G-protein-coupled inward rectifier K$^+$ channel (GIRK1-4) in the lateral septum of the rat brain (Karschin et al. 1996).

Electrophysiological studies have shown that 5-HT produces a hyperpolarizing response associated with an increase in K$^+$ conductance in neurons of the rat dorsolateral septal nucleus (DLSN) (Goto et al. 1997; Joëls and Gallagher 1988; Joëls et al. 1986, 1987) through 5-HT_1A receptors (Joëls and Gallagher 1988; Yamada et al. 2000). Much evidence has accumulated suggesting that 5-HT produces an inward rectifier K$^+$ current by directly activating G protein in neurons of the dorsal raphe nucleus and the hippocampus (Bayliss et al. 1997; Katayama et al. 1997; Pan et al. 1993; Penington et al. 1993a,b). In the DLSN, however, the 5-HT-induced hyperpolarization seems to be voltage independent in either normal artificial cerebrospinal fluid (ACSF) or high K$^+$ ACSF (Joëls and Gallagher 1988; Joëls et al. 1986, 1987). Our preliminary data showed that the 5-HT-induced K$^+$ current was accompanied by inward rectification in DLSN neurons (Yamada et al. 2000). However, Ba$^{2+}$, a blocker for GIRK channels (North 1989), produced only partial depression of the outward current mediated by 5-HT_1A receptors in DLSN neurons (Yamada et al. 2000). The purpose of the present study is to characterize, in detail, the K$^+$ conductance underlying the 5-HT-induced outward current in DLSN neurons by using whole cell patch-clamp methods.

METHODS

Brain slices containing the septal nucleus were obtained from rats in a manner described previously (Stevens et al. 1984). Male Wistar rats, 80–150 g, were killed by decapitation, and their brains were rapidly removed and immersed for 8–10 s in cooled ACSF (4–6°C) that was prebubbled with 95% O$_2$–5% CO$_2$. Transverse slices (400 µm in thickness) were cut with a Vibroslice (Campden Instruments) and left to recover for 1 h in oxygenated ACSF at room temperature.

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The slice was then transferred to a recording chamber and submerged in ACSF at 32–33°C. The composition of the ACSF was as follows (in mM): 117 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 25 NaHCO₃, 1.2 NaHPO₄, and 11 d-glucose (pH 7.4 and 295–305 mOsM). Whole cell tight-seal recordings were made from DLSN neurons using the slice patch technique (Blandon et al. 1989; Coleman and Miller 1989). Patch pipettes were filled with the following internal solution (mM): 122 K-glucuronate, 5 NaCl, 0.3 CaCl₂, 2 MgCl₂, 1 ethylene glycol-bis(β-aminoethyl ether)-N,N‘,N‘,N‘-tetraacetic acid (EGTA), 10 N-(2-hydroxyethyl)pyperazine-N‘-N′-ethanesulfonic acid (HEPES), 5 Na₂ATP, and 2 GTP-Na (pH 7.2 adjusted by KOH, 280 mOsM). The tip resistance of the whole cell patch-pipette was 4–5 MΩ. In some experiments, GTP was substituted with 300 μM GTP-Y-S and 5 mM guanosine-5’-O-(2-thiodiphosphate) (GDPβS). Membrane potential and current were recorded with an Axoclamp-2B amplifier. During the whole cell voltage-clamping, sample frequencies were between 5 and 6 kHz and the amplifier gain was 0.8–2.5 nA/mV. Voltage and current were monitored continuously with a memory oscilloscope (Nihon-Kohden, RTA-1100). The pClamp system (Axon Instruments) operating on an IBM-AX computer (Gateway 2000) was used to analyze the membrane potentials and currents.

Of the drugs used, GTP, GTP-Y-S, GDPβS, N-ethylmaleimide (NEM), tetrodotoxin (TTX), adenosine triphosphate (ATP) disodium salt, forskolin, phorbol 12-myristate 13-acetate (PMA), EGTA, N6,2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate (db-cAMP), adenosine 3′,5′-cyclic monophosphohioate Rp-isomer (Rp-cAMPs), and glibenclamide were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO). Tetraethylammonium (TEA) chloride was purchased from Tokyo Kasei. (±)-8-Hydroxy-dipropylaminotetralin hydrobromide (8-OH-DPAT) was purchased from RBI (Natick, MA). 5-HT creatinine sulfate complex was from Wako Pure Chemical Ind. (Osaka, Japan). N2-(p-bromocinnamylamino)ethyl-5-isoquinolinesulfonamide, HCl (H-89) was purchased from Calbiochem-Novabiochem (La Jolla, CA). Protein kinase C (PKC) 19–36 was from Biochem (La Jolla, CA). Glibenclamide was dissolved in dimethyl sulfoxide (DMSO) and added to the ACSF, where the final concentration of DMSO (0.1%) had no direct effect on DLSN in dimethyl sulfoxide (DMSO) and added to the ACSF, where the final concentration of DMSO (0.1%) had no direct effect on DLSN.

**Results**

5-HT causes an outward current in DLSN neurons

Neurons in the rat DLSN had resting membrane potential of −61 ± 4 mV (n = 62) and input resistance of 132 ± 11 MΩ (n = 62). DLSN neurons were voltage-clamped with a whole cell configuration at −60 mV. Bath-application of 5-HT (10 μM) caused an outward current (Iₒ,HT) in DLSN neurons (Fig. 1A). Membrane currents produced by applying either ramp potentials or step command potentials with duration of 300 ms were increased by 5-HT, suggesting an increased membrane conductance of DLSN neurons (Fig. 1A). Figure 1B shows the reversal potential of Iₒ,HT examined by changing the holding membrane potential in a DLSN neuron (Fig. 1B). Iₒ,HT increased in amplitude at depolarized membrane potentials, while it decreased when the membrane was hyperpolarized and reversed its polarity at −86 mV (Fig. 1C, ○). In this particular neuron, Iₒ,HT appeared to be voltage independent in the normal ACSF. In the same cell, the reversal potential of Iₒ,HT shifted to a hyperpolarizing membrane potential in 1 mM K⁺ solution (Fig. 1C, □). An increase in the concentration of external K⁺ to 9.7 and 20 mM shifted the reversal potential of Iₒ,HT to depolarized membrane potentials (Fig. 1C, △ and △, respectively). Iₒ,HT exhibited weak inward rectification in these high K⁺ solutions. Pooled data showed that the reversal potential of Iₒ,HT measured in ACSF (containing 4.7 mM K⁺), was −93 ± 6 mV (n = 8; Fig. 1D), which is close to the equilibrium potential of K⁺ in DLSN neurons. In ACSF containing 1, 9.7, and 20 mM K⁺, the reversal potentials of Iₒ,HT were −123 ± 5 mV (n = 6), −73 ± 4 mV (n = 4), and −53 ± 4 mV (n = 6), respectively. The relationship between the reversal potential of Iₒ,HT and the concentration of extracellular K⁺ had a slope of 54 mV for 10-fold change in external K⁺ concentration (Fig. 1D). This is identical to the expected value of the equilibrium potential for K⁺ by the Nernst equation.

Properties of the outward current induced by 5-HT

The voltage dependency of the K⁺ conductance underlying Iₒ,HT was investigated, in detail, in DLSN neurons. Figure 2A shows a sample record of Iₒ,HT obtained from a DLSN neuron.
Increased the amplitude of $I_{5-HT}$ (63%) of 97 DLSN neurons, where the amplitude of net $I_{5-HT}$ showed no obvious depression of the $I_{5-HT}$. At $-50$ mV, the amplitudes of $I_{5-HT}$ were $91 \pm 7$ pA ($n = 6$) and $80 \pm 6$ pA ($n = 6$) in the absence and the presence of Ba$^{2+}$ (100 $\mu$M), respectively. At $-130$ mV, the amplitudes of $I_{5-HT}$ were $-29 \pm 4$ pA ($n = 5$) and $-24 \pm 3$ pA ($n = 5$) in the absence and the presence of Ba$^{2+}$ (100 $\mu$M), respectively. $I_{5-HT}$ having characteristic inward rectification was seen in $20$ (21%) cells out of a total of 97 neurons in the rat DLSN (Fig. 3C). The amplitude of the inward rectifier $I_{5-HT}$ was $42 \pm 5$ pA ($n = 8$) at $-60$ mV. Ba$^{2+}$ (100 $\mu$M) preferentially depressed $I_{5-HT}$ at hyperpolarized membrane potentials. Pooled data showed that the amplitude of $I_{5-HT}$ was depressed from $52 \pm 6$ pA ($n = 5$) to $44 \pm 8$ pA ($n = 5$) by Ba$^{2+}$ (100 $\mu$M) at $-50$ mV. There was no statistically significant difference between these two data. When recorded

Effects of Ba$^{2+}$ on outward and inward rectifier currents

In $16$ (16%) of 97 neurons, the application of $5-HT$ increased the membrane conductance at potentials more positive than $-70$ mV (Fig. 3A). $I_{5-HT}$ reversed polarity at $-83$ mV in this neuron (Fig. 3Aa). The net $I_{5-HT}$ obtained by subtraction of the control $I-V$ curve from that recorded in the presence of $5-HT$ (10 $\mu$M) showed clear outward rectification (Fig. 3Ab). Figure 3B shows the effect of Ba$^{2+}$ (100 $\mu$M) on the outward rectifier $I_{5-HT}$ in a DLSN neuron. Ba$^{2+}$ (100 $\mu$M) caused no obvious depression of the $I_{5-HT}$. At $-50$ mV, the amplitudes of $I_{5-HT}$ were $91 \pm 7$ pA ($n = 6$) and $80 \pm 6$ pA ($n = 6$) in the absence and the presence of Ba$^{2+}$ (100 $\mu$M), respectively. At $-130$ mV, the amplitudes of $I_{5-HT}$ were $-29 \pm 4$ pA ($n = 5$) and $-24 \pm 3$ pA ($n = 5$) in the absence and the presence of Ba$^{2+}$ (100 $\mu$M), respectively. $I_{5-HT}$ having characteristic inward rectification was seen in $20$ (21%) cells out of a total of 97 neurons in the rat DLSN (Fig. 3C). The amplitude of the inward rectifier $I_{5-HT}$ was $42 \pm 5$ pA ($n = 8$) at $-60$ mV. Ba$^{2+}$ (100 $\mu$M) preferentially depressed $I_{5-HT}$ at hyperpolarized membrane potentials. Pooled data showed that the amplitude of $I_{5-HT}$ was depressed from $52 \pm 6$ pA ($n = 5$) to $44 \pm 8$ pA ($n = 5$) by Ba$^{2+}$ (100 $\mu$M) at $-50$ mV. There was no statistically significant difference between these two data. When recorded

The current-voltage relationship ($I-V$ curve) constructed by step command potentials increased its slope in the presence of 5-HT (Fig. 2B). The component of current activated by 5-HT (net $I_{5-HT}$) was obtained by digital subtraction of the control $I-V$ curve from that recorded in the presence of 5-HT (10 $\mu$M). The net $I_{5-HT}$ showed no obvious rectification in this particular neuron (Fig. 2C). $I_{5-HT}$ with linear $I-V$ relation was seen in 61 (63%) of 97 DLSN neurons, where the amplitude of $I_{5-HT}$ was $108 \pm 5$ pA ($n = 40$) at the holding membrane potential of $-60$ mV. Ba$^{2+}$, at a micromolar concentration, has been reported to block selectively the inward rectifier K$^+$ current in various central neurons (North 1989). Figure 2D shows the effect of Ba$^{2+}$ (100 $\mu$M) on $I_{5-HT}$ with linear $I-V$ relation in a DLSN neuron. In this neuron, Ba$^{2+}$ (100 $\mu$M) markedly depressed $I_{5-HT}$ at potentials more negative than $-100$ mV. However, the depression was less marked at depolarized membrane potentials. $I_{5-HT}$ that remained in the presence of Ba$^{2+}$ (100 $\mu$M) exhibited outward rectification (Fig. 2Da, ●). In contrast, the Ba$^{2+}$-sensitive $I_{5-HT}$ exhibited inward rectification (Fig. 2Db). The pooled data show that Ba$^{2+}$ (100 $\mu$M) decreased the amplitude of $I_{5-HT}$ from $135 \pm 8$ pA ($n = 8$) to $32 \pm 4$ pA ($n = 8$) at $-130$ mV (Fig. 2E, $P < 0.01$). At $-50$ mV, the amplitudes of $I_{5-HT}$ were $121 \pm 6$ pA ($n = 8$) and $112 \pm 6$ pA ($n = 8$) in the absence and the presence of Ba$^{2+}$ (100 $\mu$M), respectively. This difference was statistically not significant. These results suggest that $I_{5-HT}$ with linear $I-V$ relation is composed of a Ba$^{2+}$-sensitive, inward rectifier and a Ba$^{2+}$-insensitive, outward rectifier K$^+$ currents in DLSN neurons.
at $-130 \text{ mV}$, the amplitude of $I_{\text{S-HT}}$ was depressed from $-151 \pm 7 \text{ pA (n = 5)}$ to $-23 \pm 5 \text{ pA (n = 5)}$ in the presence of Ba$^{2+}$ (100 $\mu$M; Fig. 3D). Ba$^{2+}$-sensitive inward rectifier $I_{\text{S-HT}}$ has been shown previously in LSN neurons (Yamada et al. 2000).

We examined the effects of other K$^+$ channel blockers on $I_{\text{S-HT}}$ in DLSN neurons. 5-HT (100 $\mu$M) produced outward current with amplitude of $121 \pm 5 \text{ pA (n = 6)}$ at a potential of $-60 \text{ mV}$ in ACSF containing $1 \mu$M TTX, 0 mM Ca$^{2+}$ (with 2 mM EGTA), Cs$^+$ (2 mM), and 20 mM TEA. Glibenclamide (100 $\mu$M), a blocker of the ATP-regulated K$^+$ channel, and extracellular Cs$^+$ (1 mM), a blocker of nonselective cation channels ($I_Q$) (Halliwell and Adams 1982) and $I_h$ (Bobker and Williams 1989), did not depress the $I_{\text{S-HT}}$ in DLSN neurons ($n = 4$). It has been reported that the M current is an outward rectifier K$^+$ current that is controlled by muscarinic receptors in central and peripheral neurons (Brown 1990). However, hyperpolarizing command potentials (from $-40$ to $-80 \text{ mV}$) did not produce the time-dependent current relaxation that is the characteristic feature of the M current in either the presence or the absence of 5-HT (100 $\mu$M).

**Effects of 8-OH-DPAT on the membrane current in DLSN neurons**

8-OH-DPAT (30 $\mu$M), a selective agonist for the 5-HT$_{1A}$ receptor (Cervo and Samanin 1987; Kennett et al. 1987), also produced an outward current in DLSN neurons (Fig. 4A). The onset and recovery of the 8-OH-DPAT-induced outward current were slower than those of $I_{\text{S-HT}}$. The mean amplitude of 8-OH-DPAT (30 $\mu$M)-induced outward current was $58 \pm 8 \text{ pA (n = 20)}$ at $-60 \text{ mV}$. The outward current induced by 8-OH-DPAT (30 $\mu$M) also showed different voltage dependency in individual neurons (Fig. 4B). 8-OH-DPAT-induced current was associated with inward rectification in 6 neurons, outward rectification in 4 neurons, and linear I-V relation in 14 neurons of a total of 24 neurons. The proportion of the three types was similar as in the case of 5-HT.

**G protein mediates $I_{\text{S-HT}}$ in DLSN neurons**

To inhibit the activity of G-protein in DLSN neurons, the patch-pipette solution contained GTP$\gamma$S (300 $\mu$M) or GDP$\beta$S (5 mM) instead of GTP (300 $\mu$M). First, we confirmed that 5-HT consistently produced the outward current for at least 80 min under the whole cell patch-clamp recording in intact neurons (Fig. 5B). The first application of 5-HT (50 $\mu$M) produced a typical outward current with amplitude of 120 pA at $-60 \text{ mV}$ in a DLSN neuron internally dialyzed with GTP$\gamma$S (300 $\mu$M) for 5 min (Fig. 5Aa). When 5-HT was applied again 10 min after first application, $I_{\text{S-HT}}$ did not completely recover but gradually shifted in the outward direction even after removal of 5-HT from the ACSF (Fig. 5Ab) probably because of continuous diffusion of GTP$\gamma$S into the intracellular space. $I_{\text{S-HT}}$ decreased in amplitude by more than 85% of control 30 min after the first application of 5-HT (Fig. 5Ad). Intracellular application of GTP$\gamma$S for 40 min irreversibly depressed $I_{\text{S-HT}}$ (Fig. 5B). Figure 5B also shows the effect of GDP$\beta$S on the $I_{\text{S-HT}}$ in DLSN neurons. Intracellular application of GDP$\beta$S (5 mM) for 60 min via the patch pipette strongly depressed the amplitude of $I_{\text{S-HT}}$. No recovery of $I_{\text{S-HT}}$ was seen as long as whole cell recording was continued. It has been reported that
Effect of protein kinase inhibitors on \( I_{5-HT} \) in DLSN neurons

To study the contribution of PKC to \( I_{5-HT} \), DLSN neurons were dialyzed with an internal solution containing PKC 19-36 (20 \( \mu\)M), a specific peptide inhibitor for PKC that is a pseudosubstrate peptide in the regulatory domain of PKC (House and Kemp 1987). Figure 6A shows \( I_{5-HT} \) taken 5 min after dialysis of a DLSN neuron with a pipette solution containing PKC 19-36 (20 \( \mu\)M). 5-HT (10 \( \mu\)M) increased the membrane conductance at all membrane potentials tested. The net \( I_{5-HT} \) obtained by subtraction of the 2 \( I-V \) curves in Aa: pooled data for the PKC 19-36-induced depression of the \( I_{5-HT} \) recorded 5 min after the beginning of the whole cell recording. B: the PKC 19-36-sensitive outward rectifier \( I_{5-HT} \) obtained by subtraction of the 2 \( I-V \) curves in Ba: c pooled data for the amplitudes of the outward rectifier \( I_{5-HT} \) recorded at −40 mV (○) and −130 mV (■). Bc: the inward rectifier \( I_{5-HT} \) recorded 5 min (○) and 20 min (■) after beginning of the whole cell recording. The PKC 19-36-sensitive inward current component in the inward rectifier \( I_{5-HT} \) obtained by subtraction of the 2 \( I-V \) curves in Bc: pooled data for the PKC 19-36-induced depression of the inward rectifier \( I_{5-HT} \). Amplitudes of \( I_{5-HT} \) were recorded at −40 mV (○) and −130 mV (■). In A and B, number of experiments is shown in parentheses. *, the statistical significance (\( P < 0.01 \)), n.s., statistically not significant. HP, holding potential. | on each column indicates SE of mean.

net \( I_{5-HT} \) obtained by subtraction of the control \( I-V \) curve from that taken in the presence of 5-HT (10 \( \mu\)M) showed a linear \( I-V \) relation in this neuron (Fig. 6Ab). The net \( I_{5-HT} \) was also recorded in the same neuron internally dialyzed with PKC 19-36 for 20 min (Fig. 6B). 5-HT (10 \( \mu\)M) produced an inward rectifier \( K^+ \) current in this neuron at this time. The PKC-sensitive \( I_{5-HT} \), obtained by subtraction of the control \( I_{5-HT} \) from that recorded after 20 min of dialysis with PKC 19-36, exhibited outward rectification (Fig. 6C). Statistical data showed that PKC 19-36 (20 \( \mu\)M) reduced the amplitude of \( I_{5-HT} \) with linear \( I-V \) relation from 148 ± 7 (\( n = 5 \)) to 61 ± 4 pA (\( n = 5 \)) at −40 mV (Fig. 6D, \( P < 0.01 \)). In contrast, the amplitudes of linear \( I_{5-HT} \) were −163 ± 8 (\( n = 5 \)) and −158 ± 11 pA (\( n = 5 \)) in the absence and the presence of PKC 19-36 (20 \( \mu\)M), respectively, at −130 mV (Fig. 6D); there was no statistically significant difference between these two sets of data.

Figure 7A shows the effects of PKC 19-36 (20 \( \mu\)M) on the outward rectifier \( I_{5-HT} \) in a DLSN neuron. When 5-HT (10 \( \mu\)M) was applied to the bath solution 5 min after the beginning of whole cell recording, a typical outward current
with amplitude of 132 ± 6 pA (n = 5) at −40 mV (Fig. 7Aa, ○). However, in LSN neurons internally treated with PKC 19-36 (20 μM) for 20 min, 5-HT (10 μM) produced only a 23 ± 2 pA (n = 5) at −40 mV (Fig. 7Aa, ●). Thus PKC 19-36 (20 μM) in the pipette solution depressed I5-HT by about 81% (n = 5) at a potential −40 mV. The PKC 19-36-sensitive current of I5-HT showed outward rectification (Fig. 7Ab). In contrast, at the holding potential of −130 mV, amplitudes of I5-HT were −41 ± 6 pA (n = 5) and −36 ± 8 pA (n = 5) when recorded 5 and 20 min after the application of PKC 19-36, respectively. The difference between these two sets of data were statistically not significant. The effect of PKC 19-36 on the inward rectifier I5-HT was not significantly depress the inward rectifier I5-HT (Fig. 7B, a and b). Pooled data from five neurons showed that amplitudes of I5-HT at −40 and −130 mV were 194 ± 11 and 175 ± 13 pA in the presence and the absence of PKC 19-36 (20 μM), respectively. At −130 mV, they were 50 ± 4 (n = 5) and 47 ± 5 pA (n = 5) in the absence and the presence of PKC 19-36 (20 μM). There were statistically no significance between two sets of data taken at potentials of either −40 or −130 mV (Fig. 7Bc). These results suggest that PKC 19-36 preferentially depresses the outward rectifier I5-HT.

The effects of protein kinase A (PKA) inhibitors on I5-HT were examined in DLSN neurons. H-89 (10 μM), a membrane permeable and selective inhibitor of PKA (Chijiwa et al. 1990), was applied to the ACSF for 10–20 min. H-89 (10 μM) did not significantly depress the 5-HT-induced outward current in DLSN neurons (n = 4). Rp-cAMPs (300 μM), a membrane permeable cAMP analogue that is known to be a PKA inhibitor, also produced no significant depression of the amplitude of I5-HT (n = 4). Both inward and outward rectifier I5-HT were not changed by H-89 and Rp-cAMPs (n = 5).

**Effects of protein kinase activators on the membrane current in DLSN neurons**

The results obtained with PKC 19-36 strongly suggest the PKC mediated the activation of the outward rectifier by 5-HT. It may be expected, therefore that a PKC activator would turn on a K+ current with similar voltage dependence. Therefore the effect of PMA, an activator of PKC, on the membrane current was examined in DLSN neurons (Fig. 8). PMA (10 μM) was applied to the intracellular space of DLSN neurons through a patch pipette. Immediately after completion of the whole cell patch-clamp recording, bath-application of 5-HT (10 μM) produced an outward current with linear I-V relation (Fig. 8ab). In the same cell, intracellular application of PMA (10 μM) for 20 min produced an outward current with amplitude of 75 ± 5 pA (n = 6) at −60 mV. The PMA-induced current reversed polarity at −84 ± 5 mV (n = 5; Fig. 8Ba). Amplitudes of the PMA-induced current recorded at −40 and −130 mV were 134 ± 6 (n = 6) and −23 ± 7 pA (n = 6), respectively (Fig. 8C). These results indicate that PKC produces outward rectifier K+ current in DLSN neurons. By contrast, forskolin (10 μM), an activator of PKA, and db-cAMP (200 μM) did not produce visible outward current in DLSN neurons (n = 8).

**DISCUSSION**

**Properties of K+ conductance underlying I5-HT**

The present study showed that 5-HT produced an outward current (I5-HT) associated with an increase in the membrane conductance of DLSN neurons. I5-HT reversed polarity at a holding potential of −93 ± 6 mV, which is close to the equilibrium potential for K+ in ACSF containing 4.7 mM K+. The reversal potential of I5-HT changed by 54 mV per decade change in the external K+ concentration as predicted by the Nernst equation. These results indicate that I5-HT is carried exclusively by K+. It has been shown that the 5-HT-induced hyperpolarization was not obviously voltage dependent in either normal ACSF or high K+ solution in DLSN neurons (Joëls and Gallagher 1988; Joëls et al. 1986, 1987). Our preliminary report showed that although 5-HT produced an inward rectifier K+ current in some DLSN neurons, Ba2+ (100 μM) did not completely block the I5-HT (Yamada et al. 2000).
study showed three types of $I_{s,HT}$ in terms of their voltage dependencies. In 63% of neurons, $I_{s,HT}$ was associated with linear $I-V$ relation. $I_{s,HT}$ with characteristic inward rectification was seen in 21% of the neurons tested. In remaining 16% of neurons, $I_{s,HT}$ exhibited outward rectification. Ba$^{2+}$ (100 $\mu$M) almost completely depressed $I_{s,HT}$ of the inward rectifier type. Such a Ba$^{2+}$-sensitive inward rectifier $I_{s,HT}$ is probably identical to that described in raphe nuclei neurons (Bayliss et al. 1997; Katayama et al. 1997; Pan et al. 1993; Penington et al. 1983a,b). In contrast, Ba$^{2+}$ (100 $\mu$M) did not significantly affect the outward rectifier $I_{s,HT}$. In $I_{s,HT}$ with linear $I-V$ relation, Ba$^{2+}$ preferentially depressed the 5-HT current at hyperpolarizing membrane potentials. As the results, the Ba$^{2+}$-resistant component of linear type $I_{s,HT}$ clearly showed outward rectification activated at potentials more positive than $-80$ mV. These results suggest that the $I_{s,HT}$ with linear $I-V$ relation is the sum of these two component currents. Previously, we have reported that 5-HT$_{1A}$ receptors are responsible for the outward current produced by 5-HT in DLSN neurons (Yamada et al. 2000). In the present study, 8-OH-DPAT, a 5-HT$_{1A}$ receptor agonist, produced outward currents associated with inward rectification, outward rectification, and linear $I-V$ relation in DLSN neurons. However, further studies are needed to clarify the subtype of 5-HT receptors that mediate to both inward and outward rectifying $K^+$ currents in DLSN neurons.

The delayed rectifier $K^+$ current and the Ca$^{2+}$-activated $K^+$ current do not seem to be involved in $I_{s,HT}$ because 5-HT caused the outward current in an external solution containing 20 mM TEA and 0 mM Ca$^{2+}$. Joëls et al. (1987) have reported that the 5-HT-induced hyperpolarization is not sensitive to Ca$^{2+}$ in DLSN neurons. $I_{s,HT}$ was not blocked by glibenclamide (100 $\mu$M), indicating that the ATP-sensitive $K^+$ current (Schmid-Antomarchi et al. 1987; Sturgess et al. 1985) is not involved in $I_{s,HT}$. It has been shown that the $M$ current is a time-dependent outward rectifier $K^+$ current activated by acetylcholine via muscarinic receptors in autonomic and central neurons (Brown 1990). The $M$ channel may not be involved in the outward rectifier current produced by 5-HT in DLSN neurons because $I_{s,HT}$ was not associated with the time-dependent relaxation that is the characteristic feature of the $M$ current at depolarized membrane potentials.

**Signal transduction of $I_{s,HT}$**

Molecular cloning has established that 5-HT$_{1A}$ receptors belong to the superfamily of G-protein-coupled receptors (Albert et al. 1990). 5-HT$_{1A}$ receptors couple to inward rectifier $K^+$ channels via a PTX-sensitive G protein in neurons of the dorsal raphe nucleus and the hippocampus (Bayliss et al. 1997; Bobker and Williams 1989; Katayama et al. 1997; Penington et al. 1993a; Sim et al. 1997). The present study showed that intracellular application of GTP$\gamma$S and GDP$\beta$S irreversibly and almost completely suppressed $I_{s,HT}$ in DLSN neurons. NEM, an uncoupler of receptors from the PTX-sensitive G protein (Asano and Ogasawara 1986; Nakajima et al. 1990; Shapiro et al. 1994), also depressed $I_{s,HT}$ in DLSN neurons. These results suggest that a PTX-sensitive G protein mediates $I_{s,HT}$ in DLSN neurons. Previous studies have shown that a soluble second messenger is not required for the G-protein-mediated effect of 5-HT in activating inwardly rectifying $K^+$ channels in dorsal raphe neurons (Katayama et al. 1997; Pentington et al. 1993b). 5-HT activation of inward rectifier $K^+$ channels in hippocampal neurons in inside-out patches appeared to be directly mediated by G$\beta$$\gamma$ (Oh et al. 1995).

Biochemical assays have demonstrated that the rat 5-HT$_{1A}$ receptor inhibits both basal and stimulated cyclic AMP accumulation by forskolin (Fargin et al. 1989; Raymond et al. 1989). In DLSN neurons, however, db-cAMP and forskolin produced no obvious effect on the membrane current in DLSN neurons. The PKA inhibitors H-89 and Rp-cAMPS did not significantly reduce $I_{s,HT}$ with properties of either inward or outward rectification. These results suggest that PKA is not involved in the pathway that mediates $I_{s,HT}$ in DLSN neurons. In addition to the inhibitory effect on adenylyl cyclase, 5-HT, at micromolar concentration, stimulates phospholipase C activity in Hela cells with permanently expressed 5-HT$_{1A}$ receptors. This effect did not appear to be secondary to an inhibition of adenylyl cyclase, and 5-HT$_{1A}$ receptors can stimulate phosphatidylinositol hydrolysis, resulting in the activation of PKC in Hela cell (Fargin et al. 1989; Raymond et al. 1989). 5-HT$_{1A}$ receptors may be capable of coupling to multiple G-protein-associated effector systems in a single cell. The present study showed that PKC 19-36 preferentially depressed $I_{s,HT}$ with properties of the outward rectifier $K^+$ conductance, while it did not significantly affect the inward rectifier $I_{s,HT}$ in DLSN neurons. PMA, an activator of PKC, produced outward rectifier $K^+$ current in DLSN neurons. We concluded that a PTX-sensitive G protein directly mediates the 5-HT-induced inward rectifier $K^+$ current, while a soluble second messenger, such as PKC, may mediate the 5-HT$_{1A}$ receptor-activated outward rectifier $K^+$ current in DLSN neurons.

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