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Characterisation of 5-HT_3 recognition sites in membranes of NG 108-15 neuroblastoma-glioma cells with [^{3}H]ICS 205-930

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Summary. 1. The binding characteristics of [^{3}H]ICS 205-930, a potent and selective 5-hydroxytryptamine 5-HT_3 receptor antagonist, were investigated in membranes prepared from murine neuroblastoma-glioma NG 108-15 cells.

2. [^{3}H]ICS 205-930 bound rapidly, reversibly and stereoselectively to a homogeneous population of high affinity recognition sites: B_{max} = 58 ± 3 fmol/mg protein, pK_D = 9.01 ± 0.08 (n = 11). Non linear regression and Scatchard analysis of saturation data suggested the existence of a single class of [^{3}H]ICS 205-930 recognition sites on NG 108-15 cells. The binding was rapid, stable and reversible. The affinity of [^{3}H]ICS 205-930 determined in kinetic studies was in agreement with that obtained under equilibrium conditions.

3. Competition studies performed with a variety of agonists and antagonists also suggested the presence of a homogeneous population of [^{3}H]ICS 205-930 recognition sites. All competition curves were steep and monophasic and were best fit by a 1 receptor site model. [^{3}H]ICS 205-930 binding sites displayed the pharmacological profile of a 5-HT_3 receptor. Potent 5-HT_3 receptor antagonists showed nanomolar affinities for [^{3}H]ICS 205-930 binding sites with the following rank order of potency: SDZ 206-830 > ICS 205-930 > SDZ 206-792 > BRL 43694 > quipazine > BRL 24924 > SDZ 210-204 > MDL 72222 > SDZ 210-205. Metoclopramide, mCPP and mianserin showed submicromolar affinity. The rank order of potency of agonists was: 5-HT = 2-methyl-5-HT > phenylbiguanide ≫ 8-OH-DPAT > 5-carboxamidotryptamine. Drugs acting at 5-HT_1, 5-HT_2, dopamine receptors, α- and β-adrenoceptors, (methysergide, ketanserin, pindolol, spiperone, SCH 23390) showed very low affinities for [^{3}H]ICS 205-930 recognition sites.

4. The binding of [^{3}H]ICS 205-930 was not affected by guanine or adenine nucleotides (GTP, GppNHp and ATP) at 1 mmol/l. Moreover, these nucleotides did not affect the binding of agonists suggesting that 5-HT_3 recognition sites are not coupled to guanine nucleotide regulatory proteins.

5. The interactions of agonists and antagonists with [^{3}H]ICS 205-930 recognition sites were competitive in nature, as demonstrated by saturation experiments carried out with [^{3}H]ICS 205-930 in the presence and the absence of unlabelled compounds: apparent B_{max} values were not reduced whereas apparent K_D values were increased in the presence of competing ligands. There was a good agreement between apparent K_B values determined in saturation experiments with agonists and antagonists and their K_D values determined in competition experiments.

6. These findings are consistent with [^{3}H]ICS 205-930 labelling 5-HT_3 receptors on NG 108-15 cells. The pharmacological profile of the sites labelled by [^{3}H]ICS 205-930 on NG 108-15 cells is very similar to that of the 5-HT_3 sites identified on neuroblastoma N1E-115 cells (Hoyer and Neijt 1988a, b).

7. The present data demonstrate that [^{3}H]ICS 205-930 is a suitable ligand for the identification of 5-HT_3 recognition sites in membrane preparations.

Key words: 5-HT_3 Receptors – Radioligand binding – [^{3}H]ICS 205-930 – Neuroblastoma-glioma NG 108-15 cells

Introduction

Whereas an abundant literature is dedicated to the binding of various radioligands to 5-HT_1 and 5-HT_2 receptors, 5-HT_3 receptors had not up to very recently been identified with radioligand binding technique (Bradley et al. 1986). The 5-HT_3 receptor field has experienced, most recently, significant progress due to the development of potent and selective 5-HT_3 receptor antagonists: MDL 72222 (Fozard 1984a), ICS 205-930 (Richardson et al. 1985), BRL 43694 (Fake et al. 1987) and GR 38032 F (Brittain et al. 1987). 5-HT_3 receptors have been identified in the peripheral nervous system (for reviews see Fozard 1984b; Bradley et al. 1986; Richardson and Engel 1986). Their presence in the CNS has been recently suggested (Costall et al. 1987; Hagan et al. 1987), but not demonstrated functionally. Neijt et al. (1986, 1988) have pharmacologically characterized the 5-HT-induced activation of ion channels on N1E-115 neuroblastoma cells. In this murine cell line, 5-HT and 2-methyl-5-HT (a 5-HT_3 receptor agonist), induced membrane depolarization which was antagonised by nanomolar concentrations of ICS 205-930 and MDL 72222, indicating that these effects were mediated via 5-HT_3 receptors. Similar findings were made in NG 108-15 neuroblastoma cells (Neijt, unpublished

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Abbreviations: GR 38032F, (1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl)methyl]-4-one); Hepes, 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid; Tris, Tris-(hydroxymethyl)-aminomethane; G proteins, guanine nucleotide regulatory binding proteins; GTP, guanosine-5′-triphosphate; ATP, adenosine-5′-triphosphate; GppNHp, guanyl-5′-yl-imidodiphosphate

work). In both cell lines, 5-HT-induced acetylcholine release has been described but not pharmacologically characterised (Amano et al. 1972).

Recently, we were able to identify 5-HT_3 recognition sites on neuroblastoma N1E-115 cells in radioligand binding studies (Hoyer and Neijt 1988a, b). Preliminary data indicate their presence on neuroblastoma-glioma NG 108-15 cells (Hoyer and Neijt, 1987). The aim of the present report was to further characterise 5-HT_3 receptors in NG 108-15 cells with the radioligand [^{3}H]ICS 205-930, a potent and selective 5-HT_3 receptor antagonist.

Materials and methods

Cell culture. Mouse neuroblastoma-glioma cells of the clone NG 108-15 were grown in Dulbecco's modified Eagle's medium (DMEM) with Hepes (7.6 mmol/l) and sodium bicarbonate (30 mmol/l). The growth medium was supplemented with the antibiotics penicillin (100 IU/ml) and streptomycin (100 µg/ml) and with 7.5% fetal calf serum (Gibco, Basle, Switzerland). The following amino acids (in mmol/l) were included: cysteine-HCl (0.30), l-alanine (0.40), asparagine (0.45), l-aspartic acid (0.40), l-proline (0.40) and l-glutamic acid (0.40). Cells were cultured at 37° C, in closed tissue culture roller bottles (Falcon 850 cm^2, 0.75 rpm), fed every second day and subcultured every 5 days. The cells were grown to a density of $3-5\times10^8$ cells/bottle and harvested by vigorous shaking.

Membrane preparation. Harvested cells were centrifuged at 4° C at 900 *g* for 5 min. The supernatant was discarded and the cell pellet resuspended in 20–30 ml Tris buffer (20 mmol/l, pH 7.5) containing 154 mmol/l NaCl and homogenised with a Brinkman polytron (position 9, 2×15 s). The homogenate was centrifuged again at $900\times g$. The pellet was discarded and the supernatant was used for direct binding studies or kept at −70° C until used.

Radioligand binding studies. Radioligand binding experiments were performed as described (Hoyer and Neijt 1988b). Briefly, fresh or frozen membranes were diluted to approx. 2×10^6 cells/ml in Tris NaCl buffer. Binding assays consisted of 50 µl radioligand, 50 µl buffer or drug and 150 µl of membrane suspension. The experiments were started by the addition of membranes to polystyrene tubes containing radioligand and drug; tubes were then incubated at 37° C for 60 min. The incubation was stopped by rapid filtration and washing with ice-cold Tris NaCl buffer (2×10 ml) over Whatman GF/B glass fiber filters on a Brandel MR24 cell harvester. After drying under reduced vacuum, filters were added to scintillation vials containing 5 ml of Kontrogel (Kontron, Zürich). Radioactivity was counted in a Packard 4600 Tricarb β-counter, at 67% counting efficiency. Non specific binding was defined in the presence of 10 µmol/l MDL 72222. Competition (displacement) experiments were carried out with 8–12 concentrations of drug, and 2–4 nmol/l [^{3}H]ICS 205-930. Saturation experiments were performed with 12 concentrations of radioligand ranging from 0.1 to 20 nmol/l. Experiments were carried out in triplicate. Results are expressed as pK_D values (− log mol/l) ± SEM of *n* independent experiments. Protein concentrations were determined according to Bradford (1976).

Data analysis. Competition data were analysed using the non linear regression computer program SCTFIT developped by DeLean (1979). Competition curves were first analysed according to a 1 site model, then for a 2 site model. The statistical analysis was based on the "extra sum of square principle" according to Rodbard (1974) and *F*-test analysis. A 2 site model was considered to be acceptable only when $P < 0.001$. Saturation experiments were analysed with SCTFIT and according to Scatchard (1949). Some saturation experiments were performed in the presence and the absence of a given concentration of agonist or antagonist in order to assess the competitive nature of the receptor-ligand interaction. Apparent pK_B values for the competing drugs were estimated according to the equation $\log (CR-1) = \log B - \log K_B$ where *CR* is the concentration ratio (the factor by which the concentration of the radioligand has to be increased in the presence of the unlabelled compound to obtain identical binding to that observed in the absence of the unlabelled compound), *B* is the concentration of the unlabelled drug and K_B its apparent dissociation constant.

Drugs. Drugs were obtained from the following sources: quipazine (Miles, Elkhart, IN, USA), SCH 23390 (Schering Corporation, Bloomfield, NJ, USA), metoclopramide (Delagrange, Paris, France), ketanserin and spiperone (Janssen, Beerse, Belgium), phenylbiguanide (ICI, Macclesfield, Cheshire, UK) 5-HT, (5-hydroxytryptamine, serotonin; Sigma, St. Louis, MO, USA), BRL 24924, ([±endo]-4-amino-5-chloro-2-methoxy-N-(1-azabicyclo-[3,3,1]nonyl)benzamide monohydrochloride, Beecham, Harlow, Essex, UK), mianserin (Organon, Oss, The Netherlands), MDL 72222, (1αH, 3α,5αH-tropan-3-yl-3,5-dichlorobenzoate, Merrell-Dow, Strasbourg, France), 5-HTP dipeptide (N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide, Research Foundation, New York, NY, USA). The following compounds were synthesised at Sandoz: 2-methyl-5-HT; methysergide; pindolol; 8-OH-DPAT, (8-hydroxy-[2-N,N-dipropyl-amino]tetralin); 5-CT, (5-carboxamidotryptamine); mCPP, (1-(m-chlorophenyl)piperazine); ICS 205-930, ((1αH, 3α, 5αH-tropan-3-yl)1-H-indole-3-carboxylic acid ester); BRL 43694, (endo-N-(9-methyl-9-azabicyclo-[3,3,1]-non-3-yl)-1-methyl-indazol-3-carboxamide); SDZ 210-204, ((−)-(1R,2R,4S)-1H-indole-3-carboxylic acid-7-methyl-7-azabicyclo-[2.2.1]hept-2yl-ester); SDZ 210-205, (the (+) enantiomer of SDZ 210-204); SDZ 206-792, ((N-desmethyl-3-α-homotropanyl)-1H-indole-3-carboxylic acid ester); SDZ 206-830, ((3α-homotropanyl)-1-methyl-5-fluoro-indole-3-carboxylic acid ester) and [^{3}H]ICS 205-930 (specific activity, 33.9 Ci/mmole).

Results

Saturation experiments

[^{3}H]ICS 205-930 labelled with high affinity an apparently homogeneous population of binding sites on membranes of NG 108-15 cells ($pK_D = 9.01 \pm 0.08$, $B_{max} = 58.0 \pm 2.6$ fmol/mg, $n = 11$). Non linear regression analysis of the data fitted best a 1 site model (Fig. 1), in agreement with the analysis of saturation data according to Scatchard (1949). Non specific binding determined in the presence of 10 µmol/l MDL 72222 was low (10% at the K_D) and increased linearly with the free radioligand concentration.

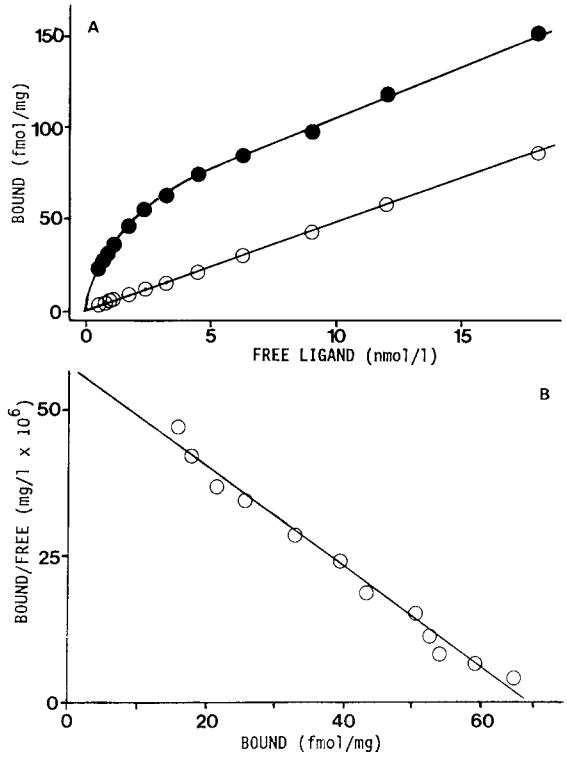

Fig. 1. Saturation experiment with [^{3}H]ICS 205-930 in membranes of NG 108-15 cells. Membranes (200–400 µg/assay) were incubated with varying concentrations of [^{3}H]ICS 205-930 in the absence (●) (total binding) and the presence of MDL 72222 10 µmol/l (○) (non-specific binding). **A** Represents bound (fmol/mg protein) versus free ligand (mmol/l). *Points* are means of triplicate determinations. The data were best fit for a 1 site model. **B** Represents the transformation of the saturation data according to Scatchard, bound/free ($1/mg \times 10^6$) versus bound (fmol/mg protein). Representative example of 11 similar experiments

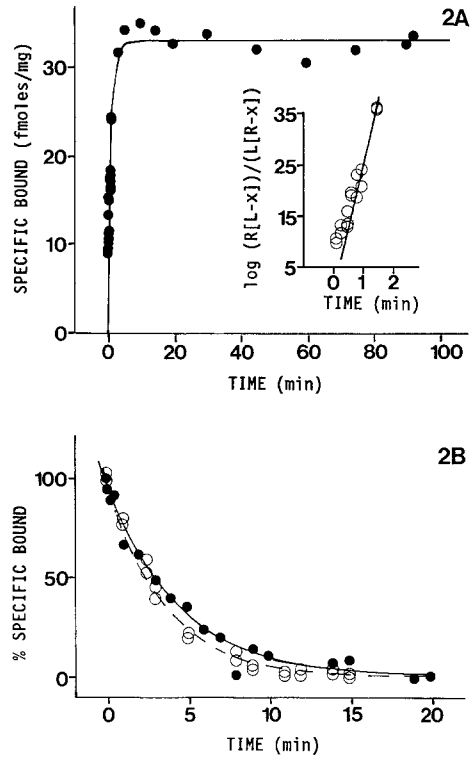

Fig. 2. Time course of association and dissociation of [^{3}H]ICS 205-930 to NG 108-15 membranes. NG 108-15 membranes were incubated with 1.7 nmol/l [^{3}H]ICS 205-930 in the absence and the presence of 10 µmol/l MDL 72222. The data are expressed as specific binding (fmol/mg) versus incubation time (min). The data points are means of triplicate or sextuplicate determinations. **A** Represents the association reaction and **B** the dissociation reaction induced after an incubation time of 45 min by the addition of an excess of 10 µmol/l MDL 72222 (○) or 100 µmol/l 5-HT (●). The inset in **A** represents a transformation of the association reaction where $\log (R[L-x])/(L[R-x]) = k_{on} \cdot t \cdot [L-R]/2.303$. R, L and x represent the concentrations of free receptors and radioligand at time 0 and bound receptor-ligand complex at time t, and k_{on} the association rate constant. k_{on} was estimated to 2.51×10^8 mol/l^{-1} · min^{-1}. The dissociation reaction was fitted according to the equation $R = R_0 \cdot \exp(-k_{off} \cdot t)$, where R and R_0 represent the concentration of receptors bound at time t and 0, and k_{off} the dissociation rate constant. k_{off} was estimated to 0.247 min^{-1} and 0.236 min^{-1} when the dissociation reaction was induced by MDL 72222 and 5-HT respectively. Representative of 2–3 similar experiments

Kinetic experiments

[^{3}H]ICS 205-930 binding to membranes of NG 108-15 cells was rapid and fully reversible (Fig. 2). Both the association and the dissociation reaction (induced by an excess of MDL 72222 or 5-HT) were monophasic. Maximal binding was reached within 10 min and remained stable for up to 90 min. The dissociation reaction was very similar whether induced by an excess of agonist or antagonist (Fig. 2B). The association rate constant k_{on} was estimated to 2.45×10^8 mol/l^{-1} · min^{-1}, and the dissociation rate constant k_{off} was 0.247 min^{-1} (when MDL 72222 was used to induce the dissociation reaction) and 0.231 min^{-1} (with 5-HT). The ratios k_{off}/k_{on} gave K_D values of 1.01 and 0.94 nmol/l, respectively, which agree with the K_D (0.98 nmol/l) obtained in saturation experiments.

Competition experiments

The binding sites labelled by [^{3}H]ICS 205-930, were characterised in competition experiments carried out with a variety of different drugs. Figure 3 shows representative competition curves obtained with potent 5-HT$_3$ receptor agonists (5-HT and 2-methyl-5-HT) and antagonists (ICS 205-930, BRL 43694 and MDL 72222). The affinity values of the tested compounds are listed in Table 1. All compounds displayed monophasic and steep competition curves. At higher concentrations all competing drugs displaced [^{3}H]ICS 205-930 binding to the same plateau as did MDL 72222 which was used to define non specific binding. These data again suggest that [^{3}H]ICS 205-930 binds to a homogeneous class of recognition sites. The binding of [^{3}H]ICS 205-930 was stereoselectively displaced by 2 stereoisomers, SDZ 210-204 and SDZ 210-205, which showed a 4–5-fold difference in affinity. 5-HT$_3$ receptor antagonists were potent inhibitors of [^{3}H]ICS 205-930 binding. Among agonists, only 5-HT, 2-methyl-5-HT and phenylbiguanide displaced [^{3}H]ICS 205-930 with micromolar affinities. In contrast, 5-HT$_1$ and 5-HT$_2$ ligands were weak inhibitors (except for quipazine and mianserin). Quipazine has been described as a potent 5-HT$_3$ receptor antagonist (Round and Wallis 1986, 1987; Ireland and Tyers 1987). Most of the compounds active at other receptors, displayed very low affinities for the site labelled by [^{3}H]ICS 205-930. The pseudo Hill coefficients of the

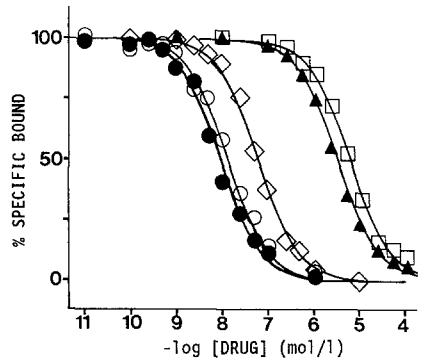

Fig. 3. Inhibition of [^{3}H]ICS 205-930 binding by various agonists and antagonists. NG 108-15 membranes were incubated as described in the presence of [^{3}H]ICS 205-930 and varying concentrations of the indicated compounds: BRL 43694 (○), ICS 205-930 (●), MDL 72222 (◇), 5-HT (▲) and 2-methyl-5-HT (□). The data points are means of triplicate determinations. The results are expressed as percent specific binding versus the drug concentrations (− log mol/l). All curves were best fit for a 1 site model. Representative examples of 4−8 independent experiments

Table 1. Affinity values of various drugs to [^{3}H]ICS 205-930 recognition sites on NG 108-15 membranes

Drug	pK_D	SEM	n
SDZ 206-830	9.35	0.24	4
SDZ 206-792	8.75	0.18	4
ICS 205-930	8.81	0.12	5
BRL 43694	8.66	0.15	5
Quipazine	8.47	0.11	3
BRL 24924	8.06	0.12	4
SDZ 210-204	8.11	0.07	5
MDL 72222	7.77	0.11	6
SDZ 210-205	7.54	0.11	4
Mianserin	6.97	0.14	3
mCPP	7.00	0.07	4
Metoclopramide	6.66	0.08	4
5-HT	6.04	0.05	8
Phenylbiguanide	5.79	0.09	4
2-Methyl-5-HT	6.01	0.11	7
SCH 23390	< 4.0		3
5-CT	4.13	0.10	3
8-OH-DPAT	4.44	0.15	3
Methysergide	< 4.0		3
5-HTP dipeptide	3.97		1
Pindolol	4.13	0.08	3
Ketanserin	3.90	0.13	3
Spiperone	3.62	0.26	3

Affinity values are expressed as pK_D's (− log mol/l, mean ± SEM of n experiments) obtained in competition experiments as described in Methods

tested drugs were not different from unity. Together, these data are compatible with [^{3}H]ICS 205-930 binding to a 5-HT_3 recognition site.

The competitive interaction between [^{3}H]ICS 205-930 and unlabelled drugs with [^{3}H]ICS 205-930 binding sites was tested by performing saturation experiments with [^{3}H]ICS 205-930 in the presence and absence of agonists or antagonists (Fig. 4). B_{max} values obtained in the presence of 5-HT and SDZ 206-830 were not significantly different from B_{max} values obtained in the absence of these compounds. In contrast, apparent K_D values were increased, suggesting a competitive interaction between the radioligand and the unlabelled compounds at the binding site. Using this method the apparent pK_B values for agonists and antagonists could be estimated (Table 2). These values are in good agreement with the pK_D values estimated from competition (displacement) curves (see Tables 1, 2; Fig. 5).

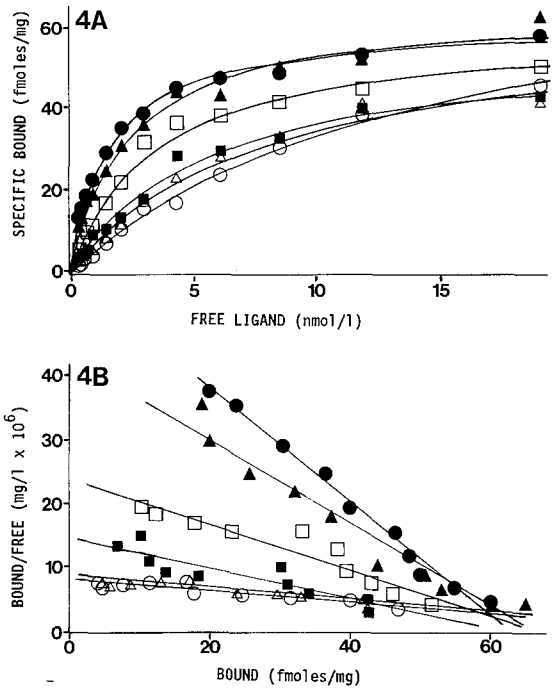

Fig. 4. Saturation experiments with [^{3}H]ICS 205-930 in the absence and the presence of agonists and antagonists. Saturation experiments were performed as described with [^{3}H]ICS 205-930 in the absence (●) and the presence of 1 μmol/l 5-HT (▲), 10 nmol/l quipazine (□), 2 nmol/l SDZ 206-830 (■), 0.1 μmol/l MDL 72222 (△) and 10 nmol/l ICS 205-930 (○). **A** Represents specific binding (fmol/mg) versus free radioligand (nmol/l). **B** Represents the same data transformed according to Scatchard, bound/free ($1/mg \times 10^6$) versus bound (fmol/mg). B_{max} values were similar in the absence and in the presence of agonists and antagonists whereas apparent K_D values were increased when compared to controls. Representative of 3−4 similar experiments

Effects of nucleotides and temperature on [^{3}H]ICS 205-930 binding

The effects of nucleotides (GTP, GppNHp and ATP, 1 μmol/l − 1 mmol/l) on [^{3}H]ICS 205-930 binding were studied in NG 108-15 membranes. None of the nucleotides, even at 1 mmol/l, produced a significant reduction of [^{3}H]ICS 205-930 binding (data not shown). Competition curves were also performed with 5-HT and 2-methyl-5-HT in the presence and the absence of 0.1 mmol/l GppNHp. The competition curves of the 2 agonists were not affected by GppNHp in the presence or absence of 10 mmol/l Mg^{2+}. pK_D values were similar to those obtained in controls, and the slopes of the competition curves remained unchanged (Table 3).

The usual incubation temperature was 37° C. Experiments were also carried out at 20° C, the temperature at which electrophysiological experiments were routinely carried out in cells. The affinity of [^{3}H]ICS 205-930 determined at 20° C ($pK_D = 9.36 \pm 0.12$, $n = 3$) remained essentially

Table 2. Apparent pK_B values obtained in saturation experiments performed in the presence of agonists and antagonists in NG 108-15 cell membranes

Drug (concentration)		Mean shift	% B_{max}	pK_B	SEM	n
MDL 72222	10^{-7}	5.0	101 ± 7	7.60	0.05	3
ICS 205-930	10^{-8}	14.4	160 ± 49	8.97	0.26	3
Quipazine	10^{-8}	2.2	100 ± 3	8.07	0.04	2
mCPP	10^{-6}	3.4	93 ± 17	6.28	0.22	3
BRL 43694	10^{-8}	3.5	109 ± 12	8.37	0.09	3
SDZ 206-792	10^{-8}	4.5	105 ± 14	8.52	0.12	3
2-Methyl-5-HT	10^{-6}	1.6	104 ± 7	5.68	0.21	3
Metoclopramide	$2 \cdot 10^{-6}$	4.3	105 ± 15	6.17	0.16	3
SDZ 206-830	$2 \cdot 10^{-9}$	2.4	84 ± 3	8.85	0.53	3
5-HT	10^{-6}	1.6	103 ± 1	5.69	0.23	3
Phenylbiguanide	10^{-6}	1.6	98 ± 4	5.73	0.16	3

The table lists apparent pK_B values [− log mol/l ± SEM (or SD in the case of quipazine) of n individual experiments] estimated from saturation experiments performed in the presence of the indicated concentrations of agonist or antagonist as described in Fig. 4. Also indicated is the mean shift in the apparent K_D for the radioligand in the presence of the competing drug and the apparent B_{max} in percent of the control experiment

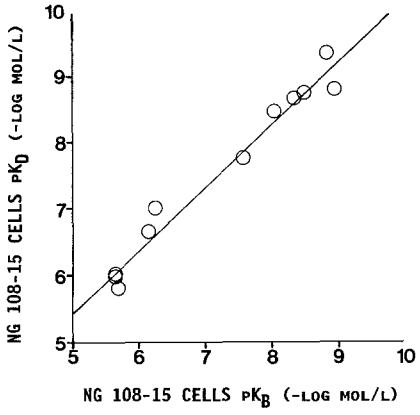

Fig. 5. Comparison between pK_D determined in competition experiments and pK_B values determined in saturation experiments for agonists and antagonists. The figure compares pK_D and pK_B values of the indicated drugs for [^{3}H]ICS 205-930 recognition sites on membranes of NG 108-15 cells. Values are from Tables 1 and 2

unchanged when compared to the affinity obtained in parallel control experiments carried out at 37° C (pK_D = 9.28 ± 0.14, n = 3). B_{max} values were slightly increased at 20° C (135 ± 7% of controls).

Discussion

The present study demonstrates that [^{3}H]ICS 205-930 labels 5-HT$_3$ recognition sites in membranes of NG 108-15 cells and extends preliminary findings (Hoyer and Neijt 1987). The study was carried out in order to better characterise the pharmacology of the 5-HT$_3$ sites present in NG 108-15 cells, a neuroblastoma-glioma cell line and to compare their profile with that of 5-HT$_3$ receptors from neuroblastoma N1E-115 cells (Hoyer and Neijt 1988a, b).

The binding of [^{3}H]ICS 205-930 was rapid, reversible, saturable and of high affinity (K_D = 1 nmol/l). The affinity

Table 3. Affinity values of 5-HT and 2-methyl-5-HT for [^{3}H]ICS 205-930 recognition sites in the presence and the absence of 100 µmol/l GppNHp

Agonist	pK_D Control	n	pK_D GppNHp	n
5-HT	6.05 ± 0.06	3	6.16 ± 0.06	3
2-Methyl-5-HT	6.15 ± 0.04	3	6.15 ± 0.06	3

Affinity values were determined from competition experiments carried out in parallel in the presence or in the absence of GppNHp

values determined for ICS 205-930 in saturation, kinetic and competition experiments were in good agreement. [^{3}H]ICS 205-930 showed rather low non-specific binding (10% at K_D value), reached apparent equilibrium within 10 min and was stable for up to 90 min at 37° C. Computer assisted analysis of saturation curves and their transformation according to Scatchard indicated the presence of a homogeneous class of [^{3}H]ICS 205-930 recognition sites on NG 108-15 cell membranes. This was also suggested by monophasic association and dissociation reactions. In addition, competition curves of all the tested compounds were steep and monophasic (pseudo-Hill coefficient not different from unity), which again suggests that [^{3}H]ICS 205-930 labels a single population of recognition sites.

The pharmacological nature of the sites labelled by [^{3}H]ICS 205-930 was investigated by performing competition experiments with a large variety of agonists and antagonists acting at different receptors. Potent 5-HT$_3$ receptor antagonists such as SDZ 206-830, SDZ 206-792, ICS 205-930 (Richardson et al. 1985), BRL 43694 and BRL 24924 (Fake et al. 1987), MDL 72222 (Fozard 1984a) and quipazine (Round and Wallis 1986, 1987; Ireland and Tyers 1987) displayed nanomolar or subnanomolar affinities for [^{3}H]ICS 205-930 recognition sites. Metoclopramide and mCPP, which act also as antagonists at 5-HT$_3$ receptors (Round and Wallis 1986, 1987; Ireland and Tyers 1987), showed submicromolar affinity. 5-HT, 2-methyl-5-HT and phenylbiguanide, which are about equipotent as agonists of 5-HT$_3$ receptors (Richardson et al. 1985; Ireland and Tyers 1987), displayed the highest affinity among agonists to these sites, whereas 8-OH-DPAT and 5-CT showed very low affinity. Compounds with high affinity for 5-HT$_1$ and 5-HT$_2$, α- and β-adrenoceptors or D$_1$ and D$_2$ dopamine receptors (except for mianserin), showed only very low affinity for [^{3}H]ICS 205-930 binding sites. The binding of [^{3}H]ICS 205-930 was stereoselectively displaced by 2 stereoisomers (SDZ 210-204 and SDZ 210-205).

The interaction of agonists and antagonists with the sites labelled by [^{3}H]ICS 205-930 was competitive and mutually exclusive. This was demonstrated in saturation experiments performed with [^{3}H]ICS 205-930 in the absence and the presence of agonists and antagonists. In the presence of these compounds saturation curves reached the same maximum as controls while apparent K_D values for the radioligand were increased, as expected from competitively interacting drugs. Apparent pK_B values calculated from these experiments for agonists and antagonists were in agreement with the pK_D values obtained from displacement experiments. These findings suggest that agonists and antagonists bind to the same recognition site which is labelled by [^{3}H]ICS 205-930. These findings are further supported by the fact that the dissociation reactions induced by an excess of MDL 72222

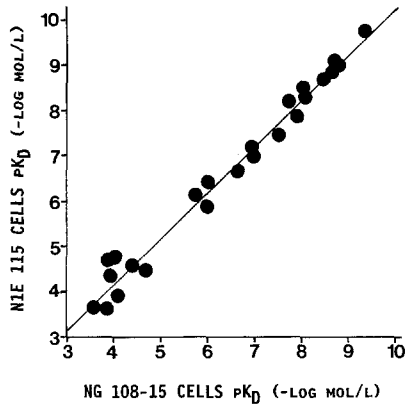

Fig. 6. Comparison between affinity values of various drugs for [^{3}H]ICS 205-930 recognition sites on membranes of NG 108-15 and N1E-115 cells. The figure compares pK_D values determined in competition experiments performed with membranes of NG 108-15 and N1E-115 cells (taken from Hoyer and Neijt 1988b)

or 5-HT were almost superimposable and monophasic. The interaction of agonists and antagonists with these sites is not modulated by guanine or adenine nucleotides; GppNHp, GTP and ATP up to 1 mmol/l had no effect, neither on [^{3}H]ICS 205-930 binding, nor on the affinity of 5-HT and 2-methyl-5-HT. There is at present no evidence for the existence of different affinity states of the agonist-5-HT$_3$ receptor complex, in contrast to the situation with receptors linked to G proteins (e.g. α- and β-adrenoceptors, dopamine, 5-HT$_1$ and 5-HT$_2$ receptors). Taken together, these experiments suggest that 5-HT$_3$ receptors are not coupled to G proteins.

The existence of subtypes of 5-HT$_3$ receptors has been proposed (Richardson and Engel 1986), based on the potency of a variety of 5-HT$_3$ receptor antagonists in different 5-HT$_3$ receptor models (Fozard 1984a; Richardson et al. 1985). For instance, MDL 72222, ICS 205-930, SDZ 206-830 and 206-792 were described as potent 5-HT$_3$ receptor antagonists in sensory neurones (vagus nerve, nodose ganglion), sympathetic (heart, superior cervical ganglion) and parasympathetic neurones (heart). By contrast, the potency of ICS 205-930, SDZ 206-830 and 206-792 was significantly lower on enteric nerves (Richardson et al. 1985), whereas MDL 72222 was almost inactive in this model (Fozard 1984a). It is too early to ascribe a subtype to the site labelled by [^{3}H]ICS 205-930 on NG 108-15 and N1E-115 cells, though it bears pharmacological similarities with the 5-HT$_3$ receptors (5-HT$_{3A}$, Richardson and Engel 1986) found on sensory and sympathetic neurones (Round and Wallis 1986, 1987; Ireland and Tyers 1987). The affinity values found in binding experiments are very close to apparent pA$_2$ or pD$_2$ values reported in electrophysiological studies (Hoyer and Neijt 1988b). It seems that the 5-HT$_3$ sites labelled on these cells are pharmacologically similar to those recently described using radioligand binding studies in rat brain (Kilpatrick et al. 1987). It is clear that the sites labelled with [^{3}H]ICS 205-930 on NG 108-15 and N1E-115 cells are different from the 5-HT$_3$ receptor found on enteric nerves (5-HT$_{3C}$), where MDL 72222 was nearly inactive (Fozard 1984a; Richardson and Engel 1986).

Although a large series of compounds was tested, there is no indication for the presence of different 5-HT$_3$ recognition sites on NG 108-15 neuroblastoma-glioma and N1E-115 neuroblastoma cells; the sites were homogeneous (saturation curves and their transformation according to Scatchard were monophasic over a large concentration range and competition curves were steep and monophasic too, although the radioligand concentration was well above the K_D value of the radioligand) and their binding profile was very closely correlated (Fig. 6). This suggests that 5-HT$_3$ receptors identified on NG 108-15 cells do not originate from the parent glioma cell (C6BU-1), but rather from the parent neuroblastoma cell (C1300) (Dawson et al. 1983). This result is disappointing: one could have expected the presence of a mixed or even different population of 5-HT$_3$ sites on NG 108-15 cells which would have suggested 5-HT$_3$ sites originating from glioma cells but this was not the case.

In conclusion, this report has characterised a 5-HT$_3$ recognition site on NG 108-15 neuroblastoma-glioma cells using the radioligand binding technique. Because of the high affinity and selectivity of [^{3}H]ICS 205-930 for 5-HT$_3$ receptors, it will hopefully prove possible to label 5-HT$_3$ receptors in various tissues using both radioligand binding and autoradiographical techniques and eventually to label subtypes of 5-HT$_3$ recognition sites which have been suggested to be present in different tissues (Richardson and Engel 1986).

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