Competitive and non-competitive effects of 5-hydroxyindole on 5-HT₃ receptors in N1E-115 neuroblastoma cells

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1 Effects of 5-hydroxyindole (5-OH-indole) (the aromatic moiety of 5-hydroxytryptamine (5-HT)) on 5-HT-evoked ion current and the nature of these effects on 5-HT₃ receptors have been investigated in whole-cell voltage clamp and radioligand binding experiments on cultured N1E-115 mouse neuroblastoma cells.

2 The amplitude of 10 μ M 5-HT-evoked ion currents was enhanced up to 150% of the control value by increasing concentrations up to 10 mM 5-OH-indole with half maximum effect of 0.8 mM. At concentrations between 10 mM and 50 mM, 5-OH-indole blocked the 5-HT-evoked ion current. Both the enhancement and the block by 5-OH-indole were accompanied by a marked slowing of the kinetics of decay of the 5-HT-evoked inward currents.

3 The blocking effect was surmounted when the 5-HT concentration was raised from 10 μ M to 100 μ M. Conversely, the increase in amplitude and the slowing of the decay of the 5-HT-evoked ion current induced by 1 mM 5-OH-indole were not reversed by the same increase of 5-HT concentration.

4 The binding of the selective antagonist [³H]-GR65630 to 5-HT₃ receptors was displaced by 5-OH-indole in a concentration-dependent manner with a pK_i of 1.96. In saturation binding experiments 10 mM 5-OH-indole reduced the affinity of [³H]-GR65630, whereas the total number of binding sites remained unaffected.

5 It is concluded that the blocking effect of high concentrations of 5-OH-indole is due to a competitive interaction with the antagonist recognition sites of 5-HT₃ receptors, whereas the potentiating effect of lower concentrations of 5-OH-indole appears to be mediated by a distinct non-competitive interaction.

Keywords: 5-Hydroxytryptamine; 5-HT₃ receptor; desensitization; block; 5-hydroxyindole; voltage clamp; ion current; radioligand binding; [³H]-GR65630; N1E-115 mouse neuroblastoma cells

Introduction

The 5-HT₃ receptor belongs to the group of ligand-gated ion channels. The direct coupling of the 5-HT₃ receptor to an intrinsic ion channel has been established by the molecular cloning of a 5-HT₃ receptor subunit, which expresses both the receptor and the ion channel on reconstitution (Maricq *et al.*, 1991). Agonist interaction with the 5-HT₃ receptor ion current is unaffected by any of the known second messenger systems (Hoyer & Neijt, 1988; Yakel & Jackson, 1988; Yakel *et al.*, 1991; Yang, 1990).

In N1E-115 mouse neuroblastoma cells the interaction of 5-HT with the 5-HT₃ receptor causes the activation of ion current, which rapidly reaches its peak value and subsequently decays to a small steady level in the continuous presence of agonist. The pharmacology of this 5-HT-evoked ion current indicates that it is carried solely by 5-HT₃ receptor-operated ion channels (Neijt *et al.*, 1988). The rate of decay of ion current depends on the concentration of 5-HT. In the continuous presence of 5-HT, the ion current decays at the same rate at which the maximum attainable 5-HT-evoked ion current is reduced. Both inward current decay and the reduction of the maximum response reflect the equilibration between so-called desensitized and non-desensitized states of the ligand-gated ion channels during exposure to the agonist (Neijt *et al.*, 1989).

Recently, we found that millimolar concentrations of tetraethylammonium ions (TEA) block 5-HT₃ receptor-operated ion current and prevent its desensitization by a competitive interaction at (part of) the agonist recognition site (Kooyman *et al.*, 1993b). Conversely, 5-OH-indole, the aromatic moiety

of the endogenous ligand 5-HT, enhances the amplitude of 5-HT₃ receptor agonist-evoked ion current in N1E-115 cells and, concomitantly, slows the kinetics of ion current decay (Kooyman *et al.*, 1993a).

Since the exact nature of the interaction of 5-OH-indole with 5-HT₃ receptors in N1E-115 cells remains to be established, we have investigated the concentration-dependent effects in whole-cell voltage clamp and radioligand binding experiments. The combined results show dual effect of 5-OH-indole on the 5-HT₃ receptor.

Methods

Cell culture

Mouse neuroblastoma cells of the clone N1E-115 (Amano *et al.*, 1972) were grown under the conditions described previously by Neijt *et al.* (1989). Subcultures of passage numbers 31–42 were used. For ligand binding studies, undifferentiated cells, grown up to a density of 60×10^6 cells per dish (Falcon, 500 cm²), were harvested by scraping the bottom of the culture dish and stored in external solution at –80°C. For electrophysiological experiments, cells were cultured in tissue culture T-flasks, up to a density of 70×10^3 cells cm^{–2} and were resuspended every five days after dissociation in phosphate buffered saline containing 1% trypsin. Subcultures were grown in 3.5 cm tissue culture dishes. On day 2 in subculture differentiation of the cells was initiated in culture medium additionally supplemented with 1 mM dibutylryl cyclic AMP and 1 mM 3-isobutyl-1-methylxanthine. This medium was refreshed about every other day. Cells were used for experiments 7–12 days after the initiation of differentiation.

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Electrophysiology

Ion currents were recorded by a suction pipette technique for whole-cell voltage clamp (Lee *et al.*, 1978) as described previously by Neijt *et al.* (1989). Cells were voltage clamped at -70 mV. Series resistance, initially estimated from the instantaneous voltage jump in response to a constant current stimulus, was compensated under voltage clamp for about 80–85%. To maintain cells at holding potential in the absence of any stimulation, a steady inward current injection was required that varied between cells in the range of 0.1–0.5 nA. Membrane currents were low-pass filtered (-3 dB at 1 kHz, 12 dB/oct), digitized (8 bits, 1024 points/record) and stored on a magnetic disc for off-line computer analysis.

Solutions

The ionic composition of the pipette solution was (in mM): K-glutamate 100, Na-HEPES 20, sucrose 120. The pH was adjusted to 7.25 with L-glutamic acid. The external solution contained (in mM): NaCl 125, KCl 5.5, HEPES 20, CaCl_2 1.8, MgCl_2 0.8, glucose 24 and sucrose 37, and the pH was adjusted to 7.3 with approximately 7 mM NaOH. The incubation buffer used in ligand binding studies was identical to the external solution.

Application of drugs

In the electrophysiological studies, cells were continuously superfused with external solution and ion currents were evoked by changing to agonist- and, optionally, drug-containing external solutions. The time constant of solution exchange was ≤ 1 s (Neijt *et al.*, 1989). Frozen concentrated stock solutions of 10 mM 5-HT and 1 M 5-OH-indole (dissolved in dimethylsulphoxide) were freshly thawed prior to the experiments. Unless the experimental protocol required otherwise, cells were repetitively exposed to the agonist at intervals of at least 100 s in order to allow complete recovery from desensitization. All experiments were performed at room temperature (20–24°C).

Ligand binding

Cells stored at -80°C were thawed and diluted in cold incubation buffer to obtain a final concentration of 4×10^5 cells ml^{-1} . Cells were homogenized with a Polytron homogenizer for 20 s at maximal setting. The radioligand [^3H]-GR65630 was used as selective 5-HT₃ receptor antagonist (Kilpatrick *et al.*, 1987; 1988; 1989; Lummis *et al.*, 1990; Sharif *et al.*, 1991). For binding assays 400 μl of cell homogenate, 50 μl [^3H]-GR65630 and 50 μl of either control buffer or compound containing buffer were incubated at room temperature (20–24°C). Experiments were started by the addition of cell homogenates to glass incubation vials that contained radioligand and drug. The incubation was stopped by rapid filtration over Whatman GF/B glass fibre filters on a Brandell MR24 cell harvester. Filters were washed three times for 5 s with ice-cold 5 mM Tris HCl buffer (pH 7.4). Filter bound radioactivity was counted for 3 min in scintillation vials containing 2.5 ml Ultima Gold MV (Packard) using a Packard instruments 2200CA liquid scintillation spectrophotometer. Non-specific binding was determined in the presence of 100 μM MDL 72222. The exact concentration of radioligand was calculated from the total radioactivity added to the incubation mixture. Protein contents were measured according to Lowry *et al.* (1951). The association rate of 0.03 nM [^3H]-GR65630 was determined from samples incubated for various periods. The dissociation rate of the radioligand was determined from samples initially incubated with the radioligand for 15 min and subsequently diluted with 100 μM MDL 72222 for various periods. Saturation binding was performed by the incubation of membranes with increasing concentrations of the radioligand for 15 min. For

competition experiments, samples were incubated for 15 min using 6 concentrations of drug at approximately 0.3 nM [^3H]-GR65630. All experiments were carried out in triplicate.

Data analysis and statistics

The dissociation rate constant (k_{-1}) was determined from the monoexponential loss of bound radioligand after dilution with 100 μM MDL 72222. The association rate constant (k_1) was calculated from the equation:

$$k_{+1} = (k_{\text{obs}} - k_{-1})/[L] \quad [1]$$

in which $[L]$ is the concentration of free radioligand and k_{obs} the rate of radioligand binding with time. K_D values were calculated from the kinetic rate constants and from saturation binding data by fitting a Langmuir adsorption isotherm. Apparent dissociation constants (K_i) were calculated from IC_{50} values according to the equation:

$$K_i = \text{IC}_{50}/(1 + [L]/K_D) \quad [2]$$

(Cheng & Prusoff, 1973) using the K_D value obtained from saturation binding with [^3H]-GR65630. Scatchard transformation of saturation binding data were tested for linearity by least-squares regression analysis. Results are expressed as means $\pm$ s.d. of n independent experiments. Concentration-effect curves were fitted according to the function:

$$i = i_{\text{max}}/(1 + \{\text{EC}_{50}/[\text{drug}]\}^n) \quad [3]$$

All non-linear curve fitting was performed using a Levenberg-Marquardt non-linear least squares algorithm (Marquardt, 1963).

Drugs

[^3H]-GR65630 ([^3H]-3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone, specific activity 79.2 Ci mmol^{-1}) was obtained from DuPont NEN, The Netherlands; tropisetron (ICS 205-930; (3 α -tropanyl)-1H-indol-3-carboxylic acid ester) from Sandoz, Basle, Switzerland; MDL 72222 (3-tropanyl-3,5-dichloro-benzoate) and 2-Me-5-HT (($\pm$)-2-methyl-5-hydroxytryptamine maleate) from Research Biochemicals Inc., Natick, U.S.A.; 5-HT (5-hydroxytryptamine creatinine sulphate) and 5-OH-indole (5-hydroxyindole) from Sigma, St. Louis, U.S.A. and TEA (tetraethylammonium bromide) from Merck-Schuchardt, Munich, Germany.

Results

Modulation of the 5-HT-evoked ion current by 5-OH-indole

Ion currents evoked by superfusion of voltage-clamped N1E-115 cells with 10 μM 5-HT changed markedly in the presence of external 5-OH-indole. In the range of 100 μM to 10 mM, 5-OH-indole enhanced the amplitude and slowed the kinetics of decay of the 5-HT-evoked inward current (Figure 1a–c). The onset of these effects was rapid, since no difference was observed between 5-HT responses evoked during continuous superfusion of 5-OH-indole and responses evoked by intermittent coapplications of 5-HT and 5-OH-indole. On removal of 5-OH-indole its effects were also readily reversed. Higher concentrations of 5-OH-indole caused block of the 5-HT-evoked ion current, but the kinetics of the remaining ion current remained slow. In addition, slow tail currents, which indicate reversal of block, were observed on washing after 5-OH-indole concentrations > 1 mM (Figure 1c–e). No effects were detected when up to 50 mM 5-OH-indole was superfused alone in otherwise normally responding cells (Figure 1f). Internal application of 10 mM 5-OH-indole neither affected the 5-HT-evoked ion current, nor caused an effect by itself (result not shown).

The concentration-dependence of the effects of 5-OH-indole on peak amplitude and on the kinetics of decay of the 5-HT-evoked ion current is shown in Figure 2. The peak amplitude of 5-HT-evoked inward current increased in a sigmoidal manner with increasing concentrations of 5-OH-indole up to 10 mM. A steep decrease of the peak inward current amplitude to a level below the amplitude of control responses, was observed when the concentration of 5-OH-indole was further increased. The concentration-effect curve fitted to the normalized peak amplitudes in the range up to 10 mM 5-OH-indole yielded an EC_{50} value of 0.8 ± 0.1 mM 5-OH-indole and a slope factor of 1.4 ± 0.2 . The effects on the time constant of inward current decay showed a similar concentration-dependence, but did not appear to saturate at the maximum potentiation of the peak amplitude observed.

The effect of 1 mM 5-OH-indole was the same, irrespective of whether ion current was evoked by the nearly maximal effective concentration of $10 \mu\text{M}$ or by the supramaximal concentration of $100 \mu\text{M}$ 5-HT (Figure 3a). In the presence of 1 mM 5-OH-indole, the peak amplitude of the $10 \mu\text{M}$ 5-HT-evoked ion current was increased to $123 \pm 11\%$ and the time constant of decay to $319 \pm 37\%$ ($n = 5$) of control values obtained in the absence of 5-OH-indole. The peak amplitude of the $100 \mu\text{M}$ 5-HT-evoked ion current in the same cells was enhanced by 1 mM 5-OH-indole to $127 \pm 11\%$ and the time constant of decay to $352 \pm 66\%$ ($n = 5$) of control values. The results obtained at the two 5-HT concentrations did not differ significantly (Student's t test, $P = 0.36-0.58$). Conversely, the blocking effect of 5-OH-indole depended on the agonist concentration. Figure 3b shows that the amplitude of the 5-HT-evoked ion current in the presence of 25 mM 5-OH-indole is significantly enhanced when the agonist concentra-

tion is raised from $10 \mu\text{M}$ to $100 \mu\text{M}$. In the presence of 25 mM 5-OH-indole, the peak amplitude of the $10 \mu\text{M}$ 5-HT-evoked ion current was $104 \pm 7\%$ ($n = 3$) and the peak amplitude of the $100 \mu\text{M}$ 5-HT-evoked current was $157 \pm 26\%$ ($n = 3$) of control values ($P = 0.03$). In some cells a simultaneous acceleration of the response kinetics was observed. These results show that the blocking effect of a high concentration of 5-OH-indole is surmounted by increasing the agonist concentration, whereas the enhancing effects of a lower concentration of 5-OH-indole cannot be surmounted.

Ligand binding to the 5-HT₃ receptor

The binding of [^3H]-GR65630 to N1E-115 cell homogenates was rapid and reversible (Figure 4a,b). The dissociation

Figure 2 Concentration-dependence of the effects of 5-hydroxyindole (5-OH-indole) on the peak amplitude (●) and on the time constant of decay (○) of the $10 \mu\text{M}$ 5-hydroxytryptamine (5-HT)-induced inward current. Data points are mean values of 3–6 independent determinations obtained from a total of 38 cells. The solid line represents the concentration-effect curve fitted to the data obtained with concentrations ≤ 10 mM 5-OH-indole. The estimated EC_{50} value was 0.8 ± 0.1 mM, the slope factor 1.4 ± 0.2 , and the maximum increase of the amplitude $56 \pm 2\%$. Both the peak amplitude and the time constant of inward current decay in the presence of 5-OH-indole are expressed as a percentage of control values obtained in the same cells.

Figure 1 Effects of 5-hydroxyindole (5-OH-indole) on 5-hydroxytryptamine (5-HT)-induced inward current in N1E-115 cells. Voltage clamped cells were superfused with $10 \mu\text{M}$ 5-HT, as indicated by the bars above the traces, in the absence and in the presence of 5-OH-indole at concentrations of $100 \mu\text{M}$ (a); 1 mM (b); 10 mM (c); 25 mM (d) and 50 mM (e). At low concentrations 5-OH-indole enhanced the amplitude and slowed the decay of the 5-HT-induced inward current (a–c), whereas at high concentrations of 5-OH-indole additional block of the inward current was observed (d–e). Note the occurrence of tail currents on washing following 5-OH-indole application at concentrations ≥ 10 mM (c–e). In contrast to 5-HT, superfusion of 50 mM 5-OH-indole (bar) did not induce a detectable response in voltage clamped N1E-115 cells (f). Each pair of traces of inward current was recorded from a single cell and the data from different cells were scaled for matching peak amplitudes of the control inward currents. Vertical calibrations: 2 nA (a,d); 5 nA (c); 10 nA (b,e,f). Horizontal calibrations 20 s (a–c,e); 10 s (d,f).

Figure 3 Dependence of the effects of 5-hydroxyindole (5-OH-indole) on agonist concentration. (a) Superimposed traces of inward current evoked in the same cell by $10 \mu\text{M}$ and $100 \mu\text{M}$ 5-hydroxytryptamine (5-HT, horizontal bar) in the continuous presence of 1 mM 5-OH-indole. Neither the amplitude nor the kinetics of inward current decay are changed by increasing the agonist concentration. Note that $100 \mu\text{M}$ 5-HT is a supra-maximal concentration. (b) Superimposed traces of inward current evoked in the same cell by $10 \mu\text{M}$ and $100 \mu\text{M}$ 5-HT (horizontal bar) in the continuous presence of 25 mM 5-OH-indole. The amplitude of the inward current is increased at $100 \mu\text{M}$ 5-HT, i.e., the blocking effect of 25 mM 5-OH-indole is surmounted by increasing the agonist concentration. Vertical calibrations: 5 nA. Horizontal calibrations: 20 s (a); 10 s (b).

Figure 4 Binding of [³H]-GR65630 to N1E-115 cell homogenates. (a) Association of the radioligand. The data represent the specific binding of 78 pM [³H]-GR65630, determined by subtracting non-specific binding in the presence of 100 μ M MDL 72222 from total binding, versus incubation time. Single exponential association curves were fitted according to the equation $B_t = B_{\infty} \times (1 - \exp(-k_{\text{obs}} \times t))$. Estimated values of k_{obs} and B_{∞} were 0.76 min⁻¹ and 2.96 fmol mg⁻¹ protein (s.e. of fit = 1.16×10^5). Imposing $k_{\text{obs}} = 0.840$ (calculated using the K_D value of 0.68 nM obtained from saturation binding and the k_{-1} value of 0.753 min⁻¹ obtained from radioligand dissociation, according to eqn. (1) yielded a fitted value for B_{∞} of 2.89 fmol mg⁻¹ protein (s.e. of fit = 1.07×10^5). (b) Dissociation of the radioligand measured at various periods after the addition of an excess of 100 μ M MDL 72222 to samples that were first incubated for 15 min with 30 pM of the radioligand. The drawn dissociation curve was fitted according to the equation $B_t = B_0 \times \exp(-k_{-1} \times t) + C$, in which B represents total binding as a function of time and C the non-specific binding, which was not displaced in the presence of MDL 72222. (c) Saturation binding of the radioligand. Samples were incubated with various concentrations of [³H]-GR65630 in the absence ($\square$) to determine total binding and in the presence of 100 μ M MDL 72222 ($\circ$) to determine non-specific binding. Specific binding (Δ) was calculated by subtracting non-specific

kinetics were fitted by a single exponential decay to a steady level of non-specific binding. The mean dissociation rate constant (k_{-1}) was 0.75 ± 0.12 min⁻¹ ($n = 3$) and the non-specific binding represented $21 \pm 7\%$ of the radioactivity bound at $t = 0$. Fitting the monoexponential association kinetics obtained in 3 experiments using final radioligand concentrations of 45, 78 and 24 pM yielded rate constants (k_{obs}) of 0.68, 0.76 and 1.71 min⁻¹, respectively. The estimated k_{obs} varied too much to calculate a reliable K_D value. However, the association data were equally well fitted by k_{obs} values which were consistent with the K_D value from saturation binding (see Figure 4 and below). This indicates that, within the range of variability of the data, the same apparent radioligand affinities can be deduced from kinetic and saturation binding experiments.

The receptor binding profile was further characterized in competition binding experiments using the selective 5-HT₃ receptor antagonists, tropisetron and MDL 72222 and by the agonists, 5-HT and 2-Me-5-HT. The affinities of these compounds to displace the binding of [³H]-GR65630 to the N1E-115 cell homogenates are listed in Table 1 and are compared to values obtained in other studies. At the highest concentrations, all competing drugs tested displaced the binding of [³H]-GR65630 to the same extent as did 100 μ M MDL 72222, which was used to monitor non-specific binding. This suggests that [³H]-GR65630 and the competing drugs act on the same population of binding sites.

Modulation of ligand binding by 5-OH-indole

The 5-HT moiety, 5-OH-indole, displaced specifically bound [³H]-GR65630 with a low pK_i value of 1.96 ± 0.53 ($n = 6$). The fraction of specifically bound radioligand that was displaced by 1 mM 5-OH-indole was $4.7 \pm 4.5\%$. It has been shown previously that external tetraethylammonium (TEA) is also a low affinity ligand of 5-HT₃ receptors in N1E-115 cells (Hoyer & Neijt, 1988). In the present experiments, TEA displaced [³H]-GR65630 with a pK_i value of 3.03 ± 0.36 ($n = 3$) (see Table 1). Although electrophysiological experiments have demonstrated that TEA is a competitive 5-HT₃ receptor antagonist (Kooyman *et al.*, 1993b), a similar conclusion with respect to the nature of the interaction of 5-OH-indole cannot yet be drawn. The nature of the interaction between the binding of the unlabelled drug 5-OH-indole and that of [³H]-GR65630 was investigated in saturation binding experiments (Figure 4c). 5-OH-indole causes an increase in the K_D but does not affect the B_{max} of the radioligand. In the absence of 5-OH-indole, the mean B_{max} and K_D values were 42.3 ± 1.9 fmol mg⁻¹ protein ($\sim 2 \times 10^4$ sites/cell) and 0.68 ± 0.10 nM ($n = 3$), respectively, whereas in the presence of 10 mM 5-OH-indole the respective values were 46.9 ± 3.8 fmol mg⁻¹ protein and 1.94 ± 0.36 nM ($n = 3$). Linear regression analysis of the Scatchard transforms (Figure 4d) showed that the two slopes, and consequently the K_D values, differed significantly at the $P < 0.001$ level. B_{max} values were within the 95% confidence limits and did not differ significantly. Both lines did not deviate significantly from linear, indicating that the radioligand labels a single affinity population of sites. Thus, 10 mM 5-OH-indole reduces the affinity of the radioligand without affecting the total number of sites, indicating a competitive interaction with the 5-HT₃ receptor.

binding from total binding. The specific binding curve represents the fitted Langmuir adsorption isotherm. (d) Scatchard transformation of the saturation binding of [³H]-GR65630 in the absence ($\square$) and in the presence (Δ) of 10 mM 5-OH-indole. Drawn lines were obtained by linear regression. All data points represent the mean values of triplicate determinations.

Table 1 Displacement of radioligand binding to 5-HT₃ receptors in N1E-115 neuroblastoma cells by various unlabelled compounds

Ligand	[³ H]-GR65630 (homogenate) ^a	[³ H]-tropisetron (homogenate) ^b	[³ H]-GR65630 (intact cells) ^c
Tropisetron	9.10 ± 0.04 (3)	9.09	9.30
MDL 72222	7.59 ± 0.07 (4)	8.21	7.91
5-HT	6.31 ± 0.70 (3)	6.42	6.25
2-Me-5-HT	5.21 ± 0.43 (3)	5.89	5.81
5-OH-indole	1.96 ± 0.53 (6)	—	—
TEA	3.03 ± 0.36 (3)	2.36	—

Affinities of the ligands are expressed as pK_i values.

^aThis report, mean ± s.d. (n).

^bAfter Hoyer & Neijt (1988).

^cCalculated from radioligand concentration, IC₅₀ and K_D values after Lummis *et al.* (1990).

Discussion

The interaction of 5-OH-indole with 5-HT₃ receptors in N1E-115 neuroblastoma cells has been investigated in whole-cell voltage clamp and radioligand binding experiments. The electrophysiological results agree with the previous observation that 1 mM 5-OH-indole potentiates the 5-HT₃ receptor-mediated ion current by enhancing the peak amplitude and by preventing desensitization (Kooyman *et al.*, 1993a). Potentiation of 5-HT₃ receptor-mediated ion current by ethanol in NCB-20 cells (Loving, 1991) and by trichloroethanol in rat nodose ganglion neurones (Loving & Zhou, 1993) has been reported before. This potentiation was associated with an enhanced rate of decay of the 5-HT-induced ion current, in contrast to the effect of 5-OH-indole.

The present description of the concentration-dependence of the electrophysiological effects of 5-OH-indole shows an additional blocking effect at high concentrations. The latter is reflected by a decrease of the peak amplitude of the 5-HT-induced inward current and by the occurrence of tail currents on washing. The electrophysiological results demonstrate that potentiation and block are distinct effects of 5-OH-indole. Firstly, the data on the concentration-dependent effects (Figure 1 and 2) show that potentiation is caused by low concentrations and block by high concentrations of 5-OH-indole. Secondly, the blocking effect is surmounted at increased agonist concentration in contrast to the potentiating effect (Figure 3). These results indicate that the two effects are due to interactions of 5-OH-indole with different sites. At intermediate 5-OH-indole concentrations, a mixture of potentiating and blocking effects is observed (Figure 1c,d). At 10 mM 5-OH-indole, enhancement of the amplitude of the 5-HT-induced ion current occurs simultaneously with a slow tail current. The tail current indicates that block has occurred in the presence of 5-OH-indole and is reversed on washing. With increasing concentration of 5-OH-indole the potentiating effect is reduced and reverses into a reduction of peak amplitude and the tail current becomes more pronounced (Figure 1c,e). These results indicate that the concentration-dependence of potentiation is distinct from that of block and that the concentration-ranges in which the two effects occur overlap. Since potentiation and block have oppositely effects on the 5-HT-induced ion current, it is not possible to determine the exact concentrations that cause half maximum potentiation and block. However, it can be inferred that half maximum block occurs at a concentration beyond 1 mM, since the absence of a tail current indicates that block does not occur at this concentration of 5-OH-indole (Figure 1b).

In order to corroborate the electrophysiological findings, the effects of 5-OH-indole were also investigated in receptor binding experiments. The radiolabelled antagonist [³H]-GR65630 labels 5-HT₃ receptors in N1E-115 cell homogenates with a K_D value of 0.68 nM (Figure 4), i.e., with similar high affinity as previously reported for intact N1E-115 cells (Lummis *et al.*, 1990) and for NCB-20 cell homogenates (Sharif *et al.*, 1991). The radioligand is also displaced by a

number of selective 5-HT₃ receptor (ant)agonists with apparent affinities which are similar to values reported for the displacement of [³H]-tropisetron in N1E-115 homogenates (Hoyer & Neijt, 1988) and of [³H]-GR65630 in intact N1E-115 cells (Lummis *et al.*, 1990) as can be seen from the compilation of data in Table 1. Half maximum displacement of [³H]-GR65630 was obtained at a concentration of approximately 10 mM 5-OH-indole, whereas at 1 mM only 5% of the specifically bound radioligand was displaced. In addition, the binding experiments showed that 10 mM 5-OH-indole displaces the radioligand in a competitive manner (Table 1; Figure 4d). A comparison of the electrophysiological and binding results shows that 5-OH-indole causes a surmountable block of the 5-HT-induced ion current and competitively displaces the radioligand with similar low affinities. Therefore, it is concluded that high concentrations of 5-OH-indole block 5-HT₃ receptors by an interaction with the ligand recognition site. The non-surmountable potentiation of 5-HT-induced ion current, which is observed at concentrations of 5-OH-indole which fail to displace the radioligand, appears to be due to a non-competitive effect.

Similar to 5-OH-indole, high concentrations of TEA displace the binding of [³H]-tropisetron (Hoyer & Neijt, 1988) as well as that of [³H]-GR65630 (Table 1). Functional competition, as between the agonist and 5-OH-indole, was previously demonstrated to occur between 5-HT and TEA (Kooyman *et al.*, 1993b). Thus, both compounds appear to interact with the ligand recognition site of the 5-HT₃ receptor, but agonistic effects of 5-OH-indole, TEA, and of the combination of the two have never been observed (A.R. Kooyman, unpublished observation). From the binding properties of chemically distinct antagonists it has been proposed that ligand binding at the 5-HT₃ receptor involves interactions with a cationic and an aromatic site (Gozlan & Langlois, 1992). It is conceivable that TEA and 5-OH-indole compete with antagonist binding at the cationic and aromatic site, respectively. The large difference between the affinity of 5-HT and the affinities of 5-OH-indole and TEA (Table 1) as well as the absence of agonistic effects of TEA and 5-OH-indole suggest that the interaction of 5-HT with its recognition site involves more than a two point interaction with a cationic and an aromatic site. A further investigation of the site at which 5-OH-indole causes the non-competitive potentiation of the 5-HT₃ response and of other ligands interacting with this site is of interest in view of its prominent modulatory role in the neuronal response to 5-HT.

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