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Ion permeation properties of a cloned human 5-HT_3 receptor transiently expressed in HEK 293 cells

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Summary. Human 5-HT_3 receptors expressed in HEK 293 cells were studied using patch-clamp techniques. The permeability ratios of cations to Na^+ were Li^+, 1.16; K^+, 1.04; Rb^+, 1.11; Cs^+, 1.11; $NMDG^+$, 0.04; Ca^{2+}, 0.49, and Mg^{2+}, 0.37. The permeability sequence of the alkali metal cations was $Li^+ > Rb^+ = Cs^+ > K^+ > Na^+$. Increased external concentrations of Ca^{2+} or Mg^{2+} decreased 5-HT-induced currents at all potentials tested in a voltage-independent manner. The single-channel conductance of human 5-HT_3 receptors measured by fluctuation analysis of whole-cell currents was 790 ± 100 fS. Differences in the basic properties of 5-HT_3 receptors between species may explain interspecies differences in pharmacological properties.

Keywords: Amino acids – Serotonin receptor – Serotonin-3 receptor – Ion channel – Ion permeability

1 Introduction

5-hydroxytryptamine type 3 (5-HT_3) receptors are ligand-gated ion channels which cause fast, depolarizing responses in neuronal cells (Yakel and Jackson, 1988; Derkach et al., 1989). The receptors are exclusively associated with neurons in both the central and peripheral nervous system, and in neuronal cell lines (Hoyer et al., 1994).

These receptors have been cloned from mice (Maricq et al., 1991; Hope et al., 1993), rats (Isenberg et al., 1993; Miyake et al., 1995), and humans (Belelli et al., 1995; Miyake et al., 1995). Each cloned 5-HT_3 receptor can form functional homo-oligomeric receptors when expressed in mammalian cells and in *Xenopus* oocytes. All the cloned 5-HT_3 receptors except human 5-HT_3 receptors exist as two splice variants. One of those splice variants lack five or six amino acids in the intracellular loop between the third and the fourth transmembrane domains. Due to a lack of splicing consensus sequence in human genomic DNA (Werner et al., 1994), the cloned human 5-HT_3

receptor was found to be the shorter splice variants of 5-HT_3 receptors (Belelli et al., 1995; Miyake et al., 1995).

The study of the pharmacological role of 5-HT_3 receptor has been facilitated by the development of selective ligands for this receptor (Greenshaw, 1993). Therapeutically, selective 5-HT_3 receptor antagonists are potentially useful in the treatment of emesis induced by cytotoxic chemotherapy or radiation (Bunce et al., 1991; Kamato et al., 1993). Recently, it has been shown that selective 5-HT_3 receptor agonists have antinociceptive and antidepressant effects in animals (Poncelet et al., 1995; Alhaider 1997). However there are pharmacological differences among species (Peters et al., 1992). The affinities of antagonists determined in isolated cervical vagus nerves from rats are generally 10 to 100-fold higher than in preparations isolated from guinea-pig tissues (Ireland and Tyers, 1987; Butler et al., 1990). Although phenylbiguanide is a potent agonist in the rat vagus nerve, it is inactive as either an agonist or antagonist in any of the guinea-pig tissues evaluated (Butler et al., 1990). Additionally, our initial report demonstrated that 2-methyl-5-hydroxytryptamine, a partial agonist for mouse 5-HT_3 receptors, was a full agonist for human 5-HT_3 receptors (Miyake et al., 1995).

The basic properties of 5-HT_3 receptors such as ion permeability and single-channel conductance affect these interspecies pharmacological differences. The elucidation of these basic properties are thus essential to understand 5-HT_3 receptor pharmacology. Endogenous 5-HT_3 receptors of mouse neuroblastoma N18 cells show a permeability sequence of alkali metal cations of $Cs^+ > K^+ > Li^+ \geq Na^+ \geq Rb^+$ (Yang, 1990). The single-channel conductances of the shorter (5-HT_3R-A_S) and longer (5-HT_3R-A_L) splice variants of cloned mouse 5-HT_3 receptors have been reported as 360 ± 70 fS (Hussy et al., 1994) and 400 ± 190 fS (Werner et al., 1994), respectively. However, little is known about human 5-HT_3 receptors. In this study, we investigated the characteristics of human 5-HT_3 receptors transiently expressed in HEK 293 cells using the patch electrode voltage clamp techniques. We report here the ion permeability of human 5-HT_3 receptor channels, the modulation of the channels with divalent cations, and the estimation of the single-channel conductance with fluctuation analysis of whole-cell currents.

2 Materials and methods

2.1 Cell culture and DNA transfection

Human embryonic kidney cells (HEK 293, ATCC CRL 1573) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 in air at 37°C. For transfections, cells were passaged the night before with trypsin/EDTA solution (0.05%/0.02%, respectively) and plated at 30% confluency in a 35 mm dish. The next day, cells were transfected using a modified calcium phosphate precipitation method (Chen and Okayama 1987) with combinations of the mammalian expression vector pEF-BOS containing the human 5-HT_3 receptor cDNA (Miyake et al., 1995) and the GFP expression vector (phGFP S65T, CLONTECH). The total amount of DNA added was 6μg. Cells expressing human 5-HT_3 receptors were

identified by GFP emitting bright green fluorescence viewed with an epifluorescence microscope (Marshall et al., 1995). Electrophysiological recording was performed 48 to 72 hours after the transfection.

2.2 Solutions

The compositions of the external and internal (inside the patch electrode) solutions used in this study are listed in Table 1. The normal solution (E1) and the CsCl internal solution (I1) were used in experiments unless otherwise noted.

In order to determine the relative permeability of human 5-HT_3 receptors to various monovalent cations, E2 was used as the external reference solution. In the test solution, all the NaCl was replaced with isomolar LiCl, KCl, RbCl, CsCl, and *N*-methyl-D-glucamine (NMDG; E3), respectively.

In divalent cation permeability experiments, isotonic external solutions containing either 100 mM of $CaCl_2$ (E4) or $MgCl_2$ (E5) were used. The NaCl internal solution (I2) was used in this series of experiments. In divalent cation modulation of the 5-hydroxytryptamine (5-HT) induced current, external solutions containing 1 mM $CaCl_2$ (E6) or 1 mM $MgCl_2$ (E8) were used with increased divalent chloride concentration from 1 to 10 mM (E7, E9).

Several ions were added to adjust the pH of the external and internal solutions. Ions introduced were included in the calculation of ion concentrations.

2.3 Drug application

In the majority of experiments, 5-HT was applied from a tapered-tip glass pipette (600 μm i.d.), positioned 2 mm from the cell being measured. The pipette was connected to solenoid valves followed with reservoirs which contained various solutions. Only one valve was open at any one time, and external solution flowed continuously between 5-HT application. At a flow rate of approximately 1 ml/min, the 10–90% exchange time (determined from current response obtained when switching an open patch electrode between solutions of different ionic composition) was less than 10 ms. Constant bath perfusion was

Table 1. Composition of solutions (mM)

	NaCl	KCl	CsCl	$CaCl_2$	$MgCl_2$	NMDG	EGTA	HEPES
External solutions								
E1	145	5	–	2.4	–	–	–	10
E2	155	–	–	1	–	–	–	10
E3	–	–	–	0.1	–	155	–	10
E4	–	–	–	100	–	24	–	5
E5	–	–	–	–	100	24	–	5
E6	125	5	–	1	–	–	–	10
E7	125	5	–	10	–	–	–	10
E8	125	5	–	–	1	–	–	10
E9	125	5	–	–	10	–	–	10
Internal solutions								
I1	–	–	150	–	–	–	5	10
I2	150	–	–	–	–	–	5	10

All external solutions contained 10 mM D-Glucose. The pH of the external solutions was adjusted to 7.1–7.4 with NaOH (*E1*, *E6–E9*), NMDG (*E2*) or HCl (*E3*, *E4*, *E5*). In E6–E9 solutin, the osmolarity was titrated to 310 mOsm with sucrose. The pH of internal solution was adjusted to 7.2 with CsOH (*I1*) or NaOH (*I2*). *NMDG N*-methyl-D-glucamine.

also maintained at a flow rate of approximately 0.5 ml/min. Cells were repetitively exposed to the agonist at intervals of at least 2 min in order to allow complete recovery from desensitization. Except in studies of the single-channel conductance, the 5-HT concentration was 100 μM. This is a saturating concentration in human 5-HT_3 receptors (Miyake et al., 1995). In fluctuation analysis of whole-cell currents, 5-HT (0.5 μM) was slowly applied by bath perfusion to achieve a slowly developing response to 5-HT.

2.4 Electrophysiological recording

The voltage clamp measurements were performed as described previously (Hamill et al., 1981). Currents were measured using whole-cell and single-channel patch-clamp configurations with an Axopatch 1D patch-clamp amplifier (Axon Instruments). Patch pipettes had resistances of 2–5 MΩ for whole-cell recording and 10–15 MΩ for single-channel recording. Series resistance was compensated for at least 80%. A coarse-tipped capillary filled with agar-3 M KCl was used as the reference electrode to minimize changes in reference electrode potential due to the various external solutions. The holding potentials were corrected for a junction potential that developed at the interface between the patch electrode and bath solutions. Data were low-pass filtered at 500 Hz (8-pole, Bessel) and sampled on-line at 1 kHz. For fluctuation analysis of whole-cell currents, DC-coupled records (low-pass filtered at 500 Hz, 8-pole, Bessel) and AC-coupled records (high-pass filtered at 1.0 Hz; low-pass filtered at 500 Hz, 8-pole, Butterworth) were digitized at 28.77 kHz with a RP880 PCM data recording system (NF Instrument, Japan) and then recorded on videotape. All recordings were done at room temperature (25°C).

2.5 Data analysis

Currents were analyzed with pCLAMP and AxoGraph software (Axon Instruments). Relative permeabilities for monovalent cations were calculated from changes of reversal potential on switching from the reference solution to the test solutions (Yang, 1990). For monovalent cations the ratios of permeabilities, P_X/P_{Na}, for the test cation X to that of Na^+ were calculated according to the Goldman-Hodgikin-Katz (GHK) voltage equation (Hodgkin 1951; Yang 1990)

$$\Delta E_r = E_{r,x} - E_{r,Na} = 2.30\frac{RT}{F}\log\frac{P_x/P_{Na}[X]_o}{[Na]_o}$$

where RT/F is 25.7 mV at 25°C, $[X]_o$ is the activity of X^+ and $E_{r,X}$ is the reversal potential in the test solution, and $[Na]_o$ is the activity of Na^+ and $E_{r,Na}$ is the reversal potential in the external reference solution.

For divalent cations, the GHK current equation was used to calculate the permeability ratios (Hodgkin 1951; Yang 1990)

$$0 = \sum_j P_j Z_j^2 \frac{E_r F^2}{RT}\frac{[j]_o - [j]_i \exp(z_j F E_r/RT)}{1 - \exp(z_j F E_r/RT)}$$

where P_j is the permeability for the jth relevant ion, Z_j is the valence, E_r is the reversal potential in the test solution, and $[j]_o$ and $[j]_i$ are the external and internal ion activities. Activity coefficients for monovalent and divalent ions were estimated from the report by Robinson and Stokes (1959).

For fluctuation analysis, AC-coupled and DC-coupled records from the videotape were play backed through the PCM and sampled at 1 kHz on two different channels. These data were divided into 1024-point segments. The variance and the mean current amplitude were calculated from the AC-coupled and DC-coupled records, respectively. Plots of the variance versus the mean current amplitude were fit to a straight line by the

least squares method. The slope of the line was used to assess single-channel conductance (Anderson and Stevens, 1973).

Data in the table and text are given as mean ± SEM (number of observation).

3 Results

3.1 Ion permeability

Voltage clamp recording revealed that application of 5-HT induced transient currents, a typical response of 5-HT$_3$ receptors (Fig. 1A). The voltage dependence of the 5-HT-induced currents (I–V relation) changed due to the species of ions in internal and external solutions (Fig. 1B). The I–V relation using the CsCl internal (I1) and NaCl external solution (E2) was inwardly rectificated and the reversal potential was −3.1 ± 0.4 mV (n = 3). When Na^+ in the

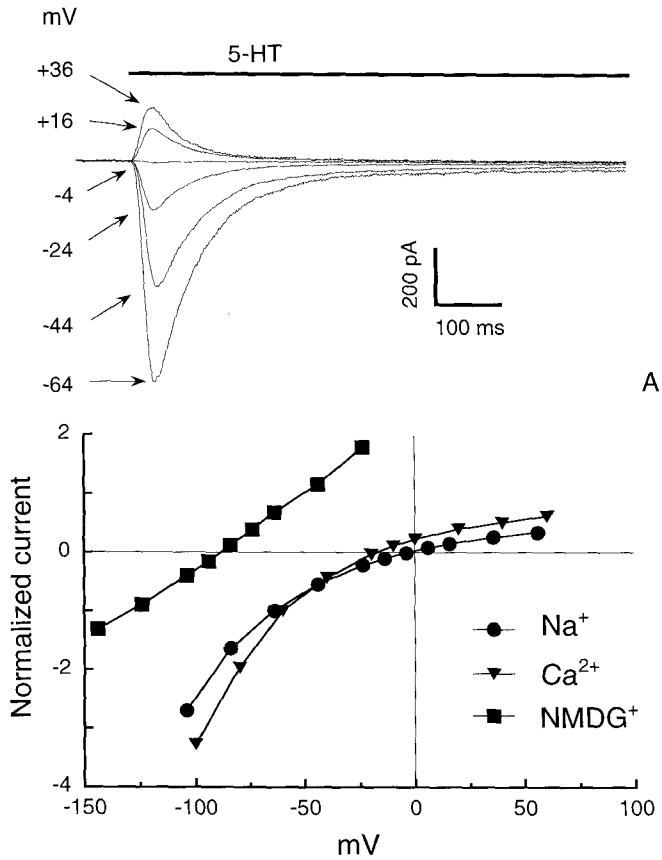


Fig. 1. Whole-cell current-voltage (I–V) relations of human 5-HT$_3$ receptors. **A** Current-responses elicited by rapid application of 5-HT (100 μM) at the various holding potentials in the Na^+ external solution (E2). Each trace is superimposed and leakage currents have been subtracted. **B** I–V relations for 5-HT-induced currents in Na^+ (E2), in $NMDG^+$ (E3), and in Ca^{2+} (E4) external solutions. The peak current amplitude was normalized to −1 at −64 mV (E2), −0.4 at −104 mV (E3), and −1 at −60 mV (E4). The reversal potential was taken as the grid (I = 0) intercept of the I–V relations. Each data point is the mean of 3 to 5 different cells. SEM of data points are smaller than the symbols

Table 2. Reversal potentials and relative permeabilities

X	ΔEr ± SEM (mV)	Er ± SEM (mV)	n	P_x/P_{Na}
Li^+	3.7 ± 0.1	–	6	1.16
Na^+	0.0	−3.1 ± 0.4	3	1.00
K^+	1.0 ± 0.3	–	6	1.04
Rb^+	2.7 ± 0.1	–	5	1.11
Cs^+	2.6 ± 0.2	–	6	1.11
$NMDG^+$	−80.5 ± 1.0	–	6	0.04
Ca^{2+}	–	−16.3 ± 0.7	6	0.49
Mg^{2+}	–	−21.9 ± 0.4	5	0.37

The value of reversal potential was determined from I–V curves with various internal- and external-solutions. Ion activities were used in all calculations.

external solution was replaced with other alkali metal cations, Li^+, K^+, Rb^+ and Cs^+, on an isomolar basis, the changes of the reversal potentials were 3.7 ± 0.1 mV (n = 6), 1.0 ± 0.3 mV (n = 6), 2.7 ± 0.1 mV (n = 5) and 2.6 ± 0.2 mV (n = 6), respectively (Table 2). The permeability ratio for Li^+, K^+, Rb^+ and Cs^+ to Na^+ were calculated according to the GHK voltage equation with resulting values of 1.16 ± 0.01, 1.04 ± 0.01, 1.11 ± 0.00 and 1.11 ± 0.01, respectively. The permeability sequence for the alkali metal cations was $Li^+ > Rb^+ = Cs^+ > K^+ > Na^+$, where ">" was used when the P value was below 0.01 (Tukey-Krammer test). This sequence is different from that of 5-HT_3 receptors endogenous to mouse neuroblastoma N18 cells; $Cs^+ > K^+ > Li^+ \geq Na^+ \geq Rb^+$ (Yang, 1990).

When Na^+ in E2 was replaced with $NMDG^+$ and the Ca^{2+} concentration was reduced from 1 mM to 0.1 mM (E3), the I–V relation became linear and the change of the reversal potential was −80.5 ± 1.0 mV (n = 6) (Fig. 1B), yielding the permeability ratio (P_{NMDG}/P_{Na}) of 0.04. This permeability ratio is much larger than that of mouse 5-HT_3 receptors (N18 cells); $P_{NMDG}/P_{Na} \leq 0.005$ (Yang, 1990), suggesting that human 5-HT_3 receptors are different from mouse 5-HT_3 receptors.

To evaluate the relative permeabilities of divalent cations, we measured the reversal potentials in the isotonic $CaCl_2$ (E4) and $MgCl_2$ (E5) external solutions with the NaCl internal solution (I2). The obtained reversal potentials were Ca^{2+}, −16.3 ± 0.7 mV (n = 6) and Mg^{2+}, −21.9 ± 0.4 mV (n = 5). Their permeability ratios relative to Na^+ were Ca^{2+}, 0.49 and Mg^{2+}, 0.37 which were calculated according to the GHK current equation. These values are slightly smaller than those of mouse 5-HT_3 receptors (N18 cells); $P_{Ca}/P_{Na} = 0.53$, $P_{Mg}/P_{Na} = 0.43$ (Yang, 1990).

3.2 Modulation of 5-HT-induced currents by Ca^{2+} and Mg^{2+}

An increase in the external concentration of Ca^{2+} or Mg^{2+} resulted in a reduction of 5-HT-induced current at all potentials examined (Fig. 2). The increase in the external Ca^{2+} concentration from 1 to 10 mM reduced the

current to 49 ± 2.0% (n = 5) of control at −64 mV. The same increase in external Mg^{2+} also reduced the current to 62 ± 3.5% (n = 5) of control at −64 mV.

When I–V relations recorded in the presence of different concentration of Ca^{2+} were normalized, they overlapped each other (Fig. 2C inset) except for the reversal potentials described later. Also, the normalized I–V relations for different concentrations of Mg^{2+} overlapped each other (Fig. 2D inset). Consequently, the inhibitory effect of Ca^{2+} and Mg^{2+} was voltage-independent. Such observations are consisted with the reports about 5-HT_3 receptors endogenous to several clonal cell lines (Peters et al., 1988; Yakel et al., 1990; Yang, 1990) and primary cultured cells (Yang et al., 1992), and cloned mouse 5-HT_3 receptor expressed cells (Gill et al., 1995).

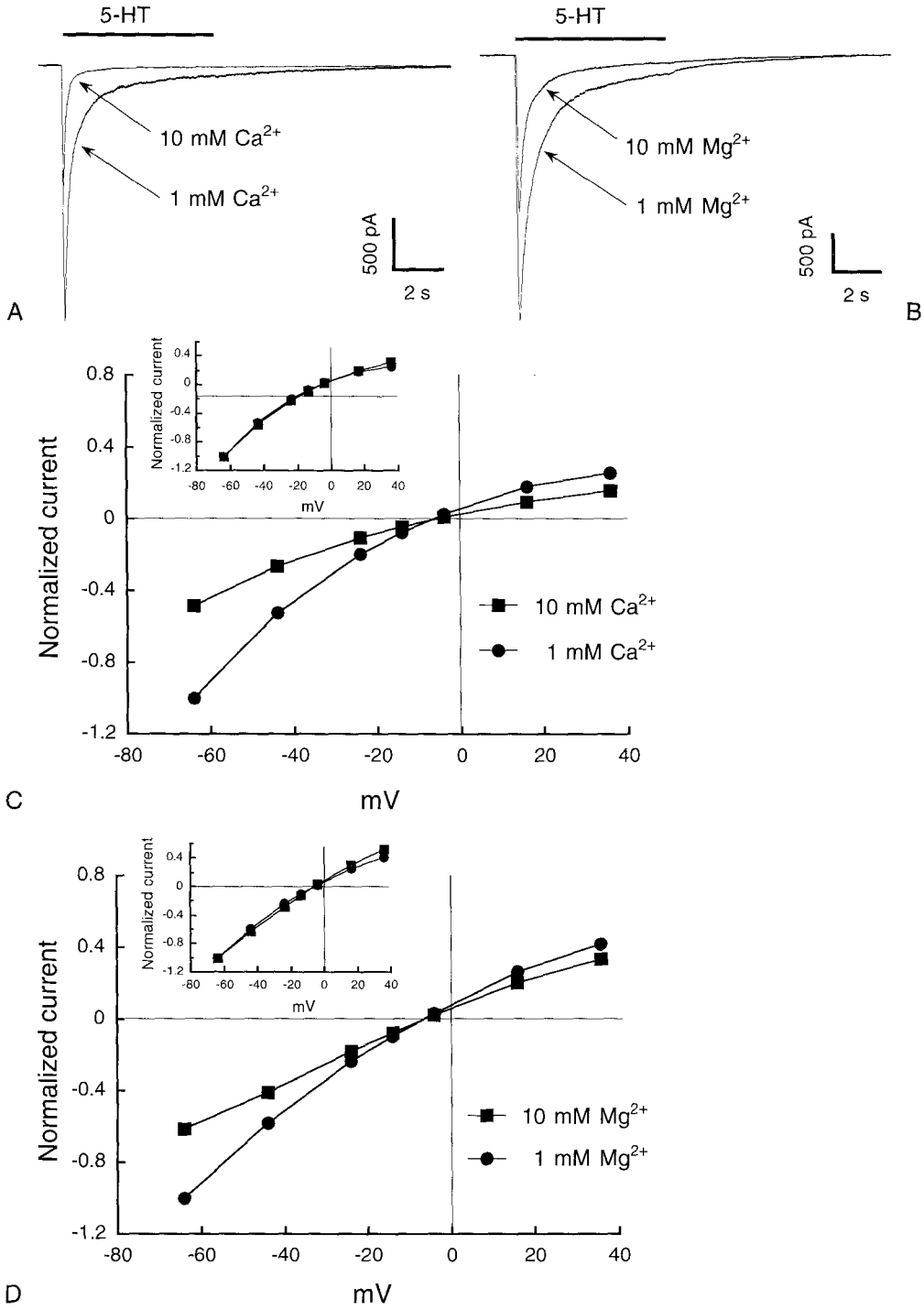
The increase of external Ca^{2+} from 1 mM to 10 mM slightly but significantly shifted the reversal potential from −6.6 ± 0.2 mV (n = 5) to −5.9 ± 0.2 mV (n = 5) ($P < 0.05$, Student's t-test). Also, the increase of external Mg^{2+} from 1 mM to 10 mM shifted the reversal potential from −6.5 ± 0.2 mV (n = 6) to −6.3 ± 0.2 mV (n = 5). The GHK current equation with the permeability ratios shown in Table 2 predicts that the shifts in reversal potential are Ca^{2+}, 0.8 mV and Mg^{2+}, 0.4 mV. The experimental results were comparable to the calculated value. Therefore, the ion permeability ratios were consistent when the 5-HT-induced currents were blocked by Ca^{2+} and Mg^{2+}.

3.3 Single-channel conductance

We attempted to observe 5-HT-induced single-channel events in excised outside-out patches from human 5-HT_3 receptors-expressing cells. However, no single-channel currents could be resolved (data not shown). In cloned mouse 5-HT_3 receptors (Hussy et al., 1994; Werner et al., 1994; Gill et al., 1995) and in 5-HT_3 receptors endogenous to several neuroblastoma cell lines (Lambert et al., 1989; Yang, 1990; Hussy et al., 1994) extremely small single-channel conductances of less than 1 pS have been reported. In such cases, single channel currents cannot be directly discerned by the patch clamp technique, but some of their properties may be determined indirectly by fluctuation analysis of 5-HT induced whole-cell currents (Lambert et al., 1989; Yang, 1990; Hussy et al., 1994; Werner et al., 1994; Gill et al., 1995).

Fig. 3A shows DC-coupled and AC-coupled records of whole-cell current induced by bath application of a low concentration of 5-HT (0.5 μM). The inward current was accompanied by an increase of current noise. Fluctuation analysis of this response yielded a linear relationship between the variance and the mean amplitude of the inward current evoked by 5-HT (Fig. 3B). From the slope of the best-fitting line, the single-channel current was estimated to be −40 fA for this cell. In the condition of the normal external solution (E1) and the CsCl internal solution (I1), the reversal potential of 5-HT-induced whole-cell currents was −2.8 ± 0.6 mV (n = 6). Holding potential in the experiment of fluctuation analysis was −64 mV. Then, dividing the

single-channel current by the driving force of −61.2 mV (the holding potential minus the reversal potential) provided an estimate for the single-channel conductance of 650 fS. From additional measurements the conductance was estimated to be 790 ± 100 fS (n = 8).



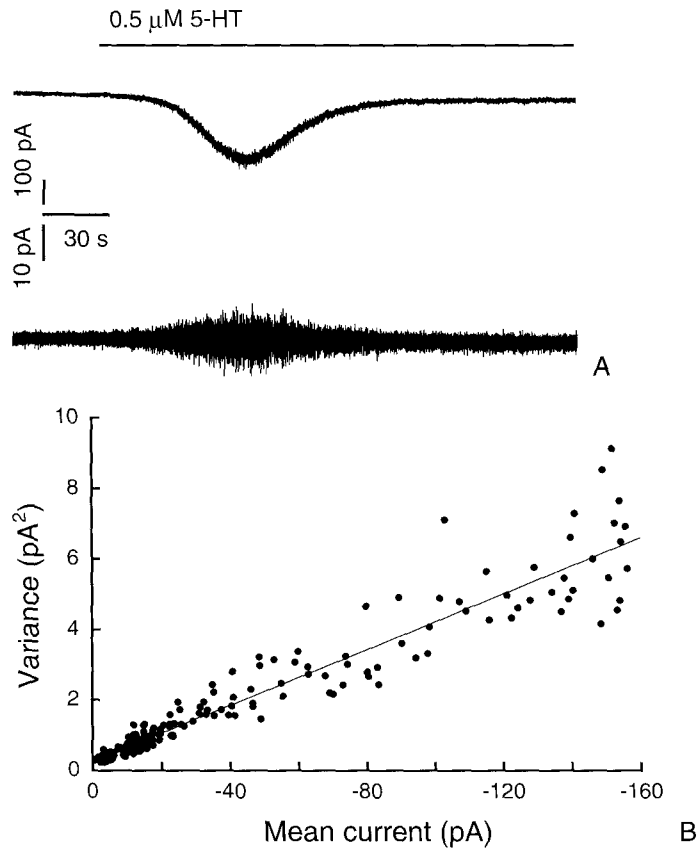


Fig. 3. Estimation of single-channel conductance by fluctuation analysis of whole-cell current. Upper trace of **A**: DC-coupled record of a whole-cell current response induced by bath perfusion of 0.5 μM 5-HT at −64 mV. Low pass filtered at 500 Hz and sampled at 1 kHz. Lower trace of **A**: AC-coupled record of the response illustrated in the upper trace of A. High pass filtered at 1 Hz, low pass filtered at 500 Hz and sampled at 1 kHz. **B** Plot of the variance of current noise vs. mean current amplitude. The variance and the mean current amplitude were calculated from 1024 point segments of the AC-coupled and DC-coupled records, respectively. The single-channel current estimated from the least-squares linear fit (γ = 0.96) was 40 fA. The single-channel conductance in this example is estimated to be 650 fS (E_r = −2.8 mV)

Fig. 2. Divalent cations modulate human 5-HT$_3$ receptors mediated current-response. **A** Typical responses influenced with extracellular concentration of Ca^{2+}. Current-responses were evoked with rapid application of 5-HT (100 μM) in the 1 mM and 10 mM Ca^{2+} containing external solution in the same cell. Each trace is recorded at −64 mV and superimposed. **B** Typical responses influenced with extracellular concentration of Mg^{2+}. Recording conditions as in **A**. **C** I–V relations were determined in the 1 mM Ca^{2+} containing external solution and then switched to the 10 mM Ca^{2+} containing solution in the same cells. The current amplitude was normalized to −1 at −64 mV in 1 mM Ca^{2+} containing solution. **C** inset: The current amplitude was normalized to −1 at −64 mV in each external solution. **D** I–V relations were determined in the 1 mM and 10 mM Mg^{2+} containing external solution. The current amplitude was normalized as in **C**. **D** inset: The current amplitude was normalized as in **C** inset. Each data point in **C** and **D** is the mean of 4 to 6 different cells. SEM of data points are smaller than the symbols

4 Discussion

The relative permeabilities of alkali metal cations in human 5-HT_3 receptors expressed in HEK 293 cells were P_{Li}/P_{Na}, 1.16; P_K/P_{Na}, 1.04; P_{Rb}/P_{Na}, 1.11, and P_{Cs}/P_{Na}, 1.11 and the permeability sequence was $Li^+ > Rb^+ = Cs^+ > K^+ > Na^+$. 5-HT-induced responses in cloned mouse 5-HT_3 receptor expressing cells (Hussy et al., 1994; Werner et al., 1994; Gill et al., 1995), in mouse SCG neurons (Hussy et al., 1994), in nodose ganglion cells (Higashi and Nishi, 1982), in cultured hippocampal neurons and NG108-15 cells (Yakel and Jackson, 1988), and in N1E-115 and NCB-20 cells (Lambert et al., 1989) also have reversal potentials near 0 mV, suggesting that the receptors are similarly permeable to several alkali metal cations. However, detailed study of ion permeabilities was performed in mouse 5-HT_3 receptors endogenous to neuroblastoma N18 cells (Yang, 1990) and the relative permeabilities were reported as P_{Li}/P_{Na}, 1.01; P_K/P_{Na}, 1.10; P_{Rb}/P_{Na}, 0.99, and P_{Cs}/P_{Na}, 1.22, yielding a permeability sequence of $Cs^+ > K^+ > Li^+ \geq Na^+ \geq Rb^+$. The result of the ion permeation sequence for human 5-HT_3 receptors was different from that of mouse 5-HT_3 receptors, although the differences of the magnitude of relative permeabilities between those receptors were small.

The permeability sequence of alkali metal cations in human 5-HT_3 receptors do not conform to any of the sequences predicted by Eisenman's theory (Hill, 1992) which explains permeability by equilibrium ion-exchange reaction. The incompatibilities with Eisenman's theory in human 5-HT_3 receptors suggests some interactions between the alkali metal cations and the pore region of the receptors (Reuter and Stevens, 1980). In 5-HT_3 receptors endogenous to mouse neuroblastoma N18 cells, the permeability sequence of alkali metal cations do not conform to any of the Eisenman's theory, also suggesting these interactions. The difference of the permeability sequences between human and mouse 5-HT_3 receptors indicates that the inner surface structure of 5-HT_3 receptor pore region is variable among species.

The I–V relation of 5-HT-induced response was inward rectificated. The extent of rectification was not altered by replacing Cs^+ in the internal solution with Na^+ or replacing Na^+ in the external solution with other alkali metal or alkali earth cations. However, the relation in the $NMDG^+$ external solution was linear. It remains to be resolved whether the rectification is an intrinsic property of the open channel or a result of voltage-dependent gating phenomenon.

In this study, an increase in the external concentration of Ca^{2+} or Mg^{2+} resulted in a reduction of the amplitude of 5-HT-induced current at all potentials examined in a voltage-independent manner. Although these blocking phenomena are different from a voltage-dependent blockade of *N*-methyl-D-aspartate-activated ion channels by Mg^{2+} (Ascher and Nowak, 1988), blockade of 5-HT_3 receptor channels by Ca^{2+} and Mg^{2+} plays an important role in the functional modulation of intracellular signaling.

The single-channel conductance of human 5-HT_3 receptors was estimated as 790 ± 100 fS (n = 8) and this value was larger than that of cloned mouse 5-HT_3 receptors; 360 ± 70 fS (Hussy et al., 1994) in 5-HT_3R-A_S, 400 ± 190 fS

(Werner et al., 1994) and 420 ± 74 fS (Gill et al., 1995) in 5-HT_3R-A_L. While the single-channel conductance of 5-HT_3 receptors native to dissociated cells from several tissues are 3.4 ± 0.8 pS (Hussy et al., 1994) in mouse SCG, 2.6 pS (Yang et al., 1992) in rat SCG, 19 pS (Peters et al., 1993) in rabbit nodose ganglion, and 15 and 9 pS (Derkach et al., 1989) in guinea pig submucous plexus neurons. The larger single-channel conductance of the native 5-HT_3 receptors than the cloned 5-HT_3 receptors may suggest that there are additional unknown subunits in native 5-HT_3 receptors (Hussy et al., 1994). Whether these unknown subunits exist in the native human cells remains to be determined, because no detailed information about the electrophysiological properties of native human 5-HT_3 receptors have been available. Future isolation of unknown subunits of 5-HT_3 receptors, if they exist, will clarify the diversity of the single-channel conductance.

The present results showed that cloned human 5-HT_3 receptors had the larger single-channel conductance than that of cloned mouse 5-HT_3 receptors. Also, human 5-HT_3 receptors appear to have a different energy barrier in the ion pathway than mouse 5-HT_3 receptors. These differences may explain, at least partially, the different pharmacology of human 5-HT_3 receptor from those of animals and would indicate an approach to rational drug designs of new antagonists (e.g. open channel blockers) for human 5-HT_3 receptors.

Additionally, ion permeation and conduction in cloned human 5-HT_3 receptors recently reported by Brown et al. (1998) also support our results.

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