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KINETICS OF THE MEMBRANE CURRENT MEDIATED BY SEROTONIN 5-HT$_3$ RECEPTORS IN CULTURED MOUSE NEUROBLASTOMA CELLS

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SUMMARY

1. Ionic currents mediated by serotonin 5-HT$_3$ receptors were studied in the mouse neuroblastoma cell line N1E-115, using suction pipettes for intracellular perfusion and voltage clamp recording. The dependence of the kinetics of the membrane current on serotonin concentration was investigated.

2. At a holding potential of -70 mV application of 5-HT (5-hydroxytryptamine creatinine sulphate) causes a transient inward current. The i–V curve of the peak amplitude is linear between -80 and 60 mV. The reversal potential is 20 ± 4 mV (mean $\pm$ S.D.). The kinetics of the transient ionic current are independent of the holding potential.

3. In the presence of 5-HT the membrane current decays to a small steady-state level with a single-exponential time course. The time constant of decay decreases with increasing concentration of the agonist, to a minimum value of $6{\cdot}5 \pm 1{\cdot}5$ s for concentrations of 5-HT $\geqq 3$ μM.

4. When the agonist is rapidly removed, single-exponential decay of the ionic current is observed. The time constant of this decay in the absence of 5-HT amounts to $6{\cdot}9 \pm 1{\cdot}5$ s and is independent of the membrane potential and of the concentration of 5-HT used.

5. In the presence of low concentrations of 5-HT the peak amplitude of the inward current evoked with a high concentration of agonist is gradually reduced. The onset of this desensitization follows the same time course as the decay of the membrane current. In the range from 0·7 to 1·5 μM-5-HT both kinetic processes show the same steep concentration dependence.

6. Recovery from desensitization, measured at variable intervals after removal of the agonist, can be fitted by a single-exponential function with a time constant of 18 ± 4 s.

7. The results show that the kinetic properties of the 5-HT$_3$ receptor-mediated ionic current can only be described by a complex, co-operative model.

INTRODUCTION

Recently, 5-HT receptors have been newly classified into 5-HT$_1$, 5-HT$_2$ and 5-HT$_3$ subtypes (Bradley, Engel, Feniuk, Fozard, Humphrey, Middlemiss, Mylecharane,

Richardson & Saxena, 1986). At present, 5-HT_3 receptors (formerly 5-HT_M) have been demonstrated in the enteric nervous system, in both sympathetic and parasympathetic autonomic neurones, in sensory neurones and in cultured neuroblastoma cells (Richardson & Engel, 1986; Neijt, Vijverberg & van den Bercken, 1986; Richardson & Buchheit, 1988). The presence of 5-HT_3 receptors in the mammalian brain has been suggested (Costall, Domeney, Kelly, Naylor & Tyers, 1987; Hagan, Butler, Hill, Jordan, Ireland & Tyers, 1987) and has recently been demonstrated using radioligand binding (Kilpatrick, Jones & Tyers, 1987).

Cultured mouse neuroblastoma cells of the clone N1E-115 constitute the first preparation in which 5-HT_3 receptors were successfully demonstrated by both functional and radioligand binding studies (Neijt *et al.* 1986; Hoyer & Neijt, 1988). Application of 5-HT to N1E-115 cells induces a membrane depolarization associated with a transient increase of the inward current. These responses are blocked by the selective 5-HT_3 antagonists ICS 205-930 and MDL 72222 in the nanomolar concentration range and are mimicked by the selective agonist 2-methyl-5-HT (Neijt, te Duits & Vijverberg, 1988). In addition, 5-HT_3 recognition sites have been characterized in N1E-115 cells with the radioligand [^{3}H]ICS 205-930 (Hoyer & Neijt, 1988).

The membrane current mediated by 5-HT_3 receptors is a fast response, which is subject to rapid desensitization. The ionic channel involved is presumed to be a fairly non-selective cation channel with a large conductance of 140 pS (MacDermot, Higashida, Wilson, Matsuzawa, Minna & Nirenberg, 1979; Guharay, Ramsey & Usherwood, 1985; Neijt *et al.* 1988). In neuroblastoma cells 5-HT_3 receptors seem not to be coupled to adenylate cyclase, guanylate cyclase or phospholipase C cascades nor to G protein-mediated mechanisms (MacDermot *et al.* 1979; Hoyer & Neijt, 1988). Further knowledge of the coupling between the 5-HT_3 receptor and its associated ionic channel is still lacking.

In order to characterize further the coupling between the 5-HT_3 receptor and the associated ionic channel, we have studied the kinetics of the ionic current and of the desensitization process in cultured N1E-115 neuroblastoma cells under voltage clamp conditions.

METHODS

Cell culture

Experiments were performed using cells of the mouse neuroblastoma line N1E-115 (Amano, Richelson & Nirenberg, 1972). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2. The culture medium used was Dulbecco's modified Eagle medium supplemented with 7·5% fetal calf serum as well as the antibiotics penicillin (100 i.u./ml) and streptomycin (100 μg/ml) and the following amino acids (mM): cysteine.HCl, 0·3; L-alanine, 0·4; L-asparagine, 0·45; L-aspartic acid, 0·4; L-proline, 0·4; and L-glutamic acid, 0·4. Cells of passages 25–50 were plated in 3·5 cm tissue culture dishes on day 1 and differentiation was initiated by supplementing the culture medium with 1 mM-dibutyryl-cyclic-AMP and 1 mM-3-isobutyl-1-methylxanthine on day 3. Cells were used for experiments between day 7 and 12. The culture medium was refreshed every 2–3 days.

Electrophysiological recordings

Ionic currents were measured under voltage clamp combined with perfusion of single cells according to methods previously developed (Lee, Akaike & Brown, 1980; Ruigt, Neijt, van der Zalm & van den Bercken, 1987). Fire-polished suction pipettes (tip diameter, 8–12 μm; resistance, 400–600 kΩ) were used to obtain tight seals (30–150 MΩ) between the pipette and the cell. The

membrane in the tip of the pipette was ruptured spontaneously or by injecting high-frequency electric current. Throughout the experiment the pressure applied to the pipette was maintained at −3 to −6 kPa using a vacuum pump. The pipette was continuously perfused with internal solution through a metal capillary inserted close to the tip allowing a rapid exchange of the internal solution of the cell. The platinized tip of the capillary also served as a current injection electrode. Membrane potential was measured by an Ag–AgCl electrode, connected to the pipette solution by a 150 mM-KCl–agar bridge. The external solution of the cell was clamped at virtual ground. The liquid junction potential at the pipette tip was compensated before each experiment and remained constant to within 2 mV. Membrane current was measured with a current-to-voltage converter incorporated in the virtual ground circuit. To hold the membrane at −70 mV a steady current of −0·1 to −2·5 nA was injected. Series resistance, estimated from the instantaneous voltage jump in response to a constant current stimulus, was compensated under voltage clamp conditions for about 85%. Membrane currents were low-pass filtered (−3 dB at 1 kHz, 12 dB/octave), digitized (8 bits, 1024 points/record) and stored on magnetic disc for off-line computer analysis.

Solutions

The ionic composition of the pipette (internal) solution was (mM): potassium glutamate, 100; Na-HEPES, 20; sucrose, 120. The pH was adjusted to 7·25 with L-glutamic acid. The external solution contained (mM): NaCl, 125; KCl, 5·5; HEPES, 20; $CaCl_2$, 1·8; $MgCl_2$, 0·8; glucose, 24; sucrose, 37. The pH was adjusted to 7·3 by addition of about 7·5 mM-NaOH.

Application of drugs

Ionic currents were induced by superfusion of the whole cell with external solution containing various concentrations of 5-HT. A capillary, with an internal diameter of 1 mm, was positioned opposite to the glass pipette at a distance of approximately 1 mm from the cell. This capillary was connected to the common outlet of a servo-motor-operated 4-way valve (Hamilton HVPD4-5). Total dead volume of the capillary and the tubes was 30 μl. At the minimum flow rate of 1 ml/min the time needed for exchange of solutions was approximately 2 s. In the figures the application of drugs indicated has been corrected for the delay caused by the dead volume of the superfusion system. Concentrated 5-hydroxytryptamine creatinine sulphate (5-HT) was diluted in the external solution prior to the experiment. Unless noted otherwise, cells were stimulated with 5-HT at an interval of at least 100 s. All experiments were performed at room temperature (20–24 °C).

Data analysis and statistics

Exponential functions were fitted to the experimental data using a Levenberg–Marquardt non-linear least-squares algorithm (Marquardt, 1963). Results are presented as mean ± standard deviation (S.D.) and were compared using the Student's t test and the Student's t test for paired observations where appropriate. Linearity was tested by least-squares regression analysis (Diem & Lentner, 1968).

RESULTS

Current–voltage relationship

Neuroblastoma N1E-115 cells, voltage clamped at negative membrane potentials, responded to 5-HT with a transient inward current. In large diameter cells ($\geqslant 40$ μm), voltage clamped at a holding potential of −70 mV, the maximum peak amplitude of the inward current evoked by concentrations of 5-HT between 10 and 30 μM was 23 ± 13 nA ($n = 25$) with a maximum of 60 nA.

The current–voltage relationship of the membrane current induced with 2 μM-5-HT was studied in five cells at holding potentials ranging from −80 to +60 mV. Examples of inward and outward currents are shown in the inset of Fig. 1. The amplitude of the responses varied from cell to cell. Therefore, the results have been normalized, i.e. the peak amplitudes of inward currents have been divided by the

peak amplitude at −70 mV for each cell, to obtain the general current–voltage relationship depicted in Fig. 1. Linear regression analysis showed that the current–voltage relationship is linear over the potential range studied ($P = 0{\cdot}63$). The reversal potential of the 5-HT-induced membrane current is 20 mV with 95% confidence limits of approximately 4 mV.

The reversal potential of the 5-HT-induced current has also been estimated by recording the membrane current while the membrane potential was changed within 2 s from −60 to 90 mV by seventy-five steps of 2 mV/step. The membrane current

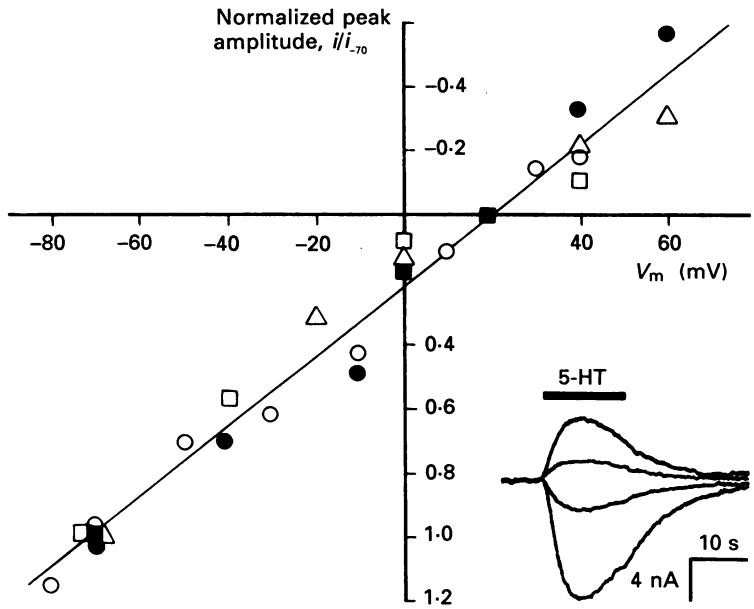

Fig. 1. The *i–V* relationship of the ionic current induced with 2 μM-5-HT in voltage clamped neuroblastoma cells. Amplitudes were normalized for each cell to the amplitude of the inward current induced at −70 mV. A different symbol is used for each cell ($n = 5$). Linear regression yielded a reversal potential of 20 mV. The inset shows a family of superimposed recordings of 5-HT-induced membrane currents at holding potentials of 60, 30, 0 and −30 mV from top to bottom, respectively. Inward currents are downward deflections.

thus obtained in the absence of 5-HT was subtracted from that recorded during a response induced with 3 μM-5-HT. The shape of the resulting curve varied with the time course of the 5-HT-induced current during the 2 s depolarization period and was considered to be of no significance. However, the intercept with the abscissa is an estimate of the reversal potential of the 5-HT-induced current. By this procedure a reversal potential of 20 ± 4 mV ($n = 5$) was calculated. This value is identical to that obtained from the data in Fig. 1.

The decay of the response

In the continued presence of 5-HT, the increase of the membrane current was always followed by an exponential decrease of the inward current (Fig. 2*A*). The

membrane current declined to a non-zero steady level, remained constant in the presence of 5-HT for as long as 13 min and rapidly decayed to base level, when the agonist was removed (Fig. 2*B*). The steady current was not always detectable and the maximum amplitude observed was 2·0 nA (Fig. 2*B*).

The time constant of exponential decay (τ_{dec}) was related to the concentration of 5-HT applied. The higher the concentration used, the more rapidly the membrane current decreased. In three cells responses induced with 0·7 μM-5-HT were recorded.

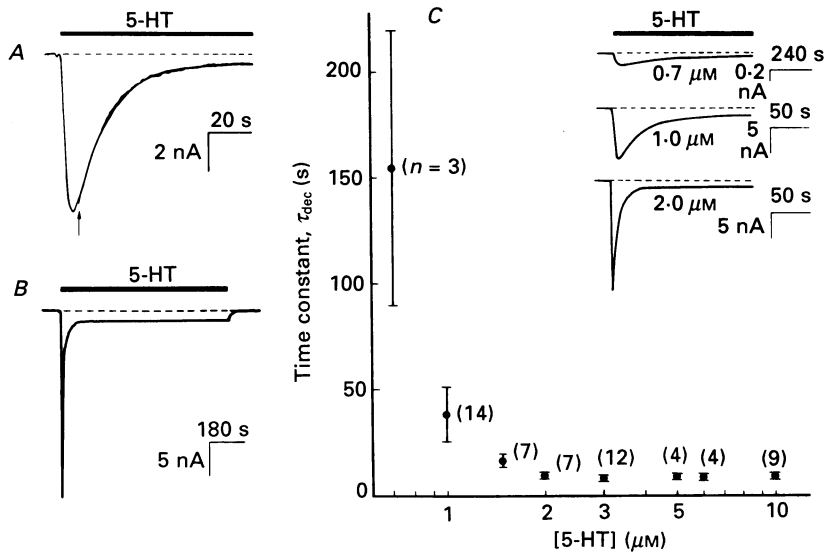

Fig. 2. Time course of the 5-HT-induced inward current and its dependence on agonist concentration. *A*, superfusion of the cell with 1·5 μM-5-HT initially induced an increase of the membrane current which subsequently declined to a small steady level. The decay of the response was fitted by a single-exponential function. The exponential curve is drawn starting at the arrow (τ_{dec} = 14 s). *B*, an inward current, induced with 3 μM-5-HT, which declined to a steady level. After 5-HT was removed the membrane current declined to base level. *C*, relationship between τ_{dec} and the concentration of 5-HT used to induce the membrane current. Shown are mean ± S.D. The number of observations is given within parentheses. At high concentrations the curve approaches a constant level. The inset shows three recordings of inward current induced with the concentrations of 5-HT indicated.

The mean peak amplitude and the mean time constant of the decay of inward currents evoked at a membrane potential of −70 mV were 3·5 ± 2·1 nA and 155 ± 65 s. At lower 5-HT concentrations the amplitude of the inward current became too small to estimate reliably the time constant of decay. Responses induced with 1·0, 1·5 and 2·0 μM-5-HT yielded a τ_{dec} of 38 ± 13 (n = 14), 16 ± 3 (n = 7) and 8·1 ± 1·7 s (n = 17), respectively (Fig. 2*C*). Concentrations of 5-HT higher than 3 μM failed to cause a further decrease of τ_{dec}. The average value of τ_{dec} obtained at these high concentrations was 6·5 ± 1·5 s (n = 29).

In four cells the effect of membrane potential on τ_{dec} was studied. The inset of Fig. 3 shows traces of an inward and an outward current evoked with 2 μM-5-HT in the same cell at holding potentials of −70 and 40 mV, respectively. The time constants (τ_{dec}) were identical within experimental error. Figure 3 also shows that

τ_{dec} was independent of the holding potential in the range of −100 to 60 mV. Regression analysis showed no deviation from linearity ($P = 0{\cdot}53$) and no regression ($P = 0{\cdot}92$).

Reversal of activation

When 5-HT was washed out, the membrane current rapidly returned to base level. This has already been shown in Fig. 2*B* and will be referred to as reversal of activation. Figure 4*A* shows that the reversal of activation can be fitted by a single-exponential function. The time constant of this process, τ_{ra}, was not significantly

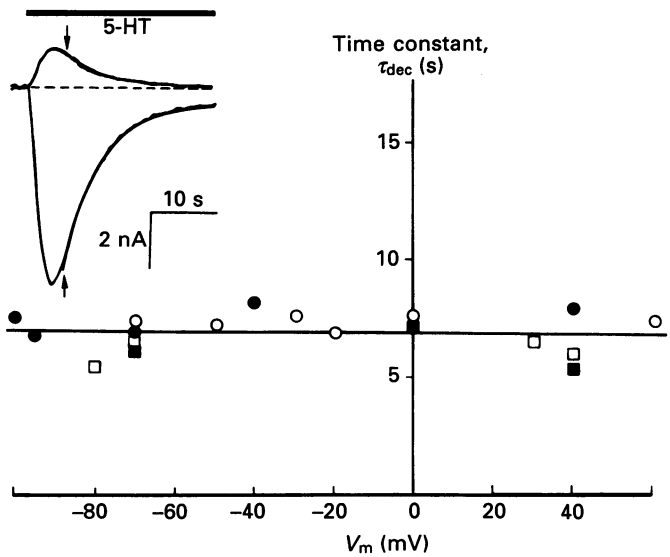

Fig. 3. Relation between inward current decay and membrane potential. The τ_{dec} of responses induced with 2 μM-5-HT is not significantly altered by changing the membrane potential within a range of −100 to 60 mV. For each cell a different symbol is used. The inset shows responses induced in a single cell at membrane potentials of −70 and 40 mV. For each response the single-exponential function was fitted and drawn starting from the arrow. Time constants were 5·4 s and 5·7 s, respectively.

affected by variation of the agonist concentration (Fig. 4*B*; *P* values for linearity and no regression were 0·90 and 0·64 respectively). Reversal of activation after application of the partial agonist dopamine (Neijt *et al.* 1986) was approximately 4 times faster than that after application of 5-HT to the same cell (not shown). This indicates that the rate of reversal of activation was related to the agonist applied and that, in the case of 5-HT, it was not limited by the rate of exchange of the external solution. In four experiments, in which 5-HT was superfused for 5–40 s, an effect of the superfusion period on τ_{ra} was not observed (*P* values for linearity and no regression were 0·35 and 0·82, respectively). Changes of the holding potential also did not affect τ_{ra} (Fig. 4*C*). The mean value of τ_{ra} was $6{\cdot}9 \pm 1{\cdot}5$ s ($n = 45$).

Onset of desensitization

Rapid desensitization is one of the characteristics of responses mediated by 5-HT_3 receptors. The rate of onset of desensitization has been investigated by superfusing

cells over different periods with low concentrations of 5-HT ($\leqq 1{\cdot}5\mu$M) and subsequently over a short period with a high concentration (5 μM). The desensitization was quantified by dividing the peak amplitude of the current evoked immediately after superfusion with a conditioning concentration of 5-HT by the peak amplitude of the control response (Fig. 5*A* and *B*). The time constant of the onset of desensitization (τ_{od}) has been obtained by fitting a single-exponential function to the normalized peak amplitudes as a function of the period of desensitization. Figure 5*C* shows

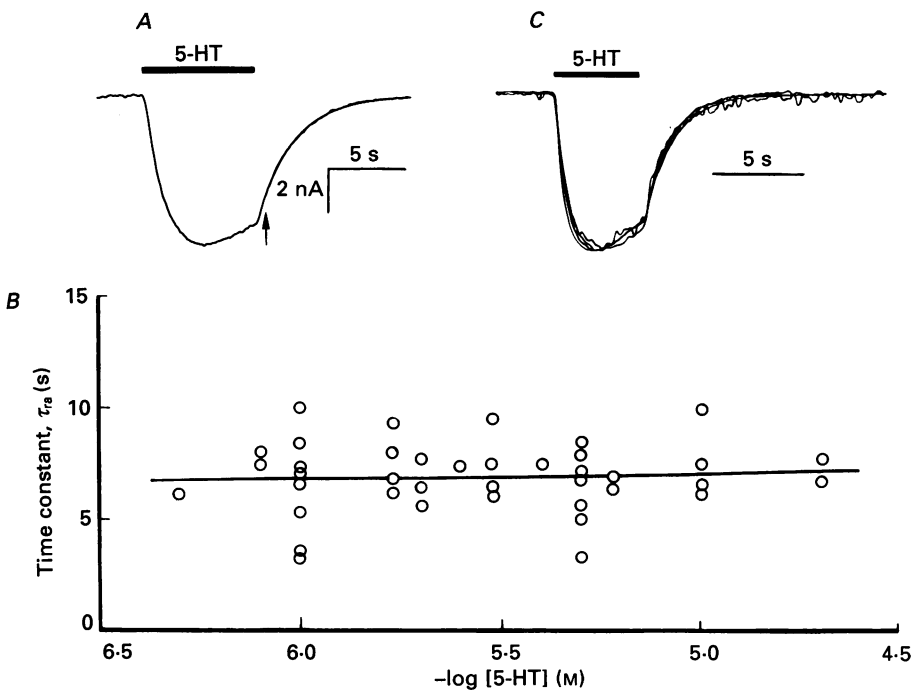

Fig. 4. *A*, a response induced with 1 μM-5-HT. Upon rapid removal of the agonist the inward current declined exponentially to base level. The fitted single-exponential curve with a time constant τ_{ra} of 3·2 s is also drawn starting from the arrow. Membrane holding potential was −60 mV. *B*, relation between the time constant of reversal of activation and the concentration of 5-HT. Results obtained from forty-five cells are depicted. The estimated mean time constant is 6·9 ± 1·5 s. *C*, four superimposed recordings of membrane currents induced in a single cell with 1 μM-5-HT. Currents were induced at membrane potentials of −90, −60, −30 and 10 mV and normalized to their peak amplitudes. This illustrates that τ_{ra} is not related to the membrane holding potential.

that the rate of onset of desensitization depended on the concentration of 5-HT. Each curve is drawn according to a single-exponential function fitted to the data of one experiment. The mean values obtained for τ_{od} in the presence of 0·3, 0·7, 1·0 and 1·5 μM-5-HT were 1130 ± 500 ($n = 3$), 260 ± 180 ($n = 3$), 59 ± 4 ($n = 3$) and 17 ± 3 s ($n = 4$), respectively. In the same cells, the concentrations of 0·7, 1·0 and 1·5 μM-5-HT not only induced desensitization, but also a transient increase of the inward current. The corresponding mean values of τ_{dec} obtained from these experiments were 155 ± 65, 57 ± 9 and 16 ± 3 s, respectively. A comparison (paired *t* test)

of these τ_{od} and τ_{dec} values revealed that there was no significant difference between the two time constants (P values ranged from 0·21 to 0·70).

Possible effects of desensitization on the decay of the inward current were also investigated. Control responses evoked with 5 μM-5-HT were compared with responses induced with the same concentration of 5-HT after cells were desensitized by 45 ± 6% ($n = 4$) by pre-exposure to 1·5 μM-5-HT. The τ_{dec} values obtained from control and partially desensitized inward currents were 7·6 ± 2·9 and 7·5 ± 2·4 s, respectively. Apparently, the rate of decay of the inward current was not affected by desensitization.

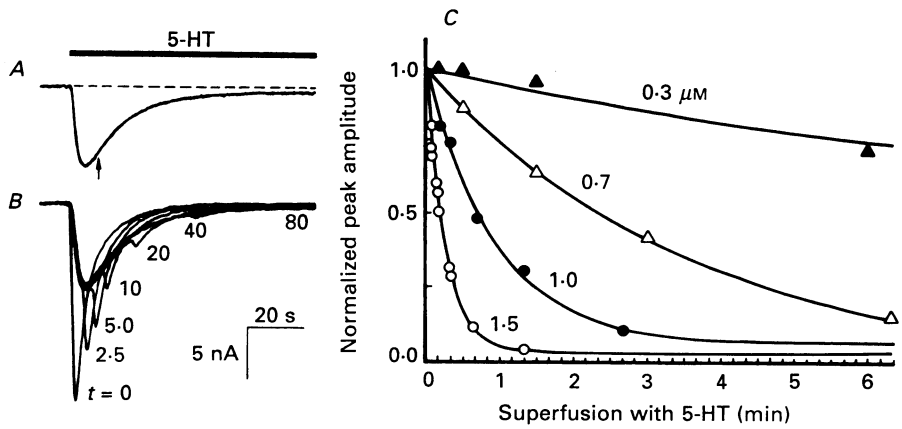

Fig. 5. *A*, inward current induced with 1·5 μM-5-HT at a membrane potential of −70 mV. The single-exponential function fitted to the decay is also drawn, starting from the arrow. The time constant τ_{dec} = 14 s. *B*, inward currents evoked with 5 μM-5-HT for 3 s immediately after superfusion with 1·5 μM-5-HT for 0, 2·5, 5·0, 10, 20, 40 and 80 s, as indicated. The seven recordings are superimposed. The single-exponential fitted to the peak amplitudes of the responses evoked with 5 μM-5-HT yielded a time constant for the onset of desensitization, τ_{od} = 15 s. *C*, the rate of onset of desensitization decreased when lower concentrations of 5-HT were used. Desensitization was induced using 1·5 (○), 1·0 (●), 0·7 (△) and 0·3 μM-5-HT (▲). The time constants (τ_{od}) obtained from the exponential curves shown are 16, 61, 210 and 1120 s, respectively.

Recovery from desensitization

Desensitization is a reversible process. Upon removal of 5-HT the receptors return to a non-desensitized state, from which they can be reactivated. After cells were desensitized by exposure to 3 μM-5-HT for at least 40 s, recovery from desensitization was enforced by superfusing with control external solution for a variable time interval. The remaining desensitization was measured by inducing a response with 3 μM-5-HT and by normalizing the peak amplitude of this inward current to that of the non-desensitized response. In Fig. 6 these normalized values are plotted as a function of the time interval during which 5-HT was washed out. After 2 min the recovery from desensitization was complete. The time constant of recovery, τ_{rd}, was obtained from the single-exponential function fitted to the data. The mean of the τ_{rd} values obtained in seven experiments was 18 ± 4 s.

The rate of recovery from desensitization was not affected by the length of the

period of desensitization. In one cell τ_{rd} was measured after 45 and 90 s of desensitization with 3 μM-5-HT. The time constants obtained were 11 and 12 s, respectively, two values which are equal within experimental error. In another four cells complete desensitization was induced for both a 45 and a 90 s period.These cells were allowed to recover from desensitization for a period of 17 s. No significant difference was observed between the amounts of recovery from a desensitization (paired t test; $P = 0{\cdot}32$). Even after a period of 900 s of desensitization, the amount of recovery obtained in a 17 s period of washing was not significantly different from the result obtained after 45 s of desensitization ($P = 0{\cdot}65$; $n = 3$). In similar experiments

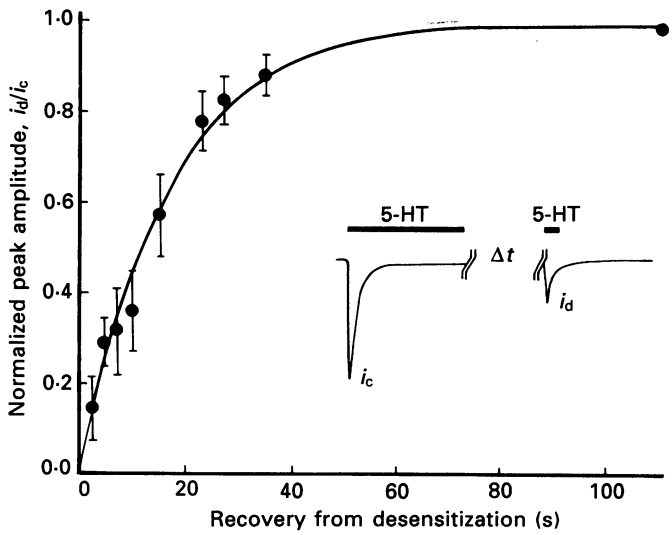

Fig. 6. Kinetics of recovery from desensitization induced by superfusion with 3 μM-5-HT for 40–90 s. After a period of recovery (Δt = 2–120 s) an inward current was evoked with 3 μM-5-HT. Desensitization was quantified by measuring the amplitude of this response, and normalizing to the control value (i_d/i_c; see inset). The results shown were obtained from seven cells. Depicted are mean $\pm$ S.D. (for Δt = 14 s, $n = 7$; otherwise $n = 3$).The single-exponential function fitted to these data is also depicted. The estimated time constant τ_{rd} = 17 s.

possible effects of the membrane potential on the rate of recovery from desensitization were investigated. The amount of recovery at $t = 17$ s, measured at holding potentials of -75 and -40 mV in one cell at -100 and -60 mV in another two cells, were identical (paired t test; $P = 0{\cdot}99$; $n = 3$). In another experiment the time constant τ_{rd} was estimated by fitting an exponential curve to the data of recovery from desensitization at variable time intervals obtained at holding potentials of -100 and -60 mV. The two values of τ_{rd} thus obtained were both 16 s.

DISCUSSION

The present results show that the kinetics of the ionic current mediated by 5-HT$_3$ receptors in neuroblastoma N1E-115 cells depend on the concentration of the agonist

and not on membrane potential. In addition, three major functional states of the ionic channel are to be distinguished: a resting closed state in the absence of 5-HT, as well as an open state and a desensitized closed state in the presence of 5-HT. From available evidence presented in the Introduction, as well as from the facts that internally perfused cells maintain a steady response amplitude and can be desensitized for long periods without adverse effects, it can be concluded that the ionic channels investigated are most probably directly coupled to 5-HT_3 receptors.

The amplitude of the ionic current is linearly related to membrane potential, which is consistent with previous findings from whole-cell voltage clamp and single-channel experiments (Guharay *et al.* 1985). The ionic current evoked by 5-HT application to N1E-115 cells is said to be mediated by non-selective cation channels, which are equally permeable to sodium and potassium ions (Peters & Usherwood, 1983; Guharay *et al.* 1985). From the present observed reversal potential of 20 mV it can be calculated (Goldman, 1943; Hodgkin & Katz, 1949) that the ionic channels are 2·4 times more permeable to sodium than to potassium ions. Conductance-ratio measurements in N1E-115 cells have shown that during activation of 5-HT_3 receptors by the partial agonist dopamine (cf. Neijt *et al.* 1986) the increase of the membrane sodium conductance is 2·7 times greater than that of the potassium conductance (Kato & Narahashi, 1983). A sodium/potassium conductance ratio of 2·3 was obtained in voltage clamped rabbit nodose ganglion cells stimulated with 5-HT (Higashi & Nishi, 1982).

Application of 5-HT causes a steep increase of the inward current, reflecting rapid opening of ionic channels. In the continued presence of a constant concentration of 5-HT the inward current subsequently decays towards a small steady-state value with a single-exponential time course. The onset of desensitization and the time constant of decay show the same concentration dependence in the range from 0·7 to 1·5 μM-5-HT. Although it was impossible to obtain paired estimates of τ_{od} and τ_{dec} outside this concentration range, the similarity suggests that the rate limiting step for the onset of desensitization is the same as that for the decay of the inward current.

Any model involving both fast transitions, which are beyond detection with the present technique, and a slow transition between the different states of the receptor and the ionic channel would predict single-exponential kinetics, i.e. would show a single relaxation time following a perturbation of the agonist–receptor equilibrium. It has been pointed out before (Katz & Thesleff, 1957; Sheridan & Lester, 1977) that sequential models, in which receptor occupation is followed by opening of the ionic channel and by desensitization, predict that the onset of desensitization is invariably faster than the recovery from desensitization. In addition, sequential models predict that the relaxation rate increases linearly with the agonist concentration and the same relation holds for simple cyclic models at low concentrations of the agonist (Katz & Thesleff, 1957; Sheridan & Lester, 1977). Figure 7 shows the relation between 5-HT concentration and the relaxation rate of the 5-HT_3 receptor-mediated inward current obtained from the measured τ_{dec} and τ_{od} values. Near the origin a sigmoid shape is clearly observed, which is at variance with the predictions for both the sequential and the simple cyclic models.

The saturation of the relaxation rate at high concentrations 5-HT in Fig. 7 shows

that the kinetics are determined by at least one agonist concentration-dependent step, i.e. receptor occupation, and one or more transitions that are independent of the agonist concentration, i.e. conformational changes or isomerization steps (Peper, Bradley & Dreyer, 1982). The sigmoid onset indicates some kind of positive co-operativity analogous to co-operative enzyme kinetics (Dixon & Webb, 1979). The same authors argue that the relative overall reaction rate can be described by the fractional saturation function used in binding studies, if the substrate binding steps remain at equilibrium during the reaction. The single-exponential kinetics indicate that the rate of occupation of 5-HT$_3$ receptors greatly exceeds the rate of desensitization, similar to results obtained with acetylcholine receptors in fast

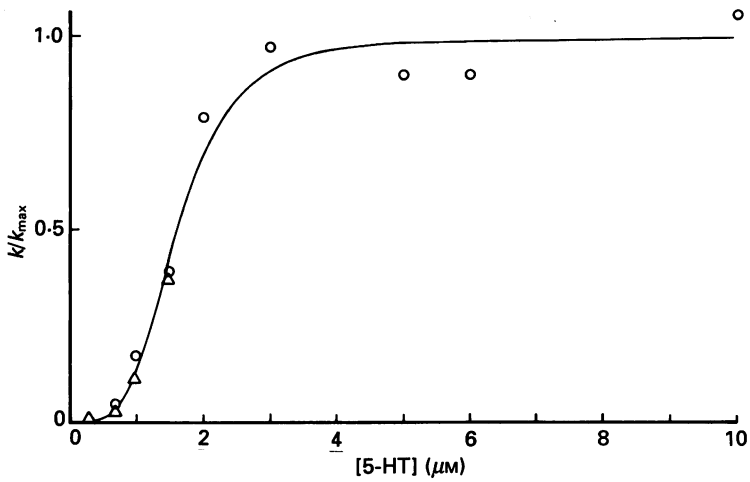

Fig. 7. Relation between the rate of desensitization and the concentration of the agonist 5-HT. Data are reciprocal mean values of the time constants of the onset of desensitization (△; see Fig. 5*C*) and of the decay of inward current (○; see Fig. 2*C*). The parameters of the line drawn according to the function $k = k_{max}/\{1+(K/[5\text{-HT}])^n\}$ are $k_{max} = 0{\cdot}16\ s^{-1}$; $K = 1{\cdot}62\ \mu M$ and $n = 3{\cdot}83$. These values were obtained by minimizing χ^2 (i.e. the sum of squares weighted by the rate values). This allowed a better fit of the sigmoid onset than by minimizing the sum of squares. The results of the latter procedure are presented in the text.

kinetic studies (Chabala, Gurney & Lester, 1985). Fitting the data by the equation $k = k_{max}/\{1+(K/[5\text{-HT}])^n\}$ (cf. Higashi & Nishi, 1982; Neijt *et al.* 1988) yielded the following parameter estimates: $k_{max} = 0{\cdot}15 \pm 0{\cdot}005\ s^{-1}$; $K = 1{\cdot}59 \pm 0{\cdot}05\ \mu M$ and $n = 4{\cdot}90 \pm 0{\cdot}80$ (mean ± estimated S.D.) with a standard error of fit of 0·009 (9 degrees of freedom). Positive co-operativity has also been observed for the dependence of the inward current amplitude on 5-HT concentration in N1E-115 cells (Neijt *et al.* 1988) and in rabbit nodose ganglia (Higashi & Nishi, 1982). Thus far, ligand-binding experiments have revealed only a single class of 5-HT$_3$ recognition sites in N1E-115 cells, but a co-operative interaction of agonists with these sites could not be excluded (Hoyer & Neijt, 1988). On the basis of the kinetic properties of the receptor-mediated ion current it can be concluded that a complex co-operative model is needed for an adequate description of the functioning of the 5-HT$_3$ receptor. Further whole-

cell voltage clamp and single-channel experiments are necessary to describe the functioning of this receptor in more detail.

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