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Different efficacy of specific agonists at 5-HT_3 receptor splice variants: the role of the extra six amino acid segment

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1 Whole cell voltage clamp electrophysiology and radioligand binding were used to examine the agonist characteristics of the two splice variants of the 5-HT_3 receptor which have been cloned from neuronal cell lines. Homo-oligomeric 5-HT_3 receptors were examined in HEK 293 cells stably transfected with either long (5-HT_3-L) or short (5-HT_3-S) receptor subunit DNAs.

2 Functional homo-oligomeric receptors were formed from both subunits, and responses to 5-HT_3 receptor agonists (5-hydroxytryptamine (5-HT), 2-methyl 5-HT and m-chlorophenylbiguanide) were qualitatively similar.

3 Maximum currents (R_{max}) elicited by the 5-HT_3 receptor agonists m-chlorophenylbiguanide (mCPBG) and 2-methyl-5-HT (2-Me-5-HT), as compared to 5-HT, differed in the two splice variants: R_{max} mCPBG/R_{max} 5-HT values were 0.68 ± 0.04 and 0.91 ± 0.01 in 5-HT_3-L and 5-HT_3-S receptors, respectively. Comparable values for 2-Me-5-HT were 0.30 ± 0.02 and 0.23 ± 0.02.

4 Radioligand binding data showed no difference in affinity of agonist or antagonist binding sites; thus the six amino acid deletion appears to cause differences in agonist efficacy.

5 The role of the 6 amino acid insertion was further investigated by use of site-directed mutagenesis to create two mutant receptors, one where serine 286 was replaced with alanine, and the second where all 6 amino acids were replaced with alanines.

6 Examination of the mutant receptors when stably expressed in HEK 293 cells revealed agonist properties resembling long and not short 5-HT_3 receptors. Thus specific amino acids in this region are not responsible for the observed differences.

7 The data show intracellular structure can have significant effects on ligand-gated ion channel function, and suggest that minor changes in structure may be responsible for differences in function observed when ligand-gated ion channel proteins are modulated intracellularly.

Keywords: 5-HT_3 receptor; splice variants; ligand-gated ion channel; patch clamp; radioligand binding; stable transfection; efficacy

Introduction

The first 5-HT_3 receptor subunit, 5-HT_3 R-A, was cloned from NCB20 mouse neuroblastoma × Chinese hamster cells (Maricq *et al*., 1991). The predicted protein shows the expected characteristics of a nicotinic acetylcholine (nACh) receptor-type ligand-gated ion channel: a large extracellular N-terminal region and four putative transmembrane domains, with a bulky intracellular loop containing putative phosphorylation sites between transmembrane segments three and four (M3 and M4). The 5-HT_3 receptor sequence shows considerable homology to other members of the family, with the greatest similarity to nACh receptor subunits (see Boess & Martin, 1994 for review). Functionally, all members of this family respond with a rapid change in conductance when challenged with the appropriate agonist; activation of the 5-HT_3 receptor results in an increased conductance to cations (Derkach *et al*., 1989). Electron microscope images of the purified 5-HT_3 receptor reveal a pentameric structure similar to that of the nACh receptor (Boess *et al*., 1995; Green *et al*., 1995).

Other 5-HT_3 subunits cloned so far are all homologues of the 5-HT_3 R-A (also called 5-HT_3-L) subunit and include two derived from related cell lines: NG108-15 (Werner *et al*., 1994 and N1E-115 mouse neuroblastoma (Hope *et al*., 1993), which exist as two splice variants, one of which is missing six amino acids in the M3-M4 intracellular loop (5-HT_3-RAs or 5-HT_3-S); interestingly only this latter version appears to exist in the human genome (Miyake *et al*., 1995). We have previously demonstrated a difference in agonist potency at 5-HT_3 receptors in N1E-115 cells as compared to those in NG108-15 cells (Sepulveda *et al*., 1991; Boess *et al*., 1992); m-chlorophenylbiguanide (mCPBG) was a partial agonist in NG108-15 cells but a full agonist in N1E-115 cells. This difference may be due to differential expression of the splice variants; developmental regulation of the two forms has been demonstrated in NG108-15 cells (Emerit *et al*., 1995). Recent work examining the characterization of the two splice variants in oocytes identified no functional differences between them, but certain characteristics of the receptor were lacking (Glitsch *et al*., 1996). We therefore generated cell lines stably expressing the long (constructed from 5-HT_3-L subunits) and short (constructed from 5-HT_3-S subunits) forms of the receptor, and examined them with whole cell voltage clamp electrophysiology and radioligand binding. As the results from these experiments suggested a difference in the efficacy of some 5-HT_3 receptor agonists, two mutant receptor subunits were constructed; one where an alanine replaced a serine in the extra six amino acid portion of the long receptor subunit, and a second where all six amino acids were replaced by alanines (Figure 1). Thus we were able to investigate whether the difference in agonist efficacy was due to phosphorylation of the receptor or to its structure.

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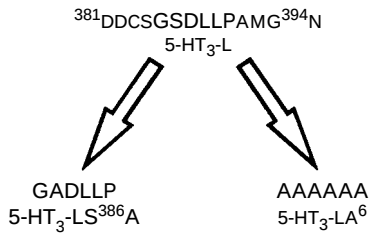

Figure 1 Part of the amino acid sequence (residues 381–394) of the 5-HT$_3$-L receptor subunit showing the extra six amino acids (enlarged). Amino acid sequence in the mutant receptor subunits, 5-HT$_3$-LS^{386}A and 5-HT$_3$-LA^6, which differ only in this six amino acid region, are also shown.

Methods

Cell culture and preparation

HEK 293 cells (ECACC, Porton Down, U.K.) were grown in 90 mm dishes in Dulbecco's F12:DMEM media supplemented with 10% foetal calf serum at 7% CO_2. When the cells reached 50–80% confluency, they were transfected by the calcium phosphate precipitation method (Chen & Okayama, 1987) with the eukaryotic expression vector pRc/CMV (Invitrogen), containing the complete coding sequence for one of four 5-HT$_3$ receptor subunit (5-HT$_3$-L, 5-HT$_3$-S, 5-HT$_3$-LS^{386}A, 5-HT$_3$LA^6). Full length 5-HT$_3$-S DNA was obtained from mouse neuroblastoma N1E-115 cell line mRNA by use of PCR and inserted into the expression vector pRc/CMV (as described in Hargreaves *et al*., 1996). Coding sequence for the additional 6 amino acids in the 5-HT$_3$-L subunit (^{385}GSDLL^{390}P) was inserted by means of site directed mutagenesis (Kunkel, 1985), and the mutants 5-HT$_3$-LS^{386}A (serine 386 replaced by alanine) and 5-HT$_3$-LA^6 (all six extra amino acids replaced by alanines) were constructed similarly (see Figure 1). Mutants were confirmed by DNA sequencing. Following transfection, cells were incubated for 48 h at 3% CO_2 and then returned to 7% CO_2. Stable cell lines were selected as previously described (Hargreaves *et al*., 1996) by diluting the transfected cells 10 fold and replating them into Selection medium, which comprised F12/ DMEM containing serum and geneticin (600 μg ml^{-1}). After 14 days, surviving colonies were individually removed and further cultured for 14 days in multiwell dishes containing 1 ml of Selection medium. The density of receptors expressed was then assayed by use of [^{3}H]-granisetron binding (see below). Colonies with the highest densities of receptors were maintained in Selection medium, and those with relatively similar densities of receptors (<5 fold difference) were further cultured. For radioligand binding the stable cells (HEK/5-HT$_3$-L, HEK/5-HT$_3$-S, HEK/5-HT$_3$-LS^{386}A and HEK/5-HT$_3$-LA^6) were prepared as described previously (Lummis *et al*., 1990; 1993). Briefly cells were grown in 90 mm dishes until confluent (3–5 days). They were then removed from plates after washing with phosphate buffered saline by gentle scraping into HEPES buffer (10 mM, pH 7.5) containing the following protease inhibitors: 1 mM EDTA, 50 μg ml^{-1} soybean trypsin inhibitor, 50 μg ml^{-1} bacitracin and 0.1 mM phenylmethylsulphonyl fluoride. They were then centrifuged for 20 min, 40,000 × *g* at 0°C, resuspended in HEPES buffer and recentrifuged twice. They were finally resuspended in HEPES buffer for assay.

Binding studies

These were performed as previously described (Lummis *et al*., 1990; 1993) with minor modifications. Briefly cell membranes were incubated at 0°C in HEPES buffer (10 mM, pH 7.5) in a final volume of 1 ml for 1 h (for [^{3}H]-granisetron binding) or 2 h ([^{3}H]-mCPBG binding). Non-specific binding was defined with 100 nM GR67730. Incubations were terminated by rapid dilutions with ice-cold buffer followed by vacuum filtration onto prewetted GF/B filters with 2 × 2 ml washes by use of a Brandel cell Harvester. All assays were performed in triplicate. Protein concentrations were determined by the method of Bradford (1976) with bovine serum albumin as the standard. Experimental data were analysed by the program EBDA (McPherson, 1985), followed by computer assisted iterative curve fitting according to the logistic equation as described by Barnes and Barnes (1993). K_d values determined by this method were found to be similar for different stable cell lines generated from the same receptor DNAs; B_{max} values varied considerably and are therefore not given in the text.

Electrophysiological procedures

These were performed as previously described (Sepulveda *et al*., 1991). Briefly, cells were grown in 35 mm dishes and patch-clamped in the giga-seal whole cell configuration by a List EPC-7 amplifier (List Medical). The culture dishes were continuously perfused (3–5 ml min^{-1}) at room temperature in a solution of the following composition (in mM): NaCl 140, KCl 2.8, $MgCl_2$ 2.0, $CaCl_2$ 1.0, glucose 10 and HEPES 10, pH 7.2. Micro electrodes (3–5 MΩ) were made from thin-walled borosilicate (hard) glass capillary tubing (Clark Electromedical GC150T-15) and filled with a solution containing (in mM): KCl 140, $MgCl_2$ 2.0, $CaCl_2$ 0.1, EGTA 1.1 (free Ca^{2+} concentration 1.0×10^{-8} M), HEPES 10, pH 7.2. Cells were routinely held at −60 mV, and agonists were applied by means of a U-tube device (Suzuki *et al*, 1990). The rate of solution exchange was calibrated as described in Sepulveda *et al*. (1991) and was complete in 75–85 ms. To allow for recovery from desensitization, 5-HT and 2-methyl-5-HT were applied after a 3 min washout, and mCBPG was applied after a 5 min washout. Initial experiments showed that these time intervals were sufficient to allow for complete recovery. In inhibition studies, the antagonist was applied for 1 min before co-application with the agonist. Currents were viewed on an oscilloscope and recorded on a DAT (Digital Audio Tape) cassette. Records were replayed on a chart recorder (Gould 1000S) and the current amplitudes elicited by agonists measured from the traces. Data from dose-response experiments were normalized to the maximum current (R_{max}) elicited by the agonist in order to use all data irrespective of a wide range of maximum responses. Curves resulting from dose-response experiments were analysed by non-linear regression curve fitting (SigmaPlot; Jandel Scientific).

Reagents

All cell culture reagents were obtained from Gibco BRL (Paisley, U.K.), except foetal calf serum which was from Sigma (Poole, U.K.). Ondansetron and tetrahydromethyl dimethylimidazole carbazol (GR6730) were gifts from Glaxo Group Research (Stevenage, U.K.). Granisetron was a gift from SmithKline Beecham Pharmaceuticals (Welwyn, U.K.). [^{3}H]-granisetron (81 Ci mmol^{-1}) and [^{3}H]-m-chlorophenylbiguanide (mCPBG; 26 Ci mmol^{-1}) were from DuPont (Stevenage, U.K.). mCPBG and 2-methyl-5-HT (2-Me-5-HT) were from Cookson Chemicals. All other reagents were obtained from Sigma (Poole, U.K.).

Results

Expression of 5-HT$_3$ receptors

Agonist-induced responses in single HEK/5-HT$_3$-L cells voltage clamped at −60 mV are shown in Figure 2. The responses obtained are similar to those elicited in cells expressing the native receptor: 5-HT, mCPBG and 2-Me-5-HT induce fast, rapidly-desensitizing inward currents. Responses obtained in HEK/5-HT$_3$-S cells were qualitatively similar (not shown). Transfected cells showed the pharmacology expected of a 5-HT$_3$ receptor (Figure 3). Responses to 5-HT (2 μM)-induced currents in HEK/5-HT$_3$-L cells were reversibly blocked by the 5-HT$_3$ receptor antagonists ondansetron (2 nM, a) and granisetron (2 nM, b). Similar effects were observed in cells transfected with 5-HT$_3$-S DNA (not illustrated). In both cases the responses were completely blocked by 10 nM of either antagonist (data not shown). Antagonists of other 5-HT receptors, e.g. ketanserin at 10 μM (c) did not inhibit the response.

Radioligand binding with [^{3}H]-granisetron, a 5-HT$_3$ receptor antagonist, and [^{3}H]-mCPBG, a 5-HT$_3$ receptor agonist, revealed no significant differences in affinity (K_d) between membranes prepared from HEK/5-HT$_3$-L, HEK/5-HT$_3$-S, HEK/5-HT$_3$-LS^{386}A or HEK/5-HT$_3$-LA^6 cells (Table 1).

Agonist-induced activation of 5-HT$_3$ receptors

Dose-response curves for mCPBG, 5-HT and 2-Me-5-HT are shown in Figure 4. The curves were constructed by measuring peak amplitude of the inward current elicited by increasing concentrations of agonist in single cells clamped at −60 mV. Since the maximum current generated by a saturating agonist concentration varied significantly between cells, the data were normalized to the response elicited by a supramaximal agonist concentration. This was routinely 2–3 fold higher than the concentration that elicited the maximal response for each cell line; concentrations higher than this resulted in truncated responses due to very rapid desensitization (as described in Sepulveda *et al*., 1991). Similar experiments in 5-HT$_3$-S cells with EC_{50} concentrations of agonists for normalization gave similar results. The parameters resulting from the best fit of the data to the Hill equation are shown in Table 2. The EC_{50} values for 5-HT and 2-Me-5-HT were not significantly different between HEK/5-HT$_3$-L and HEK/5-HT$_3$-S cells but for mCPBG the EC_{50} was approximately two fold greater in HEK/5-HT$_3$-S cells (significantly different, *t* test, $P<0.05$).

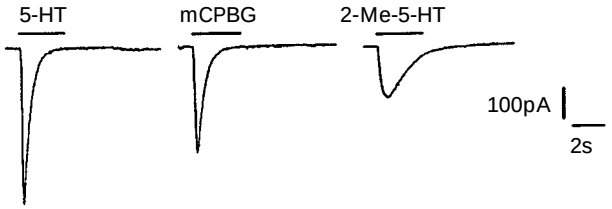

Figure 2 Typical currents elicited by 5-HT (10 μM), mCPBG (3 μM) and 2-Me-5-HT (100 μM) in an HEK293 cell stably transfected with 5-HT$_3$-L subunit DNA. Bar indicates drug application. The cell was clamped at −60 mV. Similar results were obtained with cells transfected with 5-HT$_3$-S or mutant 5-HT$_3$-L subunit DNA, although, as described in the Results and in Figure 5, relative response amplitudes differed. Untransfected cells showed no response.

Values for HEK/5-HT$_3$-LS^{386}A and HEK/5-HT$_3$-LA^6 cells were not significantly different from HEK/5-HT$_3$-L cells.

Currents evoked by a saturating concentration of mCPBG (R_{max} mCPBG) compared to the maximum response evoked by a saturating concentration of 5-HT (R_{max} 5-HT) were greater in cells expressing 5-HT$_3$-S receptors: 91±1% as compared to 68±4% in HEK/5-HT$_3$ L cells (see Figure 5). A significant but

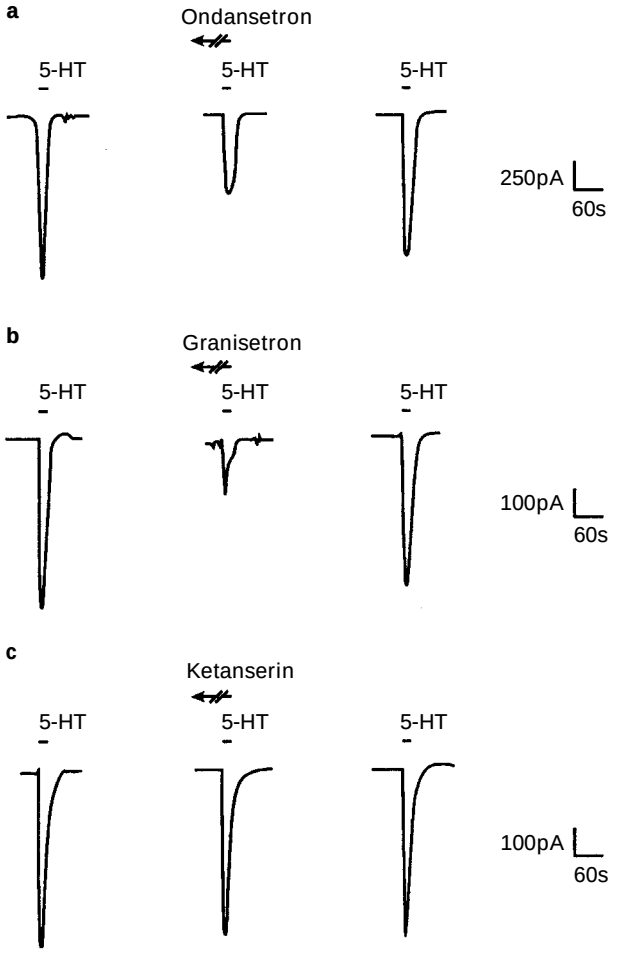

Figure 3 Responses in transfected cells show the pharmacology expected of a 5-HT$_3$ receptor. Responses to 5-HT (2 μM)-induced currents in cells transfected with 5-HT$_3$-L were inhibited by 2 nM ondansetron (a) and 2 nM granisetron (b), preapplied for 1 min. Bar indicates drug application. Similar effects were observed in cells transfected with 5-HT$_3$-S. In both cases the responses were completely blocked by 10 nM of either antagonists. Antagonists of other 5-HT receptors, e.g. ketanserin at 10 μM (c), did not inhibit the response. The data in these figures, which are typical of 5–10 experiments were obtained from small clusters of cells. As HEK293 cells are electrically coupled, this allows better signal detection. However, problems may arise from insufficient space clamping, so for quantitative studies data were collected from single cells.

Table 1 Comparison of radioligand binding affinities (K_d) obtained from HEK293 cells expressing long (5-HT$_3$-L), short (5-HT$_3$-S) or mutant 5-HT$_3$ receptors

	[^{3}H]granisetron binding K_d (nM)	*[^{3}H]mCPBG binding* K_d (nM)
5-HT$_3$-L	0.33±0.06	1.06±0.17
5-HT$_3$-S	0.36±0.06	0.92±0.21
5-HT$_3$-LS^{386}A	0.37±0.05	1.23±0.31
5-HT$_3$-LA^6	0.32±0.06	0.88±0.23

Data are presented as mean±s.e.mean, $n=3-5$.

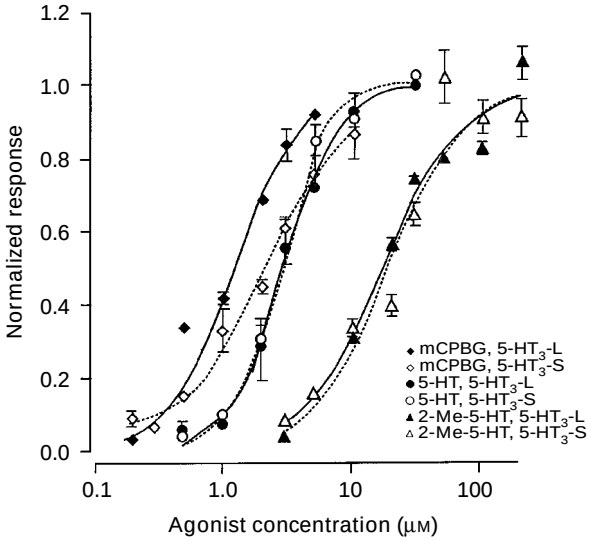

Figure 4 Concentration-dependence of mCPBG, 5-HT and 2-Me-5-HT evoked inward currents in cells transfected with 5-HT$_3$-L or 5-HT$_3$-S. All responses are expressed as a fraction of the maximum response to agonist. EC$_{50}$ values obtained from these data are shown in Table 1. Hill coefficients for mCPBG were 1.11 ± 0.08 and 1.62 ± 0.09 for 5-HT$_3$-S and 5-HT$_3$-L receptors, respectively. Hill coefficients were not significantly different for 5-HT and 2-Me-5-HT for the two splice variants. Each data point represents the mean of responses from $n \geqslant 5$ separate cells; vertical lines show s.e.mean.

Table 2 EC$_{50}$ values (μM) from HEK293 cells expressing long (5-HT$_3$-L), short (5-HT$_3$-S) or mutant 5-HT$_3$ receptors

	5-HT	*2-Me-5-HT*	*mCPBG*
5-HT$_3$-L	2.9 ± 0.2	17.2 ± 2.9	1.20 ± 0.03
5-HT$_3$-S	2.7 ± 0.2	18.1 ± 4.9	2.30 ± 0.09*
5-HT$_3$LS^{386}A	3.8 ± 0.3	20.5 ± 1.8	1.51 ± 0.19
5-HT$_3$-LA^6	2.5 ± 0.5	14.6 ± 3.6	1.27 ± 0.19

Data are presented as mean $\pm$ s.e.mean, $n \geqslant 5$. *Significantly different from 5-HT$_3$-L data, t test, $P < 0.05$.

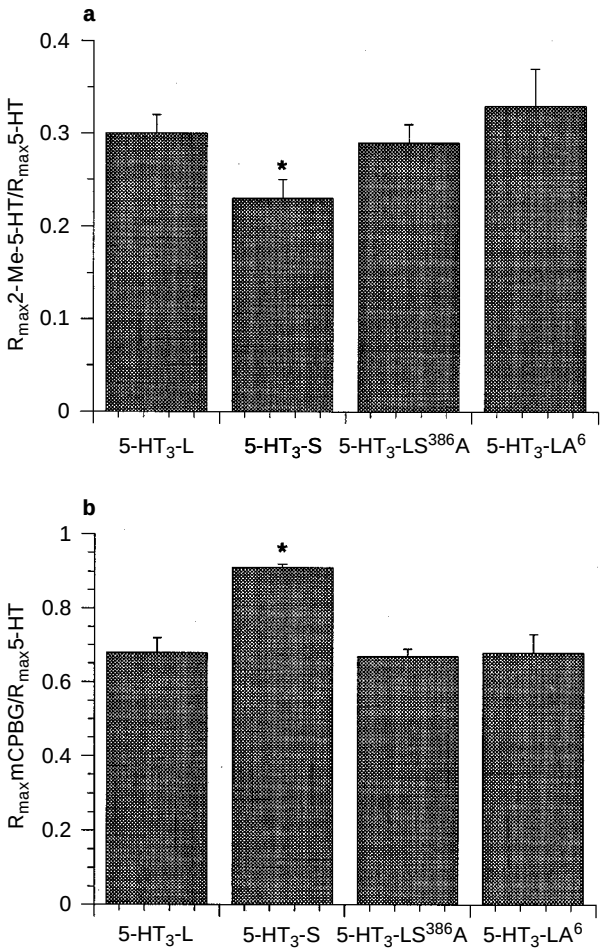

Figure 5 Maximal responses (R_{max}) generated by 2-Me-5-HT (a) and mCPBG (b) compared to maximal responses generated by 5-HT were different in 5-HT$_3$-L and 5-HT$_3$-S receptors. Mutant receptors are similar to 5-HT$_3$-L receptors. Columns show mean $\pm$ s.e.mean, $n \geqslant 5$. *Significantly different from 5-HT$_3$-L, $P < 0.05$.

smaller difference in response between the two cell lines was also detected for 2-Me-5-HT: $30 \pm 2\%$ in HEK/5-HT$_3$-L and $23 \pm 2\%$ in HEK/5-HT$_3$-S. Values for HEK/5-HT$_3$-LS^{386}A and HEK/5-HT$_3$-LA^6 cells were not significantly different from HEK/5-HT$_3$-L cells.

Discussion

The results show that the 5-HT$_3$ receptor agonists, 2-Me-5-HT and mCBPG, have differential effects on the long and short forms of the receptor. Similar but more pronounced effects for 2-Me-5-HT acting on long and short 5-HT$_3$ receptors expressed in oocytes (63% and 9% respectively) has been demonstrated (Downie *et al*., 1994), although Werner *et al*. (1994), who used the Semliki forest virus expression system in RIN cells, observed no difference in the maximal response to 2-Me-5-HT. Glitsch *et al*. (1996), using oocytes, found no differences in the characteristics of the two splice variants, but they only used two agonists, 5-HT and the much less potent 1-phenybiguanide. Interestingly Ca^{2+} entry through mCBPG-activated 5-HT$_3$ receptors was greater in HEK/5-HT$_3$-S cells than HEK/5-HT$_3$-L cells, although the maximal $[Ca^{2+}]_i$ concentrations (966 ± 129 nM and 562 ± 169 nM, respectively) were only significant at the 90% and not the 95% level, presumably due to large errors associated with the use of transient rather than stably transfected cell lines (Hargreaves *et al*., 1994). There are significant advantages in using HEK or other mammalian cells as compared to oocytes, particularly when expressing membrane proteins, as the post-translational processing in *Xenopus laevis* is not the same as that found in mammalian cells (Cooper & Millar, 1997). For whole cell studies the use of mammalian cells also has the considerable advantage of the allowing the use of devices that permit a much faster change of solution, an important consideration for rapidly desensitizing receptors such as the 5-HT$_3$ receptor. Some of the discrepant results described above may in fact be due to differences in application systems: Jones and Westbrook (1996) have modelled the responses of a fast desensitizing receptor and have shown that channel activity is not as accurately reflected by macroscopic current when slow drug application methods are used.

Thus, despite some discrepancies in the literature, we observed functional differences between long and short 5-HT$_3$ receptors. Using radioligand binding we have shown there are no significant differences in the affinities of agonist and antagonist binding sites between long and short forms of the receptor (Table 1). As the activity of a drug depends both on its affinity for the receptor and its efficacy in inducing an effect when bound to the site, the functional differences observed for 2-Me-5-HT and mCPBG are presumably due to different

efficacies. The efficacy of 2-Me-5-HT appears to be greater at 5-HT$_3$-L receptors; conversely the efficacy of mCPBG is greater at 5-HT$_3$-S receptors. This suggests that mechanism through which the binding energy is converted into channel opening may be differentially modulated for the two agonists, perhaps due to different points of contact in the 5-HT$_3$ receptor binding pocket.

The data presented here show a link between agonist binding, channel function and an intracellular region of the protein. This link has been appreciated for many years; intracellular protein phosphorylation is an important means of regulation (Huganir & Greengard, 1990). Splice variants of receptor subunits that change the number of phosphorylation sites can have significant effects on their function. Removal of serine in the γ2L subunit of the γ-aminobutyric acid (GABA$_A$ receptor, for example, affects its susceptibility to modulation by ethanol (Wafford *et al*., 1991), and phosphorylation of this residue by protein kinase C (PKC) decreases the GABA-induced response (Krishek *et al*., 1994). PKC appears to have the opposite effect on 5-HT$_3$ receptors: PKC activators potentiate 5-HT$_3$-mediated responses, while PKC inhibitors have the opposite effect (Zhang *et al*., 1995; Glitsch *et al*., 1996); this may be due to a change in the proportion of 5-HT$_3$ receptors at different conductance levels: van Hooft and Vijverberg (1995) found that stimulation of protein kinase activity enhances the probability of a high (27pS) conductance level in N1E-115 cells, whilst the PKC inhibitor staurosporin increases the probability of a lower (6pS) conductance level. The extra serine in the long form of the 5-HT$_3$ receptor is not at a consensus phosphorylation site, but this does not preclude it being phosphorylated. However, the long form of the receptor does possess a unique casein kinase 2 phosphorylation site (serine 384), that is more favourable if the extra serine (386) is phosphorylated (Pinna, 1990). The mutants that we constructed eliminated both the extra serine (in the 5-HT$_3$-LS^{386}A mutant) and the concensus phosphorylation site (by removing of aspartate 387 in the 5-HT$_3$-LA^6 mutant), and, as the mutant receptors behaved similarly to unmodified 5-HT$_3$-L receptors in terms of their behaviour to agonists (Table 1 and 2), phosphorylation cannot be responsible for the observed differences between the long and short splice variants.

The apparent difference in efficacy observed with mCPBG and 2-Me-5-HT must therefore be due to differences in protein structure. Some changes in structure are reflected by changes in neurotransmitter affinity; desensitization of ACh and GABA$_A$ receptors, for example, induces a conformational change to a form that has a high affinity for agonists (Changeux *et al*., 1987; Bristow & Martin, 1989). Thus the difference in EC$_{50}$s and Hill coefficients for mCPBG-induced activation could be interpreted as a difference in structure between long and short 5-HT$_3$ receptors. Radioligand binding studies revealed no difference in agonist affinity, but the observed difference in efficacy suggests the extra six amino acids have an effect on the transition between the closed and open states of the receptor; this would be difficult to discern from equilibrium binding parameters. Analyses of single channel data would be useful to confirm this, but we could not observe any discernable single channel events in excised patches. This suggests that under the conditions we used the conductance of the 5-HT$_3$ channel may be very small; conductance levels of $<$1pS in neuronal cell lines have been obtained previously (Lambert *et al*., 1989; Gill *et al*., 1995). However, data from other studies suggest that by increasing the driving force for Na^+ and by the use of patch-cramming, conductance levels of 6, 11, 18 and 27 pS can be observed (van Hooft *et al*., 1994; van Hooft & Vijverberg, 1995); thus it may be possible to do these experiments under certain conditions. Alternatively, the change in structure may affect phosphorylation in another part of the receptor. The intracellular loop in both splice variants has consensus sequences for protein kinases A and C. As sites of phosphorylation do not appear to be functionally equivalent (Krishek *et al*., 1994), it is possible that the different intracellular structures of the two splice variants may result in differential modulation by phosphorylation in long and short 5-HT$_3$ receptors.

Our previous studies showing differences in maximum responses caused by mCPBG (Sepulveda *et al*., 1991; Boess *et al*., 1992) can now be explained by different levels of splice variants rather than the presence of different subunits. Decreased levels of 5-HT$_3$-L mRNA upon differentiation have been observed for NG108-15 cells (Emerit *et al*., 1995), and these correlate with changes in response amplitude, half-time of desensitization and voltage-dependence of 5-HT$_3$ responses (Shao *et al*., 1991). However other studies (Glitsch *et al*., 1996; Werner *et al*., 1994) have shown no differences in current responses voltage-dependence, desensitization kinetics, ion selectivity or unitary conductance between long and short 5-HT$_3$ receptors. We also observed no difference in desensitization kinetics between NG108-15 and N1E-115 cells, which, on the basis of the maximal responses elicited by mCPBG, express mostly 5-HT$_3$-L and 5-HT$_3$-S receptors, respectively (Sepulveda *et al*., 1991). Thus the changes observed by Shao *et al*. (1991) may be due to changes unrelated to different levels of long or short receptors.

Thus we have shown that the 6 amino acids present in the long form of the 5-HT$_3$ receptor are responsible for the differences in some characteristics of this ligand-gated ion channel. So far these differences are only obvious when examining the effects of partial agonists, but may be apparent with full agonists under certain conditions *in vivo*, for example in the presence of modulatory agents. Although it is not yet clear how these receptors are modulated in the brain, a number of agents including Mg^{2+}, Ca^{2+}, alcohols and anaesthetics have been shown to modulate the function of 5-HT$_3$ receptors in isolated cells (Peters *et al*., 1988; Parker *et al*., 1996). The difference in efficacy of partial agonists, combined with previous studies showing variations in the ratios of splice variant mRNAs in rat brain and during development (Miquel *et al*., 1995), and differential expression caused by differentiation (Emerit *et al*., 1995) suggest that 5-HT$_3$ receptor splice variants do play a functional role in the nervous system, at least in rodents. As 5-HT$_3$ receptors appear to be homo-oligomers in their native state, splice variants may take the role played by different subunits in other ligand-gated ion channel receptors; for example the presence of different α subunits (α1, α4 or α6) in expressed recombinant GABA$_A$ receptors results in a number of different characteristics including different effects of modulatory agents and different efficacies to partial agonists (Wafford *et al*., 1996). Mutations in the 5-HT$_3$ receptor sequence show that it is the presence of the amino acids and not their modulation by phosphorylation, that causes the observed differences, demonstrating that intracellular structure of ligand-gated ion channel receptors affects channel function. Thus our data support the idea that changes in structure can cause significant changes in receptor efficacy.

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