Ion Permeation through 5-Hydroxytryptamine–gated Channels in Neuroblastoma N18 Cells

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ABSTRACT Ionic currents induced by 5-hydroxytryptamine (5-HT) in cultured neuroblastoma N18 cells were studied using whole-cell voltage clamp. The response was blocked by 1–10 nM 5-HT_3 receptor-specific antagonists MDL 7222 or ICS 205-930, but not by 1 μM 5-HT_1/5-HT_2 receptor antagonist spiperone or 5-HT_2 receptor-specific antagonist ketanserin. These 5-HT_3 receptors seem to be ligand-gated channels because the response (*a*) did not require internal ATP or GTP, (*b*) persisted with long internal dialysis of CsF (90 mM), AlF_4^- (100 μM), or GTPγS (100 μM), and (*c*) with ionophoretic delivery of 5-HT developed with a delay of <10 ms and rose to a peak in 34–130 ms. Fluctuation analysis yielded an apparent single-channel conductance of 593 fS. The relative permeabilities of the channel for a variety of ions were determined from reversal potentials. The channel was only weakly selective among small cations, with permeability ratios P_X/P_{Na} of 1.22, 1.10, 1.01, 1.00, and 0.99 for Cs^+, K^+, Li^+, Na^+, and Rb^+, and 1.12, 0.79, and 0.73 for Ca^{2+}, Ba^{2+}, and Mg^{2+} (when studied in mixtures of 20 mM divalent ions and 120 mM *N*-methyl-D-glucamine). Apparent permeability ratios for the divalent ions decreased as the concentration of divalent ions was increased. Small monovalent organic cations were highly permeant. Large organic cations such as Tris and glucosamine were measurably permeant with permeability ratios of 0.20 and 0.08, and *N*-methyl-D-glucamine was almost impermeant. Small anions, NO_3^-, Cl^-, and F^-, were slightly permeant with permeability ratios of 0.08, 0.04, and 0.03. The results indicate that the open 5-HT_3 receptor channel has an effective minimum circular pore size of 7.6 Å and that ionic interactions in the channel may involve negative charges near the pore mouth.

INTRODUCTION

Physiological actions of 5-hydroxytryptamine (5-HT) are mediated by three receptor subtypes, 5-HT_1, 5-HT_2, and 5-HT_3 (Bradley et al., 1986; Richardson and Engel, 1986; Peroutka, 1988). The 5-HT_1 and 5-HT_2 responses are mediated by second-messenger systems, presumably through G proteins (Peroutka, 1988). Activation of 5-HT_3 receptors, however, results in rapid excitation in many cells (Wallis and North,

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J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/90/12/1177/22 $2.00
Volume 96 December 1990 1177–1198

1978; Higashi and Nishi, 1982; Surprenant and Crist, 1988; Yakel et al., 1988). There is increasing evidence that this excitatory effect is mediated by direct opening of a cation channel that is formed by the 5-HT_3 receptor (Yakel and Jackson, 1988; Derkach et al., 1989; Lambert et al., 1989).

Functional 5-HT_3 receptors have been demonstrated in sympathetic, parasympathetic, enteric, and sensory neurons and cultured neuroblastoma cells (Richardson and Engel, 1986; Richardson and Buchheit, 1988). Their existence in the mammalian brain has also been demonstrated recently by radioligand binding (Kilpatrick et al., 1987; Peroutka and Hamik, 1988) and electrophysiological studies (Yakel et al., 1988; Yakel and Jackson, 1988). Properties of 5-HT_3 receptor channels have been studied in nodose ganglion neurons (Higashi and Nishi, 1982), cultured hippocampal neurons (Yakel et al., 1988; Yakel and Jackson, 1988), submucous plexus neurons (Surprenant and Crist, 1988; Derkach et al., 1989), and neuroblastoma N1E-115 and neuroblastoma-glioma NG108-15 cells (Yakel and Jackson, 1988; Lambert et al., 1989; Neijt et al., 1989). The single-channel conductances differ among various cells. Single-channel recordings from excised membrane patches of guinea pig submucous plexus neurons show two types of 5-HT–gated channels with conductances of 15 and 9 pS (Derkach et al., 1989), whereas fluctuation analysis in N1E-115 cells suggests a channel with a unitary conductance of only 310 fS (Lambert et al., 1989). Nevertheless, the responses in different preparations share many common properties. Notably, most reverse near 0 mV, suggesting that the channel is permeable to both Na^+ and K^+, and may be cation selective. Detailed studies on ion selectivity of the channel are still lacking.

This work provides quantitative information about ion selectivity and permeation in the 5-HT_3 receptor channel. The channel is found to be nonselectively permeable to a large number of cations and slightly permeable to small anions. The open channel appears to have a minimum pore size similar to that of the endplate nicotinic ACh receptor (AChR). A preliminary report has appeared (Yang and Hille, 1990).

METHODS

Cell Culture

Mouse neuroblastoma N18 cells were cultured as described (Catterall, 1975; Barnes and Hille, 1988). Briefly, cells were grown in 35-mm tissue culture dishes (Corning Glass Works, Corning, NY) in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum at 37°C and 7.5% CO_2. Undifferentiated cells 2–6 d in culture were used for recording. Before recording, the cells were perfused with mammalian Ringer solution (E1, Table I) for 0.5–3 h. Round cells with diameters of 20–50 μm were selected.

Solutions

Internal and external solutions are described in Table I. The normal mammalian Ringer solution (E1) and the CsCl internal solution (I1) were used in experiments other than selectivity experiments. In some experiments, 100 μM guanosine-5′-*O*-(3′-thiotriphosphate) (GTPγS) or 100 μM $AlCl_3$ and 5 mM CsF (substituting for CsCl) were added to I1 to examine the effects of activating G proteins on the 5-HT–induced response.

In monovalent cation selectivity experiments, either E2 or E3 was used as the extracellular reference solution, as indicated in Tables II and III. In the test solution all the NaCl was

replaced by an osmotically equivalent amount of the test salt. The internal solution used in most experiments was I1. In one series of experiments to measure permeabilities to large organic cations, an internal solution with a low concentration of Cl^- (I2) was used. The following salts of alkali metal ions were studied: LiCl, KCl, RbCl, and CsCl. The following organic compounds were studied as test salts: ammonium·Cl (J. T. Baker Chemical Co., Phillipsburg, NJ); dimethylamine·HCl, ethylamine·HCl, 2-methylaminoethanol, diethanolamine (Eastman Organic Div., Eastman Kodak Co., Rochester, NY); methylamine·HCl, Tris (hydroxymethyl)aminomethane, glucosamine·HCl, *N*-methyl-D-glucamine (NMDG) (Sigma Chemical Co., St. Louis, MO). When compounds were obtained as the free amine, they were titrated with HCl to pH 7.1–7.3 to form the chloride salt.

E2 was used as reference solution in divalent cation selectivity experiments. The test solutions contained either only 100 mM divalent chloride or a mixture of 20 mM divalent chloride and 120 mM relatively impermeant NMDG·Cl. They also contained 12 mM glucose and 5 mM L-histidine, pH 7.4 (J. T. Baker Chemical Co.). Internal solution I3 was used in this series of experiments.

TABLE I

Composition of Solutions

	NaCl	KCl	CsCl	$CaCl_2$	$MgCl_2$	Na-Glu	Tris-Glu	NMDG-Cl	EGTA	HEPES	His	D-Glucose
External solutions												
E1	140	5	—	1.5	1	—	—	—	—	10(Na^+)	—	10
E2	150	—	—	2	—	—	—	—	—	—	5	10
E3	150	—	—	—	—	—	—	—	—	—	5	12
E4	—	—	—	—	—	15	145	—	—	—	—	5
Internal solutions												
I1	—	—	135	0.5	2	—	—	—	5(Cs^+)	5(Cs^+)	—	—
I2	14	—	—	—	—	130	—	—	—	5(Na^+)	—	—
I3	135	—	—	0.5	2	—	—	—	5(Na^+)	5(Na^+)	—	—
I4	15	—	—	—	—	—	—	129	—	5	—	—

All concentrations are in millimolar. The pH of the internal solutions and external solution E1 was adjusted to 7.4 with either CsOH or NaOH, as indicated in HEPES. The pH of other external solutions was adjusted with HCl to 7.1–7.4. Glu, glucuronate; NMDG, *N*-methyl-D-glucamine; His, L-histidine.

The control extracellular solution for studying anion permeation was E4, which contained a large anion glucuronate (glu) as the only anion. In the test anion solution, glucuronate was replaced with nitrate, chloride, or fluoride by replacing Na-glucuronate with equivalent amounts of their sodium salts and by titrating the Tris solution with respective acids to pH 7.1. The final concentration of the anions was calculated using $pK_a = 8.1$ for Tris (Table IV). The internal solution used in these experiments was I4.

Application of Agonists and Antagonists

5-HT (Research Biochemicals Inc., Natick, MA) was prepared in 10 mM stock solution in distilled water and then diluted to its final concentration in appropriate solutions and kept frozen. In monovalent cation selectivity experiments 5-HT was diluted in the glucosamine test solution (150 mM glucosamine·Cl, 5 mM L-histidine, and 12 mM D-glucose), whereas in divalent cation selectivity experiments 5-HT was dissolved in appropriate Ca^{2+} test solutions. In anion selectivity experiments, however, 5-HT was diluted in the glucuronate reference solution (E4). The agonist was applied to cells in one of three ways. In most experiments 5-HT

(10–20 μM) was delivered by pressure ejection from a PV 820 picopump (World Precision Instruments, Inc., New Haven, CT) with pressure pulses of 4–6 psi and 50–200 ms. The drug pipette was ~10 μm from the cell. In experiments designed to maximize the speed of application, 5-HT was delivered ionophoretically by passing an 80-nA, 0.5–1-ms positive current pulse through a high-resistance electrode (50–100 MΩ) filled with 100 mM 5-HT in Ringer solution. A backing current of 1.5 nA reduced the leakage of 5-HT and the pipette was held 2–3 μm from the cell. In fluctuation analysis experiments a low concentration of 5-HT (0.5 μM in Ringer solution) was applied by bath perfusion to permit slow activation of the response with minimal desensitization.

Antagonists were applied by bath perfusion. 3-Tropanyl-indole-3-carboxylate (ICS 205-930), 3-tropanyl-3,5-dichlorobenzoate (MDL 72222), and spiperone (Research Biomedicals, Inc.) were prepared in 1 mM stock solution in DMSO. Ketanserin-(+)-tartrate (Research Biochemicals Inc.) and D-tubocurarine·Cl (Sigma Chemical Co.) were prepared in 1 mM stock solution in Ringer solution. All drugs were dissolved to their final concentration in Ringer solution before use. The concentration of DMSO in those solutions was <0.1%. 0.1% DMSO alone had no effect on the 5-HT–induced responses.

Current Recording

Current was recorded using the whole-cell voltage-clamp configuration (Hamill et al., 1981) with an EPC-7 patch clamp amplifier (List Electronics, Darmstadt, FRG). The capacitive and series-resistance compensation (~80%) were usually used. Series resistances ranged from 2.5 to 10 MΩ. The recording pipettes pulled from VWR micropipettes (75-μl purple; VWR, Seattle, WA) had resistances of 1.3–3 MΩ when filled with solution I1. After leak compensation, the current signal was either low pass filtered at 500 Hz (−3dB point, 4-pole Bessel) and digitized with the BASIC-FASTLAB system (Indec Systems Inc., Sunnyvale, CA) in an IBM AT computer (IBM, Armonk, NY) or filtered at 10 kHz, digitized with a modified Sony PCM-501 (Unitrade, Philadelphia, PA), and stored on video tapes. The signals from the video tape were later played back through the PCM, refiltered at 10–1,000 Hz (−3dB), and sampled into the computer. The current was also recorded on a chart recorder (Brush Mark 280; Gould Inc., Cleveland, OH).

Cells were constantly perfused with bath solutions in a 100-μl sylgard chamber with a flow rate of 1–1.3 ml/min. In ion selectivity experiments cells were bathed in the reference solution most of the time and between each test solution. Reversal–potential measurements in the test solutions were usually made 1–2 min after switching from the reference solution to the test solutions. Complete exchange of bathing solutions was accomplished within 30–45 s, as judged from junction potential changes between the bath and recording pipette. The bath was connected to ground with an agar bridge (4% wt/vol in Ringer solution E1). In one series of anion selectivity experiments a 3-M KCl agar bridge was used. Cells were held at −75 mV unless specified otherwise. All experiments were done at room temperature (20–22°C).

Data Analysis

Analysis was performed off-line using the BASIC-FASTLAB program on an IBM AT computer. For fluctuation analysis the signals from the video tape were played back through the PCM, refiltered at 500 Hz (−3 dB, 4-pole Bessel), and sampled at 1 kHz. The digitized record was fragmented into a series of 1-s sections and those with obvious artifacts were discarded. Useable sections of data were then fitted with a second-order polynomial curve that was subtracted from the record to remove slow curvatures due to slow rising of the current, and the variance was calculated. The apparent single-channel current (i) was estimated from

the current noise using the relation (Neher and Stevens, 1977):

$$i = \sigma/I(1 - p) \tag{1}$$

where I is the mean current, σ is the current variance, and p is the channel open probability.

For selectivity analysis the current signal from the EPC-7 amplifier was filtered at 500 Hz (−3dB) and sampled at 1 kHz. Reversal potentials (E_r) for 5-HT–induced currents in various solutions were determined by applying a complex voltage pulse (Fig. 5 *B*, *bottom*) once every 600 ms before and during the 5-HT–induced response. The voltage pulse consisted of 18 symmetrical steps, each 10 ms long, first depolarizing then repolarizing, or vice versa, in intervals of 7–10 mV near the reversal potentials. Currents measured at the same voltage in one pulse were averaged and normalized by the current at the most negative step. In most experiments currents measured from three to five such voltage pulses were averaged and plotted versus voltage. The reversal potential was interpolated by fitting five to six points straddling E_r with a second-order polynomial. Since reversal potentials measured in various solutions were different, the range of the voltage steps was changed to obtain clear inward and outward currents in all solutions. Reversal potential measurements usually started 5 min after the whole-cell recording was established to allow sufficient time for the pipette solution to equilibrate with the cytoplasm. The reversal potential in the reference solution was measured between each test solution, and if E_r changed by as much as 4 mV the test measurement was discarded.

Relative permeabilities for monovalent cations and anions were calculated from changes of reversal potential upon switching from the reference solutions to the test solutions (Hille, 1971; Adams et al., 1980; Dwyer et al., 1980). 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 equation

$$\Delta E_r = E_{r,X} - E_{r,Na} = 2.30RT/F \log \frac{P_X/P_{Na}[X]_o + P_{Cl}/P_{Na}[Cl]_i}{[Na]_o + P_{Cl}/P_{Na}[Cl]_i} \tag{2}$$

where $2.30RT/F$ is 58.2 mV at 20°C, $[X]_o$ is the activity of X^+ in the test solution, $[Na]_o$ is the activity of Na^+ in the external reference solution, and $[Cl]_i$ is the activity of CL^- in the internal solution. Chloride was included in the equation because it had measurable permeability in the channel. In calculating the relative permeability for NMDG an upper limit of 0.0087 was obtained if CL^- was assumed to be impermeant.

The permeability ratios for anions were calculated using the equation

$$\Delta E_r = E_{r,X} - E_{r,glu} = 58.2 \log \frac{[Na]_i}{[Na]_i + P_X/P_{Na}[X]_o} \tag{3}$$

where $[Na]_i$ is the activity of Na^+ in the internal solution and $[X]_o$ is the activity of anion X^- in the external test solution. In deriving the equation it was assumed that the reference anion, glucuronate, was impermeant and that the contribution from NMDG was negligible since it was almost impermeant. Indeed, identical values of $P_{Cl}/P_{Na} = 0.04$ were obtained whether NMDG was taken into account or not.

For divalent ions and mixtures of divalent ions with NMDG, the following generalized Goldman-Hodgkin-Katz (GHK) equation (Goldman, 1943; Hodgkin and Katz, 1949) was used to calculate the permeability ratios,

$$0 = \sum_j P_j Z_j^2 \frac{E_r F^2}{RT} \frac{[j]_o - [j]_i \exp(z_j F E_r/RT)}{1 - \exp(z_j F E_r/RT)} \tag{4}$$

where RT/F is 25.3 at 20°C, P_j is the permeability for the jth relevant ion, Z_j is the valence, and $[j]_o$ and $[j]_i$ are the external and internal ion activities. All values given in the tables are apparently permeability ratios taking into account activity coefficients.

Activity coefficients for LiCl, NaCl, KCl, RbCl, CsCl, NH_4Cl, and the divalent salts in solutions containing only one electrolyte were interpolated from the tables of Robinson and Stokes (1965). Since the activity data for the other organic cations tested are not available, their activity coefficients were assumed to be equal to that of Na^+. The activity coefficients for the divalent salts (20 mM) in the mixtures were assumed to be equal to that of $CaCl_2$, which was ~0.533 as interpolated from the data in Table I of Butler (1968). Single-ion activity coefficient of a divalent ion X^{2+} was defined as the square of the mean activity coefficient of the salt according to the Guggenheim convention (Butler, 1968).

Membrane potentials and all reversal potential measurements were corrected for junction potentials between the recording pipette and bath solution, and between the bath solution and bath electrode. Junction potentials were measured with a ceramic junction, saturated KCl reference electrode (Corning Glass Works) using the EPC-7 Amplifier in the current clamp mode.

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

RESULTS

General Properties of the 5-HT-induced Currents

5-HT-induced currents. N18 cells were found to have a large current from 5-HT_3 receptors. Local application of 5-HT (10–20 μM) elicited rapid inward membrane current in >95% of cells. Peak currents ranged from 0.2 to 4 nA at −75 mV. The response was accompanied by a large increase in the membrane conductance (Fig. 1 *A*). During continued application of 5-HT the response showed rapid desensitization (Fig. 1 *B*). In most cells the time course of desensitization (with 10 μM 5-HT) could be fitted with a single exponential with a time constant of 3.9 ± 0.5 s (n = 32), similar to the minimum value of 6.5 s observed in N1E-115 cells (Neijt et al., 1989). In some cells, however, the decaying phase was better fitted with an additional, slower component.

The current–voltage (*I-E*) relation of the 5-HT–induced currents was studied in five cells with CsCl as the internal solution (I1). Currents elicited by brief (10 μM) 5-HT "puffs" at various holding potentials clearly reversed sign between −4 and 6 mV (Fig. 1 *C*). The interpolated E_r from the *I-E* relation in Fig. 1 *D* was −3.2 mV. The mean E_r measured this way for the five cells was −1.6 ± 0.8 mV. The peak *I-E* relation in Fig. 1 *D* shows moderate inward rectification, which persisted in the absence of internal divalent ions and when internal Cs^+ was replaced with K^+ or Na^+ (data not shown).

Pharmacological characterization of the 5-HT receptor. Currents elicited by 5-HT were completely blocked or significantly decreased by low concentrations (1–10 nM) of the 5-HT_3 receptor–specific antagonists ICS 205-930 (Fig. 2 *A*) or MDL 7222 (Fig. 2 *B*). The suppression was slowly reversible. Thus the response recovered partially 15 min after washout of MDL 72222 (Fig. 2 *B*) in one cell and fully recovered 30 min after washout of ICS 205-930 in another cell (Fig. 2 *A*). Curare, which blocks 5-HT_3 receptor–mediated responses in cultured hippocampal neurons and NG108-15 cells (with $IC_{50} \leq 1.5$ nM; Yakel and Jackson, 1988), also completely

blocked the 5-HT responses in N18 cells at 10 nM ($n = 4$). In contrast, the 5-HT$_1$/5-HT$_2$ receptor antagonist spiperone ($n = 8$) and the selective 5-HT$_2$ receptor antagonist ketanserin ($n = 5$) had little effect on the 5-HT response at concentrations of 1–1.3 μM (Fig. 2, *C* and *D*). A selective 5-HT$_3$ receptor agonist, 2-methyl-5-HT, elicited similar but smaller rapid responses at 100 μM (data not shown). These results indicate that the responses in N18 cells were mediated by 5-HT$_3$ receptors.

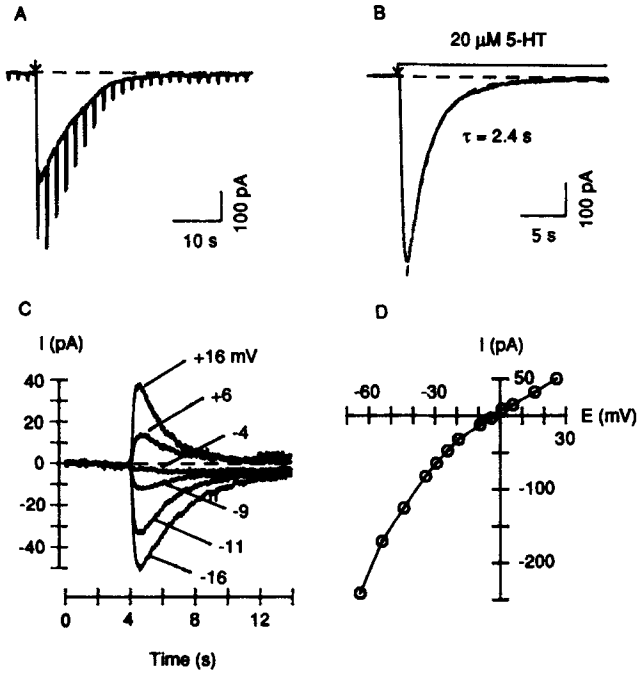

FIGURE 1. 5-HT–induced inward current. 10 μM 5-HT was delivered by pressure ejection (*arrow*). (*A*) Current induced by a brief (4 psi, 100 ms) application of 5-HT. The response was accompanied by a large increase in membrane conductance as reflected in the increase in current amplitude elicited by 300 ms, −20 mV constant negative voltage pulses. Holding potential (HP) = −75 mV. (*B*) Desensitization in the prolonged presence of 5-HT. The dashed curve is a single exponential fit with $\tau = 2.4$ s. HP = −75 mV. (*C*) 5-HT–induced currents at various holding potentials. The current reversed sign between +6 and −4 mV. (*D*) Peak current–voltage relation for the same cell as in *C*. Internal solution, I1; external solution, E1. Filtered at 10 Hz, sampled at 20 Hz.

Fast activation of the 5-HT response. Activation of the receptor-mediated currents was rapid, as expected if the receptor complex incorporates an ion channel instead of acting through second messenger pathways. With pressure application of agonist, the response developed with a delay of 25–200 ms and a time-to-peak of 50–1,000 ms. With ionophoretic application of 5-HT the current developed more rapidly with a delay of ~9 ms and reached a peak in <50 ms (Fig. 3). The average delay and time-to-peak of the response in four cells were 9.7 ± 1.8 and 63 ± 23 ms.

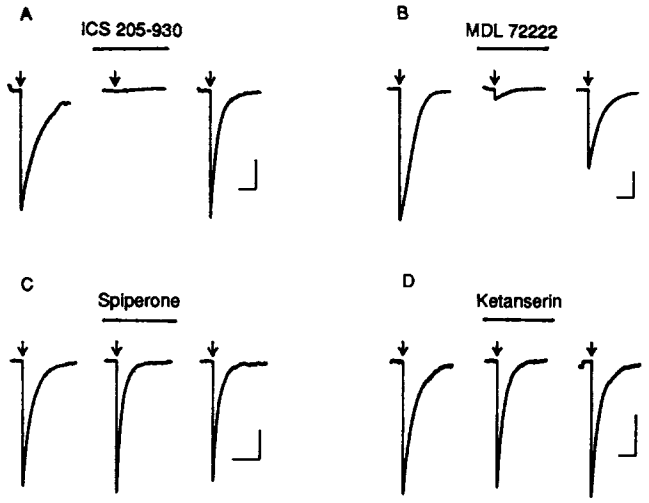

FIGURE 2. Pharmacological identification of the 5-HT receptor. 10 μM 5-HT was delivered by brief pressure pulse (*arrows*). The response was reversibly blocked by the 5-HT_3 receptor–specific antagonists (*A*) 2 nM ICS 205-930 or (*B*) 4 nM MDL 72222, but not by 5-HT_1 and 5-HT_2 receptor antagonists (*C*) 1 μM spiperone or (*D*) 1.3 μM ketanserin. The response recovered fully 30 min after washout of ICS 205-930 or partially 15 min after washout of MDL 72222. HP = −75 mV. Filtered at 10 Hz, sampled at 20 Hz. Same solutions as in Fig. 1. Calibrations: 400 pA (*A*, *B*, and *D*) or 200 pA (*C*)/20 s.

Several observations in addition suggest that no second messengers or G proteins were involved. The amplitude of 5-HT–induced currents remained relatively constant for 30 min to 2 h in cells recorded with internal solutions lacking ATP and GTP ($n = 20$). In some experiments cells were dialyzed with internal solutions containing 90 mM CsF ($n = 20$), 100 μM AlF_4^- ($n = 6$), or 100 μM GTPγS ($n = 6$) to activate all G proteins. This procedure produced no inward current by itself, yet the cells still responded to repetitive 5-HT application for up to 2 h.

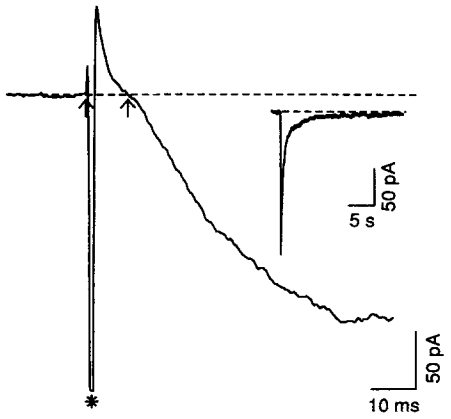

FIGURE 3. Time course of activation when 5-HT was delivered by ionophoresis from a high resistance electrode (~100 MΩ) filled with 100 mM 5-HT. * marks the artifact produced by the ionophoretic pulse (80 nA, 1 ms). The time measured between the arrows, which was 9.1 ms in this cell, was taken as the delay of the response. HP = −75 mV. Filtered at 1 kHz, sampled at 10 kHz. The inset shows the same response on a compressed time scale. Same solutions as in Fig. 1.

Fluctuation Analysis

The apparent single-channel conductance of the channel was estimated using fluctuation analysis, assuming that the whole-cell currents arise from identical and independent channels. To obtain a steady, nondesensitizing response suitable for noise analysis, a low concentration (0.5 μM) of 5-HT was applied by bath perfusion (Fig. 4 *A*). The current could be completely blocked by 10 nM ICS 205-930 ($n = 2$, data not shown). The rising phase of the response was accompanied by a small increase in current noise as revealed by the high-gain, AC-coupled records (Fig. 4 *B*). The variance of the current fluctuation increased linearly with the mean current

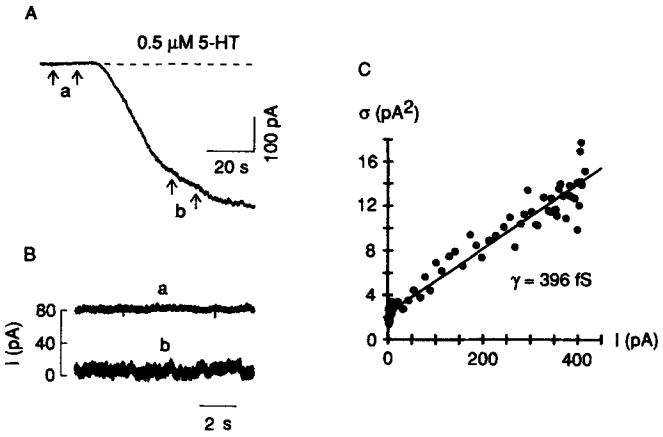

FIGURE 4. Estimation of single-channel conductance from fluctuation of the 5-HT–induced whole-cell current. (*A*) DC-coupled record of inward current induced by bath perfusion of 0.5 μM 5-HT. Arrows indicate the sections of the record shown in *B*. HP = −75 mV. Filtered at 10 Hz, sampled at 20 Hz. (*B*) High-gain, AC-coupled records (*a*) from control and (*b*) during the rising phase of the 5-HT current which was accompanied by an increase in membrane noise. Both records were filtered at 500 Hz, sampled at 1 kHz, and then digitally filtered to 500 Hz. (*C*) Plot of the variance of current noise vs. mean current. The single-channel current estimated from the least-squares linear fit ($r = 0.97$) was 0.029 pA, which corresponds to a single-channel conductance of 396 fS ($E_r = -1.6$ mV). The variance at the origin of the abscissa is the control variance in the absence of agonist. Same solutions as in Fig. 1.

(correlation coefficient $r = 0.97$), indicating that the open probability (p) was still low (Fig. 4 *C*). The single-channel current estimated from the slope of the variance–mean current plot was 29.1 fA, yielding a single-channel conductance of 396 fS ($E_r = -1.6$ mV). The same analysis done in eight cells ($r = 0.65$–0.97) gave single-channel conductances ranging from 346 to 1,040 fS and a mean of 575 ± 89 fS.

Ion Selectivity

The permeability ratios for a variety of ions were determined from reversal potetials, E_r, measured using the protocol illustrated in Fig. 5. The response shown in Fig. 5 *A*

(with E3 as external solution and I1 as internal solution) was elicited by a brief puff of 20 μM 5-HT mixed in the glucosamine solution. Fig. 5 *B* shows selected, superimposed current records in response to the voltage pulse drawn underneath. The currents reversed sign around the −5-mV voltage step. The average reversal potential interpolated from the *I-E* relationship in Fig. 5 *C* was −5.3 mV.

A concern for determining reversal potentials using this method is that the solution in the drug pipette, which was ejected together with 5-HT, would change the composition of the bathing solution and cause drift in the reversal potential. This

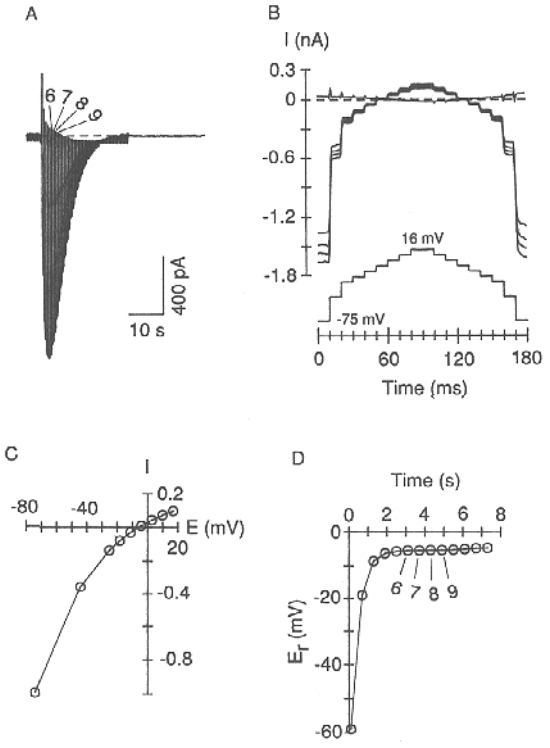

FIGURE 5. Method for measuring reversal potentials in ion selectivity experiments. (*A*) A complex voltage pulse (shown at the bottom of *B*) was given before and during the 5-HT–induced response every 600 ms. Each "spike" such as those numbered represented a current in response to the voltage pulse on a much compressed time scale. The first spike after the 5-HT puff was numbered 1. (*B*) Selected current records (numbers 6–9) in response to the voltage pulse are shown on an expanded time scale. The averaged control sweep is also shown. The voltage pulse started from −75 mV, depolarized step by step (each 10 ms long) to +16 mV, and then repolarized back to −75 mV. The 5-HT–induced current reversed sign near −5 mV. Filtered at 500 Hz, sampled at 1 kHz. (*C*) Averaged current–voltage relation obtained from records in *B*, as described in Methods. (*D*) Plot of reversal potential as a function of time after the 5-HT puff. The first four points were caused by transient dilution of the sodium bath solution by the glucosamine puff solution. HP = −75 mV. Internal solution, I1; external solution, E3.

was indeed observed as shown in the plot of the reversal potentials measured at different times after the 5-HT puff (20 μM 5-HT in glucosamine solution) (Fig. 5 *D*). The reversal potential measured immediately after the puff was −59 mV, close to that measured when glucosamine was the test solution (Table III). However, as the puff solution was washed away by superfusion with the Na^+ solution (E3), the reversal potential quickly approached its final value. Thus, in <2 s the reversal potential reached a value of −6.3 mV, and stabilized near −5.3 mV ~3 s after the puff. The effect of this transient drift was therefore minimized by starting the

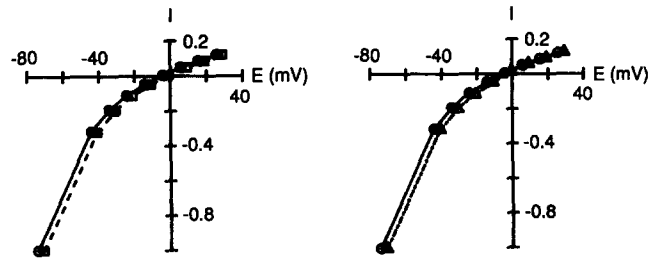

FIGURE 6. Current–voltage relations in reference Na^+ solution (O) and in Cs^+ (□) or K^+ (△) test solutions. *I-E* curves in both test solutions were shifted toward right. Reversal potential changed from −5.5 mV in Na^+ reference solution to −2.1 mV in the Cs^+ solution and −3.3 mV in the K^+ solution.

reversal potential measurements 3 s after 5-HT application. The average reversal potential of 43 cells measured under control ionic conditions was −5.6 ± 0.3 mV, which is close to the value of −1.6 mV measured with the holding potential method (Fig. 1, *C* and *D*). Theoretical calculation from the GHK equation for these ionic conditions gave a reversal potential of −2.7mV, using the permeability ratios for Cs^+ and Cl^- given in Tables II and V. The *I-E* relationship shown in Fig. 5 *C* also exhibited modest inward rectification similar to that in Fig. 1 *D*. Taken together, these results indicate that the voltage–pulse method described above gave fairly good measurements of reversal potentials.

Alkali metal ions are highly permeant. The 5-HT$_3$ receptor channel in N18 cells was basically nonselective among monovalent metal ions. There were only small poisitive shifts in the reversal potentials of the 5-HT–induced currents upon switching from the Na^+ reference solution to the Cs^+ and K^+ test solutions (Fig. 6), indicating that Cs^+ and K^+ were slightly more permeant than Na^+. The responses in the test solutions also exhibited inward rectification similar to that in the reference solution. Reversal potential changes and the calculated permeability ratios relative to Na^+ (Eq. 2) are listed in Table II. When concentration was used in the calculation, the permeability ratios for Cs^+, K^+, Li^+, and Rb^+ were 1.18, 1.08, 1.04, and 0.97. The selectivity sequence for the metal ions was $Cs^+ > K^+ > Li^+ \geq Na^+ \geq Rb^+$.

Many organic cations are permeant. I have examined permeabilities for nine saturated ammonium cations with molecular weights ranging from 18.0 to 196.2.

TABLE II

ΔE_r and Permeability Ratios for Alkali Metal Ions

X	ΔE_r ± SE	n	P_x/P_{Na}
	mV		
Cs^+	4.0 ± 0.2	7	1.22
K^+	1.9 ± 0.5	6	1.10
Li^+	0.9 ± 0.4	8	1.01
Na^+	0.0	—	1.00
Rb^+	−0.7 ± 0.8	6	0.99

Test and reference solutions: 150 mM XCl, 2 mM $CaCl_2$, 5 mM L-histidine, and 10 mM D-glucose, pH = 7.4. Internal solution, I1. P_X/P_{Na} calculation assumes $P_{Cl}/P_{Na} = 0.04$.

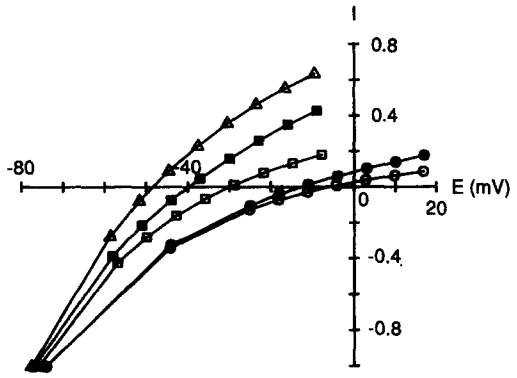

FIGURE 7. Current–voltage relations in different monovalent organic cation solutions. E_r = −5.5 mV in the Na^+ reference solution (○). The *I-E* curves were shifted more and more towards the left as the molecular weight of the test ion increased. Symbols and interpolated E_r: ●, dimethylamine, −12.8 mV; □, methylethanolamine, −30.0 mV; ■, diethanolamine, −39.8 mV; △, Tris, −48.5 mV. All curves are from one cell.

The reversal potential changes (Fig. 7), which were larger than with alkali cations, and the calculated relative permeabilities are summarized in Table III. Ammonium and methylamine were more permeant than Na^+. Six other organic cations were measurably permeant, with permeability ratios in the range of 0.08–0.81. Table III also gives the molecular weight for each test ion, which correlates well with the mean molecular size of the ion. The relative permeability decreases with increasing size of the ion.

Since Ca^{2+} and other divalent ions were quite permeant in this channel (see below), reversal potential measurements for large organic cations such as Tris, glucosamine, and NMDG were made in divalent cation–free test solutions. One series of experiments used a low Cl^- internal solution to minimize the contribution

TABLE III

ΔE_r and Permeability Ratios for Organic Cations

$\Delta E_r \pm$ SE	n	P_x/P_{Na}	M.W.	X
mV				
25.5 ± 0.8	7	2.84	18.0	Ammonium*
8.4 ± 0.4	9	1.41	32.1	Methylamine*
−5.0 ± 0.5	9	0.81	46.1	Ethylamine*
−8.3 ± 0.6	6	0.71	46.1	Dimethylamine*
−24.5 ± 1.1	5	0.36	76.1	Methylethanolamin‡
−36.2 ± 1.2	5	0.21	106.2	Diethanolamine‡
(−40.1 ± 1.7)	4	(0.20)	122.1	Tris (pH = 7.0)‡
−41.3 ± 0.8	4	0.17		
(−64.0 ± 0.8)	6	(0.076)	180.2	Glucosamine (pH = 6.3)‡
−59.2 ± 0.3	2	0.062		
(−120 ± 8.7)	3	(≤0.005)	196.2	*N*-Methyl-D-glucamine‡

*Reference solution: E2, pH = 7.2. Test solutions: 150 mM XCl, 2 mM $CaCl_2$, 5 mM L-histidine, 10 mM D-glucose, pH = 7.2. ‡Reference solution: E3, pH = 7.2. Test solutions: 150 mM XCl, 5 mM L-histidine, 12 mM D-glucose, pH = 7.2 or as indicated. Internal solution: I1, but E_r and P_X/P_{Na} in parentheses were obtained with I2 as internal solution. Calculation of P_X/P_{Na} assumes $P_{Cl}/P_{Na} = 0.04$.

from Cl^-, which was also slightly permeant. The relative permeabilities for Tris and glucosamine with different internal Cl^- concentrations were similar, indicating that possible artifacts from Cl^- were small. The errors from other sources (e.g., incomplete solution exchange) were negligible, since the reversal potential could be shifted by more than −120 mV in the NMDG test solution.

Divalent cations are fairly permeant. Relative permeabilities for Ca^{2+}, Mg^{2+}, and Ba^{2+} were examined in either 100 mM divalent chloride solutions or 20 mM divalent chloride and 120 mM NMDG chloride mixtures. 5-HT–induced currents were always small in these solutions, a phenomenon similar to that observed in the nicotinic AChR (e.g., Adams et al., 1980; Imoto et al., 1986). Extracellular concentrations of Ca^{2+} as low as 2 mM reduced the current (Fig. 8 *A*). Both the amplitude and duration of the response were greatly enhanced when extracellular Ca^{2+} was

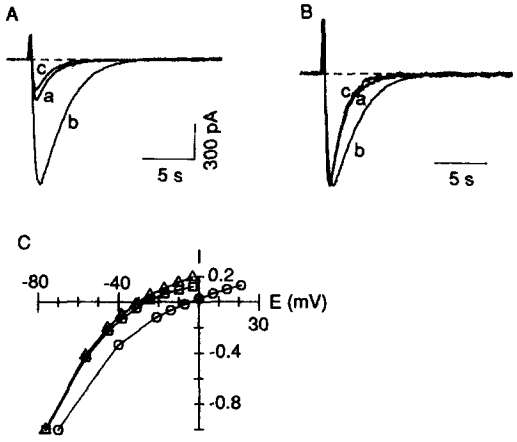

FIGURE 8. (*A*, *B*) Effect of external Ca^{2+} on the 5-HT–induced response. (*A*) 5-HT–induced currents in extracellular solutions with 2 mM Ca^{2+} (*a*), no added Ca^{2+} (*b*), and again with 2 mM Ca^{2+} (*c*). The amplitude of the response increased by ~210% in the absence of external Ca^{2+}. No other divalent cations were added in the solutions. (*B*) The same responses were scaled to the same size to compare the decay phase. The decay time constant increased by 80% in the absence of external Ca^{2+}. Internal solution, I1. Filtered at 10 Hz, sampled at 20 Hz. (*C*) Current–voltage relation in the Na^+ reference solution (○) and in 100 mM Ca^{2+} (□) or 100 mM Mg^{2+} (△) test solutions. The interpolated reversal potentials were −25.9 mV and −29.7 mV for Ca^{2+} and Mg^{2+}, respectively.

removed (Fig. 8 *A*). The prolongation was evident when the responses were scaled to the same peak size (Fig. 8 *B*). On average, the amplitude and decay time of the 5-HT–induced currents were increased by 90 ± 15% (n = 10) and 68 ± 12% (n = 4), respectively. Similar blocking effect of divalent cations has also been reported in N1E-115 neuroblastoma cells (Peters et al., 1988). Block of the 5-HT$_3$ receptor channels by Ca^{2+} appeared to be voltage independent, since inward rectification of the current–voltage relations of the responses was similar in the presence (e.g., Figs. 1 *D* and 6) or absence of 2 mM external Ca^{2+} (e.g., Fig. 5 *C*). The reversal potential of the 5-HT–induced response in Ca^{2+}-free solution was −5.2 ± 0.4 mV (n = 10), almost identical to the value of −5.4 ± 0.4 mV obtained in solutions with 2 mM Ca^{2+}, indicating that the selectivity of the channel was not changed by such low concentrations of Ca^{2+}.

Despite their blocking effects, divalent cations were quite permeant by the reversal-potential criterion. N18 cells have both T- and L-type–like calcium channels, which could be partially activated by the voltage protocol used to measure reversal potentials of the 5-HT–induced currents. To minimize the contamination from current in calcium channels, 300 μM $CdCl_2$ was included in the $CaCl_2$ and $BaCl_2$ test solutions to block the L-like calcium channels, and a 100-ms prepulse to 0 mV was used to inactivate the T-like calcium channels. The current–voltage relations for 5-HT–induced currents in Na^+ reference solution and 100 mM Ca^{2+} or Mg^{2+} test solutions, with I3 as internal solution, showed similar inward rectification (Fig. 8 *C*). The reversal potentials for the divalent ions were more negative than for Na^+. The mean absolute reversal potentials and calculated permeability ratios (using ion activities) in mixtures of 20 mM divalent and 120 mM NMDG chloride or in 100 mM divalent ion solutions are compared in Table IV. For comparison with other work

TABLE IV

E_r and Permeability Ratios for Divalent Cations

X	20 mM X*			100 mM X‡		
	$E_r \pm$ SE	n	P_x/P_{Na}	$E_r \pm$ SE	n	P_x/P_{Na}
	mV			*mV*		
Ca^{2+}	−38.1 ± 0.6	8	1.12	−23.3 ± 1.0	8	0.53
	(−41.0)			(−20.0)		
Ba^{2+}	−44.4 ± 0.5	7	0.79	−29.8 ± 0.3	7	0.39
	(−48.0)			(−27.0)		
Mg^{2+}	−45.9 ± 1.0	6	0.73	−26.5 ± 1.1	6	0.43
	(−47.0)			(−25.0)		

Reference solution: E2, pH = 7.4. Internal solution: I3. *Test solutions: 20 mM XCl_2, 120 mM NMDG-Cl, 5 mM L-histidine, and 10 mM D-glucose, pH = 7.4. ‡Test solutions: 100 mM XCl_2, 5 mM L-histidine, and 10 mM D-glucose, pH = 7.4. Calculation of P_X/P_{Na} assumes $P_{Cl}/P_{Na} = 0.04$. E_r in parentheses are theoretical values calculated as described in the Discussion for the model with assumed negative surface charge density of 0.0025e/Å^2 and intrinsic $P_{Ca}/P_{Na} = 0.37$, $P_{Mg}/P_{Na} = 0.27$, and $P_{Ba}/P_{Na} = 0.25$.

permeability ratios were also calculated using concentrations. The relative permeabilities for Ca^{2+}, Ba^{2+}, and Mg^{2+} were 0.42, 0.30, and 0.27 in the mixtures and 0.19, 0.13, and 0.16 in the pure solutions. These values are very close to those reported for the endplate AChR channel where concentrations were used in the calculations (Adams et al., 1980). The difference in apparent permeabilities when divalent ions were diluted from 100 to 20 mM is examined in the Discussion.

Small anions are slightly permeant. Relative permeabilities for small anions such as F^-, Cl^-, and NO_3^- were also examined by measuring reversal potential changes when the external anion was changed. Glucuronate, which has a molecular size slightly greater than that of glucosamine, was assumed to be impermeant and served as the reference anion. As would be expected if small anions were slightly permeant, the measured reversal potential changes were much larger than predicted from the small activity differences of the cations (Table V). In another series of experiments the relative permeability of Cl^- was further examined when the junction potential

difference between the reference solution and test solution was reduced from −16 mV to −1 mV using a 3-M KCl agar bridge as the bath electrode. The reversal potential change relative to the reference solution obtained from these experiments was −8.2 ± 1.9 mV (n = 6), yielding a permeability ratio (P_{Cl}/P_{Na}) of 0.04. This is identical to that obtained with the normal bath electrode. Therefore, P_{Cl}/P_{Na} of 0.04 was used in calculations of all the cation permeability ratios already discussed.

DISCUSSION

Time Course of the Response

5-HT$_3$ receptors mediate rapid responses in a variety of cells (Wallis and North, 1978; Higashi and Nishi, 1982; Surprenant and Crist, 1988; Yakel and Jackson, 1988; Neijt et al., 1989). My results indicate that N18 neuroblastoma cells also have a large 5-HT$_3$ receptor–mediated current, since the pharmacological profile satisfies the three criteria proposed by Bradley et al. (1986) for 5-HT$_3$ receptor–mediated responses. The time course was fast compared with that mediated by 5-HT$_1$ or 5-HT$_2$

TABLE V

ΔE_r and Permeability Ratios for Anions

X	[X]	ΔE_r ± SE	n	P_x/P_{Na}
	mM	*mV*		
NO_3^-	147	−14.4 ± 1.9	7	0.08
Cl^-	147	−8.3 ± 0.6	7	0.04
F^-	147	−6.1 ± 1.1	5	0.03

Reference solution: E4, pH = 7.1. Internal solution: I4. Calculation of P_X/P_{Na} assumes that glucuronate is impermeant. ΔE_r was corrected for junction potentials, which ranged from −11.6 to −16 mV (pipette negative) when the normal agar bath electrode was used. However, ΔE_r was not corrected for the small activity differences of Na^+ and Tris ions among the solutions.

receptors (VanderMaelen and Aghajanian, 1980; Colino and Halliwell, 1987; Davies et al., 1987; Yakel et al., 1988). When 5-HT was applied rapidly the response could be activated with a minimal delay of 7.5 ms and a rise time of 35 ms. Some of the delay was probably due to diffusion of the agonist both because the drug pipette was usually 2–3 μm away from the cell and because the receptors are distributed diffusely over the cell. Therefore the delay and rise time obtained in my experiments must underestimate the speed of activation of the receptors. A similar time course has been reported for 5-HT$_3$ receptor–mediated responses in NG108-15 cells (Yakel and Jackson, 1988). These fast kinetics suggest that a multistep activation by second messengers or G proteins is unlikely. The fastest such response described so far is the activation of cardiac inward rectifying K^+ channels by muscarinic receptors, which are believed to be coupled to the channels through a G protein (Pfaffinger et al., 1985; Yatani et al., 1987; Logothetis et al., 1988). Although no apparent soluble second messengers are involved in this response, there is still a minimal delay of ~30 ms and a slow rise time of >200 ms (Osterrieder et al., 1981). Activation of the cardiac Ca^{2+} channels by β-adrenergic receptors, which are also believed to be

coupled to the channels by a direct G protein pathway, had similar time courses with a delay of 29 ms and an activation time constant of 150 ms (Yatani and Brown, 1989). The fast opening of the 5-HT_3 receptors, therefore, suggests that these receptors are ligand-gated channels. The observations that 5-HT–induced responses did not require internal nucleotides and persisted with internal dialysis of G protein activators are also consistent with this suggestion.

Comparison with Other 5-HT_3 Receptor Channels

The 5-HT_3 receptor channels in N18 cells are quite selective for cations over anions, but nonselective among small cations. 5-HT–induced responses 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 permeability to both Na^+ and K^+. Lambert et al., (1989) estimated a permeability ratio of 1.09 for both K^+ and Cs^+ in 5-HT–gated channels in N1E-115 cells, which is quite close to those in N18 cells (Table II). In contrast, K^+ is apparently more permeant than Na^+ in submucous plexus neurons with a P_K/P_{Na} of ~2.3 (Derkach et al., 1989).

My results provide the first direct evidence that 5-HT_3 receptor channels have measurable permeability to Ca^{2+} with a permeability ratio similar to that in the endplate AChR channel (Lewis, 1979; Adams et al., 1980). Substantial whole-cell current was observed in 20- and 100-mM $CaCl_2$ solutions. Therefore, there should be a small influx of Ca^{2+} under physiological conditions. Since Ca^{2+} regulates many intracellular processes, Ca^{2+} influx through 5-HT_3 receptor channels may be physiologically important. In the frog neuromuscular junction, for example, Ca^{2+} flowing through the AChR channels can regulate desensitization of the ACh receptors (Cachelin and Colquhoun, 1989).

The current–voltage relation of 5-HT–induced response showed modest inward rectification (e.g., Figs. 1 *D*, 5 *C*, and 6). The extent of rectification was not altered by replacing Cs^+ in the internal solution with either K^+ or Na^+, or by removal of internal Ca^{2+} ions. This suggests that the rectification was not a result of voltage-dependent block of the 5-HT–gated ion channel by Cs^+ or Ca^{2+} from the inside. Inward rectification persisted in various extracellular solutions, even in solutions containing 100 mM divalent cations (Fig. 8). It remains to be determined whether the rectification is an intrinsic property of the open channel or a result of voltage-dependent gating. Similar rectification has been reported for 5-HT_3 receptor–mediated responses in cultured hippocampal neurons and NG108-15 cells (Yakel and Jackson, 1988), and in N1E-115 and NCB-20 cells (Lambert et al., 1989).

Despite similarities among various 5-HT_3 receptors, there is a major difference in single-channel conductance between neuroblastoma cells and enteric neurons. My fluctuation analysis in N18 cells suggested a single-channel conductance of 575 fS, close to the value of 310 fS reported in N1E-115 neuroblastoma cells using the same method (Lambert et al., 1989). In contrast, single-channel recordings from outside-out membrane patches from guinea pig submucous plexus neurons showed channels with conductances of 15 and 9 pS (Derkach et al., 1989). An underestimation of single-channel conductance using fluctuation analysis could occur if the channel has several subconductance levels or fast flickerings (Fenwick et al., 1982). In addition,

the existence of multiple types of channels is not excluded. Nevertheless, the significant differences in single-channel conductance and ion selectivity between neuroblastoma cells and enteric neurons suggest the existence of different types of 5-HT$_3$ receptor channels in the two cells. It would be interesting to know the biophysical properties of 5-HT–gated ion channels in other systems.

Ion Selectivity and Ion Permeation

5-HT$_3$ receptor channels in N18 cells discriminate weakly among small monovalent metal ions with a selectivity sequence of $Cs^+ > K^+ > Li^+ \geq Na^+ \geq Rb^+$. Although this sequence is not exactly the order of free-solution mobilities of the ions, the permeability ratios are so close to each other that they suggest that the channel is a large, water-filled pore. This notion is further supported by the observation that large organic cations such as Tris and glucosamine are substantially permeant (Table III). The experiments with organic cations suggest that the size of the ion is the major determinant of permeability. Fig. 9 plots my permeability ratio measurements

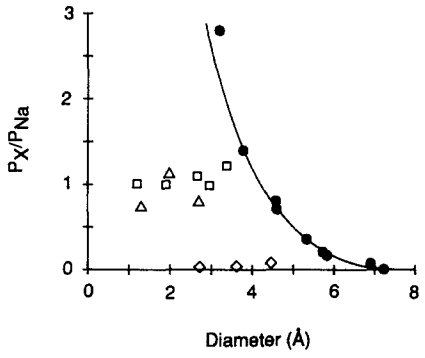

FIGURE 9. Relation between relative permeability and mean ionic diameter for permeant cations and anions. Ionic crystal radii are from Pauling (1960). Geometric mean diameter for organic cations were obtained from space-filling molecular models. The solid line represents Eq. 5. It was scaled according to the permeability ratio for methylethanolamine, and assumes a 7.6 Å diameter of the open pore. □, Monovalent metals; △, alkaline earths; ●, organic cations; ◊, anions.

for all tested ions against the ionic crystal diameter for metal ions and anions (Pauling, 1960) or the geometric mean diameter for organic cations (from space-filling molecular models). Cations up to the size of glucosamine are permeant. If the 5-HT–gated channel is assumed to be a cylindrical pore then the minimum pore size of the open channel should be bigger than the diameter of glucosamine, which is ~7.1 Å. The minimum pore size has been estimated for other ion channels by measuring relative permeabilities for ions of different sizes and modeling the channel as a cylindrical pore (Hille, 1971, 1973; Dwyer et al., 1980; McCleskey and Almers, 1985; Bormann et al., 1987; Vyklicky et al., 1988). With this approach and a pore with a diameter of d permeated by spherical ions of diameter a, the permeability decreases because the area available for diffusion becomes smaller by $(1 - a/d)^2$ (the "excluded volume effect"), and the frictional forces become larger in proportion to $1/a$ (Dwyer et al., 1980). According to this theory, the relative permeabilities are described by

$$P_X/P_{Na} = \frac{21.3}{a}\left(1 - \frac{a}{7.6}\right)^2 \quad (5)$$

The function was plotted in Fig. 9 (solid line) to fit the measured data for the organic cations. The open pore size deduced from the fit is 7.6 Å, similar to the value of 7.4 Å for the endplate AChR channel (Dwyer et al., 1980).

Divalent cations were quite permeant in the 5-HT_3 receptor channel with relatively small differences in their permeabilities (Table IV). This is consistent with a large water-filled pore. However, the apparent permeability ratios for divalent cations appeared to decrease as the concentrations increased (Table IV), a phenomenon that has been observed in the AChR channel at the neuromuscular junction and described as an effect of the negative surface potential (Lewis, 1979; Adams et al., 1980). The variation of the permeability ratios for divalent ions in the 5-HT–gated channels can also be partially accounted for by the surface potential theory proposed by Lewis (1979). Thus if a negative surface charge density of $0.0025e/Å^2$ is assumed, the surface potential changes from −24 mV in 100-mM divalent chloride solutions to −33 mV in 20-mM divalent and 120-mM NMDG chloride mixtures, as calculated from the Grahame (1947) equation using ion concentrations. The reversal potentials in parentheses in Table IV were calculated from the GHK equation modified for the effect of surface potentials (Lewis, 1979). To fit the measured reversal potentials best, intrinsic permeability ratios of 0.37, 0.27, and 0.25 are assumed for Ca^{2+}, Mg^{2+}, and Ba^{2+}. Although the theoretical reversal potentials did not fit the experimental data perfectly, they were much closer to the measured values than those predicted with no assumed surface charge. The existence of a negative local potential is also consistent with the result that the channel prefers cations over anions.

A weak discrimination between monovalent and divalent metal ions suggests that the narrow portion of the channel wall lacks high field strength charges (Hille, 1984). However, the divalent permeability sequence of $Ca^{2+} > Ba^{2+} \geq Mg^{2+}$, which is different from the aqueous mobility sequence $Ba^{2+} > Ca^{2+} > Mg^{2+}$, suggests some interactions between the divalent ions and the channel. One possibility is that the divalent ions bind to the negative charges in the channel wall with different affinities, thereby reducing the negative surface potential to different extents (Hille et al., 1975).

Comparison with Other Ligand-gated Channels

The selectivity of the 5-HT–gated ion channels for cations is very similar to that of the AChR channel at the neuromuscular junction (e.g., see Adams et al., 1980 and Dwyer et al., 1980): both channels discriminate poorly among alkali metal ion, are similarly permeable to divalent cations, and have a very similar deduced open pore size. However, there are some major differences between the two channels. Whereas the single-channel conductance of the nicotinic AChR ranges from 40 to 60 pS in mammalian muscles (Sakmann et al., 1985; Mishina et al., 1986), the unitary conductance of the 5-HT_3 receptor channel in N18 cells is only ~0.6 pS. Furthermore, anions are not measurably permeant in the nicotinic AChR channel (e.g., Adams et al., 1980), whereas small anions are slightly permeant in the 5-HT–gated channel.

Why does the 5-HT_3 receptor channel have such a small conductance yet a large open pore size? Many factors can influence the conductance of an ion channel

beside the minimum open pore size, such as the length of the narrow region of the pore, the net charge on the channel wall near the mouth of the pore (Imoto et al., 1988), and the size and shape of the vestibules (Dani, 1986). Thus a long narrow region of the pore, less net negative charge on the channel wall near the narrow region, and a long and narrow vestibule could all contribute to the small single-channel conductance of the 5-HT–gated channel in N18 cells. Indeed, rings containing negatively charged amino acid residues were found to be major determinants of conductance in *Torpedo* nicotinic AChR channel (Imoto et al., 1988). Reducing the total number of net negative charges in the rings reduced the single-channel conductance. Perhaps in the 5-HT$_3$ receptor channel there are fewer negatively charged amino acid residues, or they are placed unfavorably near the mouth region of the pore. This would also explain the slight permeability to small anions in the 5-HT–gated channel, since less repulsion would be experienced by the permeant anions. A small, nonselective conductance is not unique to the 5-HT$_3$ receptor channels in N18 cells. Kainate-activated channels, which in some cells have low conductances of 0.14–4 pS (Ascher and Nowak, 1988; Cull-Candy et al., 1988), have in cultured neurons cation permeabilities and open pore size similar to that of the endplate nicotinic AChR channel (Vyklicky et al., 1988).

In summary, my results indicate that the 5-HT$_3$ receptors in N18 cells are ligand-gated channels with very low unitary conductance. The channel has similar cation selectivity and open pore size to that of the endplate nicotinic AChR. Ionic interactions in the 5-HT–gated channel may also be similar to those in the nicotinic channel. However, the small single-channel conductance and the slight anion permeability of the 5-HT–gated channel suggest that distinct structural differences may exist between the two channels.

I am grateful to Dr. Bertil Hille for advice, assistance, and support during all stages of this work, to Drs. David Beech, Laurent Bernheim, Martha Bosma, Alistair Mathie, Amy Tse, and Lonnie Wollmuth for their helpful discussions and comments on the manuscript, to Ms. Lea Miller for secretarial help, to Don Anderson for technical assistance, and to Ms. Laurie Maechler and Dr. William A. Catterall for help with and facilities for culturing cells.

This work was supported by National Institutes of Health research grant NS-08174 and a Research Award from the McKnight Endowment for the Neurosciences to Dr. Bertil Hille.

Original version received 16 April 1990 and accepted version received 18 June 1990.

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