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Ligand-gated ion channels opened by 5-HT in molluscan neurones

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1 5-Hydroxytryptamine (5-HT) activated a fast (70 ms to half maximum) and desensitizing inward current through non-selective channels conducting predominantly monovalent cations in neurones of *Helix aspersa*.

2 α-Methyl-5-HT was equipotent with 5-HT in activating this current, but the known selective agonists at vertebrate 5-HT_3 receptors, 2-methyl-5-HT and arylbiguanides were ineffective (<100 μM). 5-Methoxytryptamine which is inactive on vertebrate 5-HT_3 receptors was a very weak agonist.

3 The responses were antagonized by the specific vertebrate 5-HT_3 receptor blocker MDL-72222 ($IC_{50}=1$ μM), but were only weakly affected by ondansetron (10 μM). The 5-HT_2-type antagonist, ketanserin (<5 μM), had no effect. The responses were also antagonized by the non-specific antagonists (+)-tubocurarine and strychnine.

4 Unitary currents through channels non-selective for monovalent cations, and with a conductance of 2pS, could be activated repeatedly by 5-HT or α-methyl-5-HT in outside-out patches from neurones exhibiting the fast 5-HT-activated current (I[5-HT]$_{fast}$), even in the presence of 500 μM GDP-[βS] in the recording pipette. This strongly supports direct-gating of these channels by 5-HT. The properties of these unitary currents resembled those of I[5-HT]$_{fast}$.

5 The pharmacological properties of this molluscan 5-HT-operated, ligand-gated channel differed sufficiently from known vertebrate 5-HT_3-type receptors to suggest that it represents a new class of 5-HT receptor.

Keywords: 5-HT_3 receptor; ligand-gated; molluscan neurones; 5-hydroxytryptamine

Introduction

Early work showed that synaptically released, and exogenously applied, 5-hydroxytryptamine (5-HT) can exert several different actions on molluscan neurones, including a fast depolarizing response (see e.g. Gerschenfeld & Stefani, 1966; Cottrell & Macon 1974; Gerschenfeld & Paupardin-Tritsch 1974; Vehovszky & Walker, 1991). Since then, studies on vertebrate species have demonstrated the existence of many types of 5-HT receptor based on mechanistic information, drug susceptibilities, and structure (reviewed in Hoyer *et al.*, 1994). One of these, the 5-HT_3 receptor, is known to be a ligand-gated ion channel (Yakel & Jackson 1988; Derkach *et al.*, 1989; Maricq *et al.*, 1991; Peters *et al.*, 1992).

Like the response mediated by vertebrate 5-HT_3 receptors, the fast depolarizing response induced by 5-HT in molluscan neurones rapidly desensitizes, results from an increased permeability to monovalent cations and is blocked by (+)-tubocurarine. Here we have investigated whether these rapid 5-HT-induced responses in molluscan neurones also result from the direct activation of a ligand-gated ion channel, and we have tested the effectiveness of some known agonists and antagonists of vertebrate 5-HT receptors, with a particular emphasis on those which interact with the 5-HT_3 receptor type.

Methods

Experiments were carried out on neurones in the medial and caudal corner of the visceral ganglion of *Helix aspersa*. We have mainly used two neurones at the locations of E5 and E6 as described by Kerkut *et al.* (1975) (which we refer to as E5 and E6), and also for some fewer recordings E4. Each of these neurones shows large depolarizing responses to 5-HT. Most experiments were done on E5, which is distinguished by a particularly fast and completely desensitizing response to 5-HT. Neurones were dissected free from connective tissue. For patch clamp experiments they were exposed to 0.1% trypsin to facilitate giga seal formation.

Currents from perikarya were recorded by discontinuous single electrode voltage-clamp method using a Dagan 8100. Unitary currents were recorded with standard techniques and an Axopatch-200 (Axon Instruments) integrating amplifier. Unitary current amplitudes were measured manually on a Nicolet digital storage oscilloscope. The integrated size of multichannel responses following 5-HT application to outside-out patches was calculated as the area under the trace and above the mean basal current level.

The physiological solution had the following composition (mM): NaCl 90 (or 94.5), KCl 5 (or 0.5), $MgCl_2$ 5, $CaCl_2$ 7, HEPES 20, with the pH adjusted to 7.4 with NaOH. Reduction of $[K^+]_o$ to 0.5 mM for patch clamp experiments was found to be advantageous for reducing interference from spontaneous unitary current activity. For experiments with a low sodium solution, NaCl was omitted and the solution was buffered with 20 mM Trizma mixture. The osmolarity was maintained by adding sucrose. The bath was continuously perfused throughout the experiments, with complete exchange taking about 2 min. The standard pipette solution for the patch clamp experiments (high Cs^+) contained (mM): NaCl3, CsCl 90, $MgCl_2$ 1, EGTA 5, HEPES 20, with the pH adjusted to 7.4 with CsOH (used where not stated otherwise). For the high sodium and high potassium pipette solutions, CsCl was replaced with either NaCl or KCl and the pH adjusted with NaOH or KOH as appropriate. In some experiments, GTP-[γS] or GDP-[βS] (Sigma), prepared as a stock solution of 10 mM in distilled deionized water and kept frozen at −10°C, was added to the patch pipette solution to yield a final con-

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centration of 500 μM. Stock solutions of all drugs were prepared in distilled deionized water, kept frozen and diluted with physiological solution just before use.

5-HT creatinine sulphate and α-methyl-5-HT maleate were applied by pressure ejection (175 kPa for 2–20 ms) of various concentrations up to 100 μM. Antagonists were applied to isolated patches by local perfusion from a separate pipette placed nearby, and to whole cells by the same technique or by bath perfusion. When applying antagonists locally the micropipette which contained the antagonist was removed immediately prior to pressure ejection to avoid direct interference with the flow of agonist over the cell.

Results

5-HT responses in E4, E5, and E6

Application of 5-HT to neurones E4, E5 and E6 of *Helix* gave rise to large (up to 10 nA) inward current responses resembling the fast A-type response described by Gerschenfeld and Paupardin-Tritsch (1974). The tendency of the responses to desensitize varied significantly. In neurones E4 and E6 the response only partially desensitized when repeated doses of 5-HT were given at 5 s intervals, whilst in E5 this protocol led to essentially completely desensitization of the response. The rate of onset also differed. In E5 the time to half maximum amplitude was relatively brief (70 ms in some cases mean 122±9.3 ms (s.e.mean), $n=10$), whilst in E4 and E6 the onset of the response was somewhat slower (363±42.5 ms (s.e.mean), $n=8$) (Figure 1a and 1b). It is unlikely that the rise time of the response is significantly limited by the rate of drug delivery since (i) many receptors are on the neuronal somata and therefore readily accessible to the applied drug, and (ii) responses recorded under similar conditions, to dopamine applied by pressure ejection in another *Helix* neurone had significantly faster rise times than was ever seen with 5-HT in this study (30 ms to half maximum amplitude, Green *et al.*, 1996).

As we wished to investigate whether the fast response (which we refer to as I[5-HT]$_{fast}$) results from activation of ligand-gated ion channels similar to the 5-HT$_3$ receptors of vertebrate neurones, we undertook most experiments on E5 which showed responses with the most rapid rise time, essentially complete desensitization, and apparently little contamination by other non-desensitizing current components (e.g. the cell in Figure 1a).

Ionic-dependence of I[5-HT]$_{fast}$

The current/voltage relationship of I[5-HT]$_{fast}$ indicated an equilibrium potential close to 0 mV with standard physiological solution, and with potassium acetate in the recording electrode (Figure 1c). When external sodium was iso-osmotically replaced by sucrose, I[5-HT]$_{fast}$ was eliminated except at the most hyperpolarized values of holding potential as would be expected for currents through non-selective cation channels. When calcium was removed from the external solution, with either magnesium elevated to 12 mM, or sucrose added, to maintain the osmolarity, I[5-HT]$_{fast}$ was reduced to 60% of the control. This could be due either to a significant permeability of the channels to calcium, or an effect on binding of 5-HT to its receptor. This is in contrast to the 5-HT$_3$ receptor in neuroblastoma cell lines where both calcium and magnesium ions

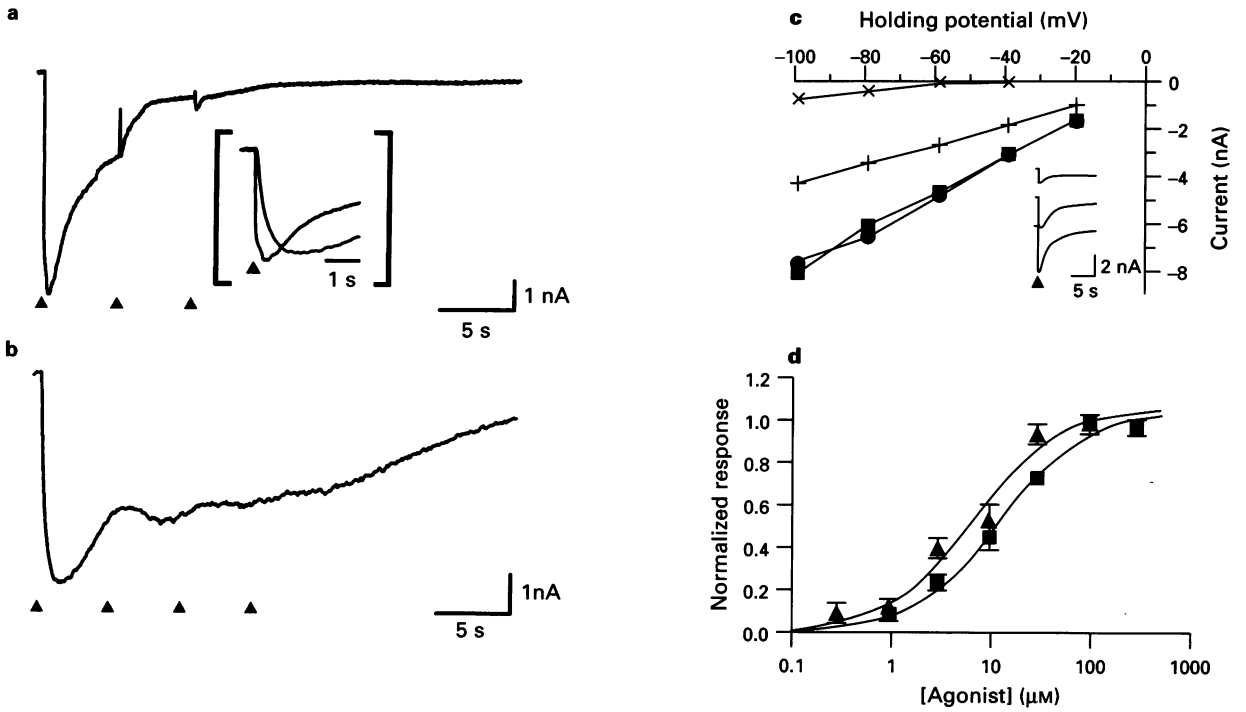

Figure 1 (a) and (b) illustrate voltage clamp recordings of responses to repeated applications of 10 μM 5-HT to two different cells, E5 and E4 respectively. The responses are much more desensitized on the latter two applications for the cell E5 (a). Also the initial response rise time was faster for the cell in (a) and the response decayed more quickly (see inset on an expanded time scale). (c) The current-voltage relation for currents recorded from two different cells, both E5 (● and ■). For the same two cells, (×) and (+) are currents recorded in physiological solution containing zero sodium and zero calcium respectively. Also shown are currents recorded from E5 with standard solutions at holding potentials of −20mV, −40mV, and −60mV (from top to bottom); (▲) under the traces indicates the points of application of 5-HT. (d) The mean concentration-response relationship for pressure applications of various concentrations of 5-HT (●) and α-methyl-5-HT (▲) (3 cells each, ±s.e.mean). The response amplitudes were normalized for each cell to the maximum response obtained in each case.

produce a voltage-independent block of 5-HT$_3$-mediated currents (Peters *et al.*, 1988; Lovinger, 1991).

Effects of 5-HT agonists

I[5-HT]$_{fast}$ could be activated by α-methyl-5-HT as effectively as by 5-HT itself. α-Methyl-5-HT is generally thought of as being a selective agonist at 5-HT$_2$-type receptors on vertebrate neurones. Determination of the concentration-response relationship for 5-HT and α-methyl-5-HT from least squared fits to the data in Figure 1d gave similar EC_{50} values of 7 μM and 10 μM respectively, and a submicromolar threshold concentration for both. The rise time of responses to 10 μM α-methyl-5-HT were as fast or faster than those to 5-HT (94±9.1 ms (s.e.mean) to half maximum, $n=10$).

1-Phenylbiguanide, *m*-chlorophenylbiguanide, and 2-methyl-5-HT have all been used as relatively selective agonists for 5-HT$_3$ receptors in a range of vertebrate species and cell types. On *Helix* neurones, none of these substances acted as agonists of I[5-HT]$_{fast}$ at concentrations up to 100 μM. 5-HT-induced responses were also not mimicked by application of 10 μM 5-methoxytryptamine, a substance which has so far been shown to activate all vertebrate 5-HT receptors except 5-HT$_3$. At a higher concentration of 100 μM, 5-methoxytryptamine did activate a small, slow, and poorly desensitizing current.

Effects of 5-HT antagonists

MDL-72222 (3-tropanyl-3,5-dichlorobenzoate), a relatively specific antagonist of vertebrate 5-HT$_3$ receptors, was found to inhibit I[5-HT]$_{fast}$ potently with a threshold concentration of 0.1 μM and a calculated IC_{50} of 1 μM. Similarly MDL-72222 reversibly inhibited the fast response to the application of 10 μM α-methyl-5-HT (Figure 2, a and b). In cell E6 in which response desensitization was incomplete, the blocking effect of similar concentrations of MDL-72222 was transient and restricted to the initial part of the response. MDL-72222 was also able to inhibit the small current response evoked by high concentrations of 5-methoxytryptamine. Ondansetron, another very potent and selective antagonist at vertebrate 5-HT$_3$ receptors, only weakly inhibited I[5-HT]$_{fast}$ in *Helix* neurones decreasing it to 80% of control at a concentration of 10 μM.

Although I[5-HT]$_{fast}$ was readily activated by α-methyl-5-HT, the 5-HT$_2$ receptor blocker, ketanserin was ineffective at blocking these currents at a concentration of 5 μM (Figure 2a). In some other cells a partial block of the 5-HT-induced currently by this concentration of ketanserin could be due to an effect on receptors other than those responsible for I[5-HT]$_{fast}$.

I[5-HT]$_{fast}$ was reversibly inhibited by (+)-tubocurarine, and also by strychnine, both substances which block other fast depolarizing responses mediated by non-selective cation channels, including those to dopamine and acetylcholine in invertebrate neurones (Ascher, 1972; Carpenter *et al.*, 1977). Strychnine was found to be more potent with an IC_{50} of 1 μM as compared with 10 μM for (+)-tubocurarine (Figure 2, a and b).

Unitary currents

Application of 5-HT to outside-out patches from E5 cells evoked unitary inward currents with an amplitude of 0.2 pA at −60 mV. Such responses could also be recorded in patches from cell E6 where the total cell current response was only partially desensitized with repeated applications of 5-HT. Figure 3a shows examples of unitary currents recorded over a range of holding potentials with the high caesium pipette solution. These unitary currents could be recorded for a prolonged period of time after patch isolation (20 min, usually determined by the patch stability), depending on the concentration of 5-HT applied and the frequency of applications. Similar unitary currents could be activated by α-methyl-5-HT in the same patches, but were not activated by 2-methyl-5-HT. Figure 3b shows the current-voltage relationship which indicated a reversal potential close to 0 mV consistent with non-selective cation channels. The conductance of these channels (2pS) was independent of whether the predominant cation in the recording pipette was potassium, sodium, or caesium.

Other, smaller (0.1 pA at −60 mV) 5-HT-activated unitary currents were seen in a few patches which were extremely prone to irreversible rundown even with low concentrations of 5-HT (2 μM), with usually only one or two responses being obtained from a particular patch. These unitary currents were not activated by application of α-methyl-5-HT or 2-methyl-5-HT, and it is possible that they underlie the non-desensitizing slower component of the compound response observed in many cells, and that they may require GTP, or some other intracellular mediator, for their continued activation. We have not investigated them further here.

Unitary current responses could also be evoked by continued microperfusion of a low concentration of 5-HT (1 μM) over a patch from a separate pipette positioned nearby (Figure

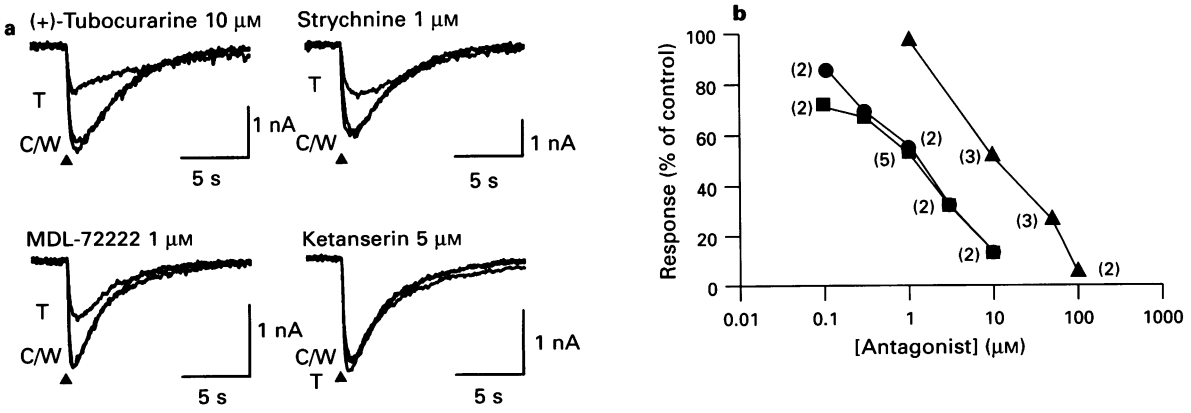

Figure 2 (a) Traces illustrate the influence of the antagonists (+)-tubocurarine (10 μM), strychnine (1 μM), and MDL-72222 (1 μM), on the inward current response of an E5 neurone to 10 μM α-methyl-5-HT. Note the lack of effect of ketanserin (5 μM). In each case the initial control response (C), the response in the presence of the antagonist (T), and the response after washing (W) are shown superimposed; (▲) under the traces indicates the points of application of α-methyl-5-HT. (b) Concentration-dependence of the antagonism of responses to 10 μM 5-HT or 10 μM α-methyl-5-HT in E5. The symbols refer to strychnine (■), MDL-72222 (●), and (+)-tubocurarine (▲).

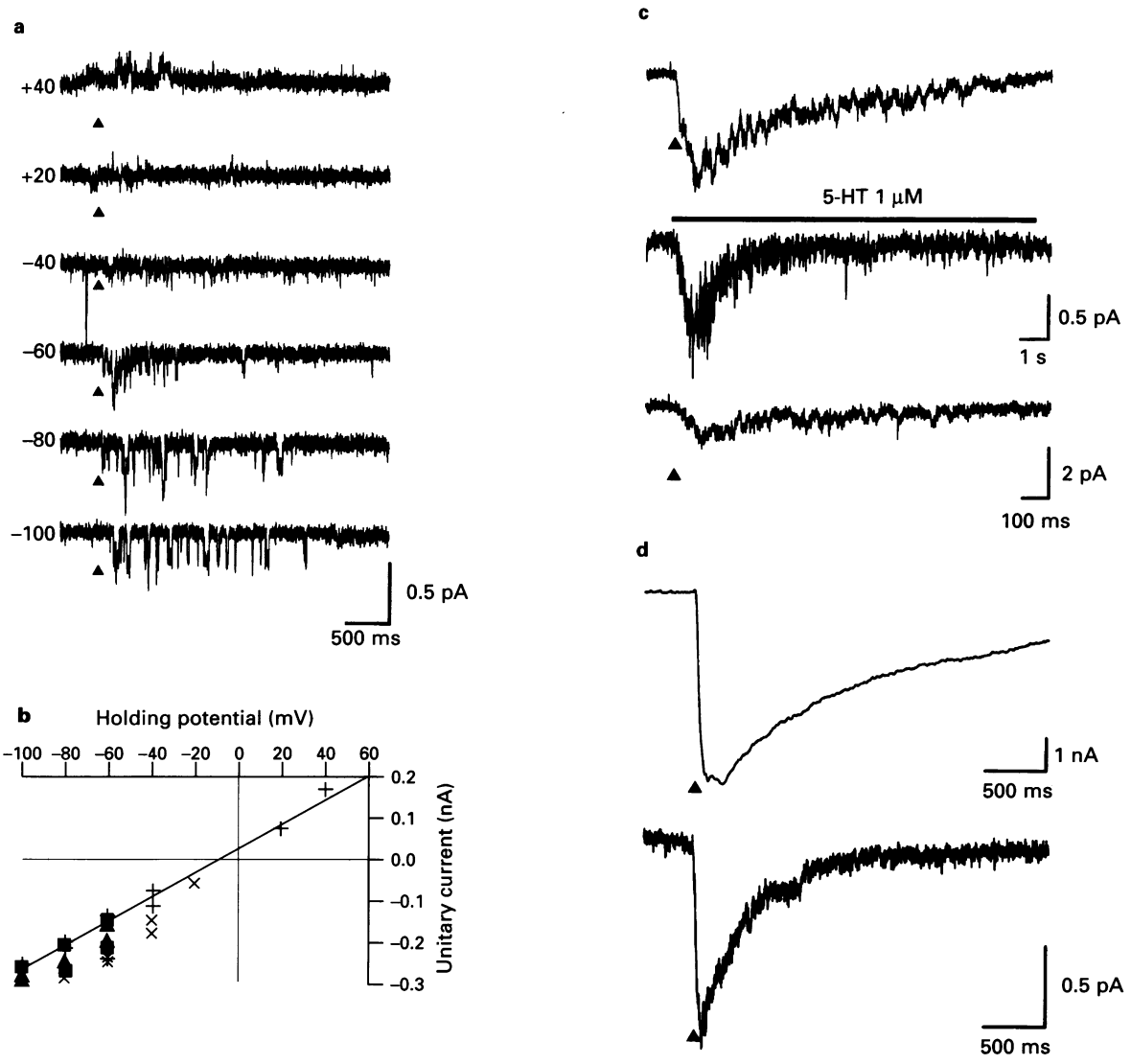

Figure 3 (a) Unitary currents following application of 2 μM 5-HT to an outside-out patch, recorded with the high caesium pipette solution over a series of holding potentials. (b) Current-voltage relationship for unitary currents activated by 5-HT in outside-out patches with the pipette solution containing high potassium (▲), high sodium (×), or high caesium (+). A linear regression has been made to the data obtained with the high caesium pipette solution. (c) Multichannel responses recorded from one patch following pressure application of 50 μM 5-HT (uppermost and lowermost traces), and prolonged local perfusion of 1 μM 5-HT over the same patch from a separate pipette nearby (middle trace). The holding potential was −40 mV and the recording pipette contained the high sodium pipette solution. (d) Comparison of the time course of the fast and desensitizing response to 5-HT recorded from the whole cell (upper trace) with the averaged unitary current response recorded from 40 patches (lower trace); (▲) under the traces in (a), (c), and (d) indicates the points of application of 5-HT.

3c). With such a prolonged (10 second) application, some desensitization was evident even with this low concentration, as is evident from the transient nature of the activation in the continued presence of 1 μM 5-HT.

In Figure 3d the mean response recorded from 40 separate outside-out patches from E5 and E6 at a holding potential of −60 mV is compared with that of the whole cell response in E5. The rise time to half maximum amplitude was similar (70 ms), but the decay of the mean unitary current response was faster reflecting the readier access of the washing solution to the membrane surface of the isolated patch. Application of 5-HT by pressure ejection was within 50–100 μM of the isolated patches and did not limit the rise time since responses to other substances recorded under the same conditions were notably faster (e.g. Green *et al.*, 1996).

Responses to 5-HT application onto outside-out patches were concentration-dependent (Figure 4a) as was their tendency to desensitize (Figure 4b). For the unitary currents in Figure 4b, repeated doses of 2 μM 5-HT evoked consistent responses. Application of 100 μM 5-HT to the same patch stimulated a much larger response initially. However, the response progressively decreased in magnitude over the first four applications, leaving a stable residual response with an amplitude only slightly larger than that which was intially seen with 2 μM 5-HT. Lower concentrations of 5-HT were however able to give rise to significant desensitization when applied for long periods of time (see Figure 3c).

Unitary current responses in outside-out patches from cell E5 or E6 could be repeatably and reversibly antagonized by the specific vertebrate 5-HT$_3$ receptor blocker MDL-72222 at a concentration of 1 μM. (+)-Tubocurarine and strychnine (both 10 μM) also antagonized unitary current responses to 5-HT (or α-methyl-5-HT) reversibly (Figure 5a 5-HT5; b and c).

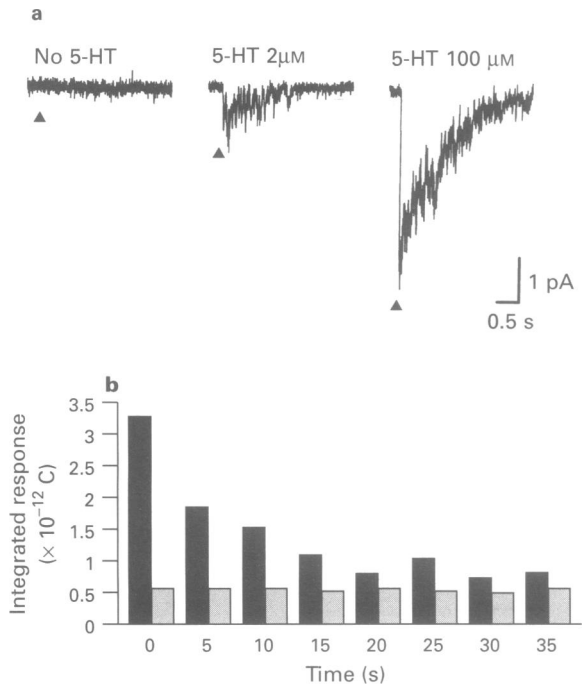

Figure 4 (a) Comparison of the response to pressure applications of physiological solution containing no 5-HT, 2 μM 5-HT, and 100 μM 5-HT to the same outside-out patch. (b) Histogram showing the integrated size measured for responses for the patch in (a) to 2 μM 5-HT (grey columns), and 100 μM 5-HT (solid columns), with repeated applications at 5 s intervals; (▲) under the traces indicates the points of application of 5-HT (or physiological solution only).

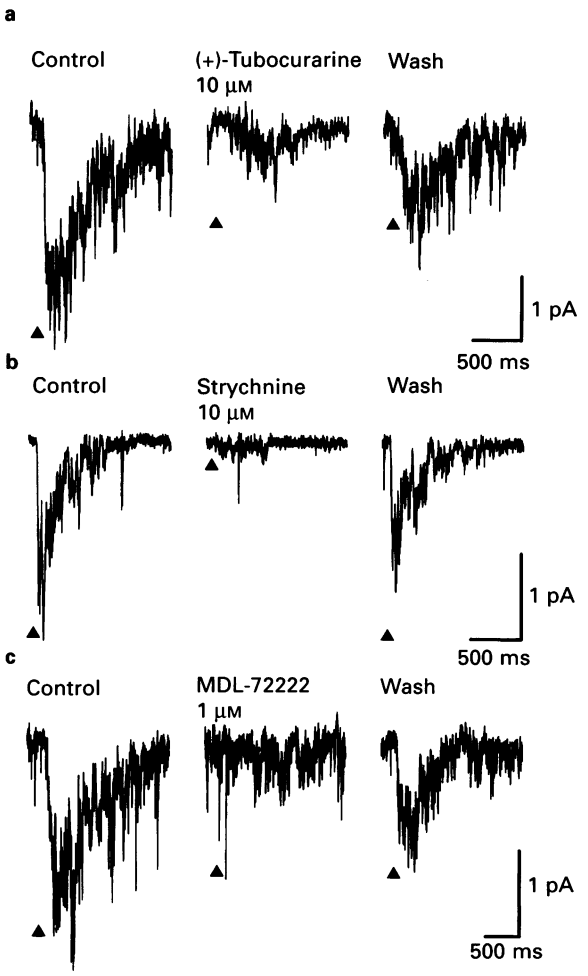

Figure 5 The effects of blocking agents on multiple unitary current responses to 10 μM 5-HT application (a and b), and 10 μM α-methyl-5-HT application (c). (a), (b), and (c) respectively show inhibition of the unitary responses by local perfusion over the patches of 10 μM (+)-tubocurarine, 10 μM strychnine, and 1 μM MDL-72222; (▲) under the traces indicates the points of application of 5-HT or α-methyl-5-HT.

Are G-proteins involved in I[5-HT]$_{fast}$

Since some membrane-delimited G-protein-coupled responses can be fast (< 100 ms) (Hille, 1994), we have used the GTP analogues GTP-[γS] and GDP-[βS] which are known to interfere with G-protein-coupled responses, in order to test whether such a mechanism is involved. Figure 6a shows examples of responses to 10 μM α-methyl-5-HT recorded from E5 over a long period of time with 500 μM GTP-[γS] in the recording electrode. After 20 min there was no significant change in the response from that which was recorded immediately on entering the cell, and this was still true even after 80 min. In cell E5 there was also no significant inhibitory effect of inclusion of 500 μM GDP-[βS] in the pipette solution, and after 10 min the response amplitude was still 82% of the previously recorded control response without GDP-[βS] in the recording pipette (Figure 6b). For comparison in E4 when the recording pipette contained 500 μM GDP-[βS], the evoked current responses were progressively reduced in size down to 58% of the initial amplitude over a short time (2 min), and to 46% after 10 min, indicating that in this cell one or other of the receptors involved in the 5-HT response is regulated by, or regulates its effector channel via a G-protein (Figure 6c).

These data indicate that the receptors mediating the fast 5-HT depolarizing response on cell E5 are not G-protein coupled. In support of this conclusion, unitary currents were also not blocked by inclusion of 500 μM GDP-[βS] in the recording pipette solution and were as persistent as in control patches.

Discussion

The results strongly support activation by 5-HT of a ligand-gated, non-selective channel conducting mainly monovalent cations. The decreased response seen on removal of external calcium might also indicate a significant contribution of calcium ions to the current. However, allosteric effects of elevated extracellular calcium leading to potentiation of neuronal nicotinic ACh receptor currents through non-selective cation channels have been reported (Vernino, *et al.*, 1992), and such an effect could also explain our data. It is also possible that a relatively small calcium entry through the 5-HT channels could activate other channels such as calcium-dependent non-selective-cation channels (Partridge *et al.*, 1994) which might contribute to the response, and be significantly reduced with decreased external calcium.

Ligand-gated ion channels are mainly responsible for carrying the fast 5-HT-activated inward current in cell E5. In other neurones, for example E6, these channels also occur, since they can be recorded in patches isolated from this cell. However, the overall response is not as fast in onset, since it consists also of an apparently separate, slower and non-desensitizing component which contributes significantly to the rise time of the current. This is confirmed by the partial and transient blocking effect of MDL-72222 in these cells, as compared with the near complete block in cell E5. The slower component probably corresponds at least in part to the A′ current reported in some molluscan neurones by Gerschenfeld & Paupardin-Tritsch (1974).

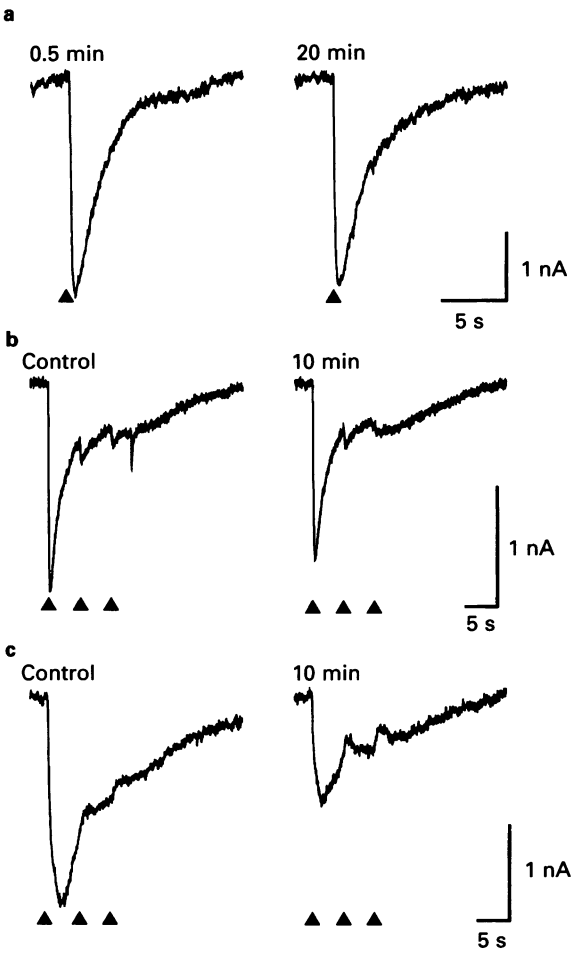

Figure 6 Current responses to application of α-methyl-5-HT (a) or 5-HT (b and c), in E5 (a and b), and E4 (c); (a) shows examples of responses to 10 μM α-methyl-5-HT recorded from E5 over a long period of time with 500 μM GTP-[γS] included in the recording pipette. In (b) and (c) for the control response the recording electrode was filled with 1 M potassium acetate only (left most traces). The following responses were recorded at different times after reimpalement of the same cells with a recording electrode containing GDP-[βS] at a concentration of 500 μM; (▲) under the traces indicates the points of application of 5-HT or α-methyl-5-HT.

The time course and pharmacological properties of the 2pS unitary currents generally recorded from isolated patches is consistent with them carrying I[5-HT]$_{fast}$. The fact that I[5-HT]$_{fast}$ was unaffected by the presence of GTP-[γS] or GDP-[βS] in the recording electrode, and that the 2pS unitary currents remained in the presence of 500 μM GDP-[βS], supports the conclusion that they are ligand-gated. In contrast the smaller unitary currents, although initially clearly activated by the 5-HT application, rapidly became inactive in isolated patches, thus demonstrating their dependence on some intracellular factor for their continued activity.

The potent activation of I[5-HT]$_{fast}$ by α-methyl-5-HT, a selective agonist for vertebrate α-methyl-5-HT$_2$ receptors, and the very weak effect of ondansetron suggests fundamental differences between the 5-HT$_3$ receptor of vertebrate and invertebrate neurones. Also arylbiguanides act as partial agonists at most vertebrate 5-HT$_3$ receptors but had no effect on the *Helix* neurones. The lack of activity of these substances, or 2-methyl-5-HT at the *Helix* 5-HT$_3$-like receptor, is not without precedent however, since subtypes of vertebrate 5-HT$_3$ receptor, for example those in guinea-pig tissues, are known not to respond to the arylbiguanides (Kilpatrick & Tyers, 1991), although they do respond to the 2-methyl-5-HT. (+)-Tubocurarine which is a rather non-specific antagonist of various molluscan responses which are mediated by non-selective cation channels, blocks 5-HT$_3$ receptors by a mechanism not involving channel blockade. It remains to be determined whether that is also true for the molluscan fast 5-HT response.

These data demonstrate the existence of a 5-HT receptor in molluscan neurones which is a ligand-gated channel. The pharmacological properties of this receptor differ significantly from those of known vertebrate 5-HT$_3$ receptors, which are also ligand-gated channels. No other data on pharmacological specificity or other properties of other ligand-gated 5-HT-operated ion channels in invertebrate tissues are available for comparison to see whether this is a general difference from vertebrates, and justifies addition of a new class to the present classification of 5-HT receptors.

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References

ASCHER, P. (1972). Inhibitory and excitatory effects of dopamine on *Aplysia* neurones. *J. Physiol.*, **225**, 173–209.

CARPENTER, D.O., SWANN, J.W. & YAROWSKY, P.J. (1977). Effect of curare on responses to different putative neurotransmitters in *Aplysia* neurones. *J. Neurobiol.*, **8**, 119–132.

COTTRELL, G.A. & MACON, J.B. (1974). Synaptic connections of two symetrically placed giant serotonin-containing neurones. *J. Physiol.*, **236**, 435–464.

DERKACH, V., SURPRENANT, A. & NORTH, R.A. (1989). 5-HT$_3$ receptors are membrane ion channels. *Nature*, **339**, 706–709.

GERSCHENFELD, H.M. & PAUPARDIN-TRITSCH, D. (1974). Ionic mechanisms and receptor properties underlying the responses of molluscan neurones to 5-hydroxytryptamine. *J. Physiol.*, **342**, 427–546.

GERSCHENFELD, H.M. & STEFANI, E. (1966). An electrophysiological study of 5-hydroxytryptamine receptors of neurones in molluscan nervous system. *J. Physiol.*, **185**, 684–700.

GREEN, K.A., HARRIS, S.J. & COTTRELL, G.A. (1996). Dopamine directly activates a ligand-gated channel in snail neurones. *Pflügers Arch.*, **431**, 639–644.

HILLE, B. (1994). Modulation of ion-channel function by G-protein-coupled receptors. *Trends Neurosci.*, **17**, 531–535.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P.A. (1994). VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, **46**, 157–204.

KERKUT, G.A., LAMBERT, J.D.C., GAYTON, R.J., LOKER, J.E. & WALKER, R.J. (1975). Mapping of nerve cells in the suboesophageal ganglion of *Helix aspersa*.. *Comp. Biochem. Physiol.*, **50A**, 1–25.

KILPATRICK, G.J. & TYERS, M.B. (1991). Inter species variants of the 5-HT$_3$ receptor. *Biochem. Soc. Trans.*, **20**, 118–121.

LOVINGER, D.M. (1991). Inhibition of 5-HT$_3$ receptor-mediated ion current by divalent metal cations in NCB-20 neuroblastoma cells. *J. Neurophysiol.*, **66**, 1329–1337.

MARICQ, A.V., PETERSON, A.S., BRAKE, A.J., MYERS, R.M. & JULIUS, D. (1991). Primary structure and functional expression of the 5-HT$_3$ receptor, a serotonin-gated ion channel. *Science*, **254**, 432–437.

PARTRIDGE, D.L., MÜLLER, T.H. & SWANDULLA, D. (1994). Calcium-activated non-selective channels in the nervous system. *Brain Res. Rev.*, **19**, 319–325.

PETERS, J.A., HALES, T.G. & LAMBERT, J.J. (1988). Divalent cations modulate 5-HT$_3$ receptor-induced currents in N1E-115 neuroblastoma cells. *Eur. J. Pharmacol.*, **151,** 491–495.

PETERS, J.A., MALONE, H.M. & LAMBERT, J.J. (1992). Recent advances in the electrophysiological characterization of 5-HT$_3$ receptors. *Trends Pharmacol. Sci.*, **13,** 391–397.

VEHOVSZKY, A. & WALKER, R.J. (1991). An analysis of the 5-hydroxytryptamine (serotonin) receptor subtypes of central neurones of *Helix aspersa*. *Comp. Biochem. Physiol.*, **100C,** 463–476.

VERNINO, S., AMADOR, M., LUETJE, C.W., PATRICK, J. & DANI, J.A. (1992). Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. *Neuron*, **8,** 127–134.

YAKEL, J.L. & JACKSON, M.B. (1988). 5-HT$_3$ receptors mediate rapid responses in cultured hippocampus and a clonal cell line. *Neuron*, **1,** 515–621.

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