0026-895X/88/030303-07$02.00/0

MOLECULAR PHARMACOLOGY, 33:303-309

Identification of Serotonin 5-HT$_3$ Recognition Sites in Membranes of N1E-115 Neuroblastoma Cells by Radioligand Binding

DANIEL HOYER and HANS C. NEIJT

Preclinical Research, SANDOZ Ltd, CH-4002 Basel, Switzerland (D.H.) and Department of Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht (H.C.N.), NL-3508 TD Utrecht, The Netherlands

Received October 6, 1987; Accepted December 21, 1987

SUMMARY

[^{3}H]ICS 205-930 recognition sites were analyzed in membranes prepared from murine neuroblastoma N1E-115 cells. [^{3}H]ICS 205-930 bound rapidly, reversibly, and stereoselectively to a homogeneous population of high affinity recognition sites: B_{max} = 40 ± 5 fmol/mg of protein, pK_D = 9.20 ± 0.05 (n = 11). Nonlinear regression and Scatchard analysis of saturation data suggested the existence of a single class of [^{3}H]ICS 205-930 recognition sites on N1E-115 cells. The affinity of [^{3}H]ICS 205-930 determined in kinetic studies was in agreement with that obtained under equilibrium conditions. Competition studies carried out with a large variety of agonists and antagonists also suggested the presence of a homogeneous population of [^{3}H] ICS 205-930 recognition sites. [^{3}H]ICS 205-930-binding sites displayed the pharmacological profile of a 5-HT$_3$ receptor. Potent 5-HT$_3$ receptor antagonists showed nM affinities for [^{3}H]ICS 205-930-binding sites with the following rank order of potency: SDZ 206-830 > SDZ 206-792 > ICS 205-930 > BRL 43694 > quipazine > BRL 24924 > MDL 72222 > GR 38032F. Methiothepine, mCPP, and metoclopramide showed sub-μM affinity. The rank order of potency of agonists was: 5-HT > phenylbiguanide = 2-methyl-5-HT ≫ 5-methoxytryptamine = 5-carboxamidotryptamine. All antagonist competition curves were steep (pseudo-Hill coefficients not lower than 1), monophasic, and best fit for a one-site model; 5-HT and 2-methyl-5-HT produced pseudo-Hill coefficients of 1.2–1.4. Drugs acting at 5-HT$_1$, 5-HT$_2$, α- and β-adrenergic, dopaminergic, and histaminergic receptors (methysergide, ketanserin, propranolol, phentolamine, sulpiride, SCH 23390, cimetidine) were essentially inactive at 10 μmol/liter. The binding of [^{3}H]ICS 205-930 was not affected by guanine and adenine nucleotides (GTP, GppNHp, and ATP) at 1 mmol/liter. These nucleotides did not affect the binding of agonists, suggesting that 5-HT$_3$ recognition sites are not coupled to G-proteins. 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 unlabeled compounds: apparent B_{max} values were not reduced, whereas apparent K_D values were increased in the presence of competing ligands. 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. These findings are consistent with previous electrophysiological experiments where 5-HT$_3$ receptors were demonstrated in N1E-115 cells.

Based on functional experiments, serotonin 5-HT receptors were initially subdivided by Gaddum and Picarelli (1) into 5-HT D and 5-HT M receptors. *D*ibenzyline (phenoxybenzamine) was found to be an antagonist of 5-HT at 5-HT D receptors located on smooth muscle in the guinea pig ileum, while *m*orphine was used as an antagonist at 5-HT M receptors located neuronally in the same preparation. Radioligand binding studies have identified a multiplicity of 5-HT recognition sites in mammalian brain. Peroutka and Snyder (2) identified initially two types of 5-HT recognition sites: 5-HT$_1$ sites labeled by [^{3}H] 5-HT and 5-HT$_2$ sites labeled by [^{3}H]spiperone. [^{3}H]LSD was shown to label both populations of sites. More recently, 5-HT$_1$ recognition sites were further subdivided (3–12). To date, four different 5-HT$_1$ recognition sites have been identified in mammalian brain. 5-HT$_{1A}$ sites are labeled with [^{3}H]8-OH-DPAT (7, 10, 13, 14), [^{3}H]ipsapirone, and [^{3}H]PAPP (15, 16); these sites were initially defined by their high affinity for spiperone. 5-HT$_{1B}$ sites were identified using [^{125}I]cyanopindolol (6, 7, 17)

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); LSD, lysergic acid diethylamide; PAPP, (1-[2-(4-aminophenyl)-ethyl]-4-(3-trifluoromethyl)-phenylpiperazine; 8-OH-DPAT, (8-hydroxy-[2-*N*,*N*-dipropylamino] tetralin; ICS 205-930, (3α-tropanyl)1-H-indole-3-carboxylic acid ester. MDL 72222, 1αH, 3α, 5αH-tropan-3-yl-3,5-dichlorobenzoate; [^{125}I]MIL, N1-methyl-2-[^{125}I]lysergic acid diethylamide; 5-CT, 5-carboxamidotryptamine; RU 24969, (5-methoxy-3-(1, 2, 3, 6-tetrahydro-4-pyridinyl)-1H indole; 5-MeOT, 5-methoxytryptamine; mCPP, 1-(*m*-chlorophenyl)piperazine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BRL 43694, (endo-*N*-(9-methyl-9-azabicyclo-[3, 3, 1]-non-3-yl)-1-methyl-indazol-3-carboxamide; GR 38032 F, (1, 2, 3, 9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl)methyl]-4-one); SCH 23390, *R*-(+)-8-chloro-3-methyl-5-phenyl-7-ol-benzazepine; BRL 24924, [±-endo]-4-amino-5-chloro-2-methoxy-*N*-(1-azabicyclo[3,3,1]non-yl)-benzamide monohydrochloride; SDZ 210-204, (−)-(1*R*,2*R*,4*S*)-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; GppNHp, guanyl 5′-yl imidodiphosphate.

and [^{3}H]5-HT (3, 7, 10, 14) in rat, mouse, and guinea pig brain; they show high affinity for RU 24969, cyanopindolol, and other indole β-blockers. 5-HT_{1C} sites were characterized with [^{3}H] mesulergine (5, 7), [^{125}I]LSD (18, 19), and [^{125}I]MIL (20); 5-HT_{1C} sites display high affinity to mianserin, methergoline, and other ergolines. 5-HT_{1D} sites were identified very recently with [^{3}H]5-HT in bovine (11), pig, and human brain membranes (12) which appear not to possess 5-HT_{1B} recognition sites (7, 9, 11, 21). 5-HT_{1D} sites show high affinity for 5-CT, 5-MeOT, and methysergide, but low affinity for 5-HT_{1A}-, 5-HT_{1B}-, and 5-HT_{1C}-selective drugs. 5-HT_2 recognition sites are labeled by [^{3}H]spiperone (2, 22), [^{3}H]ketanserin (23), [^{3}H]mesulergine (24), [^{125}I]LSD (25, 26, 37) and [^{125}I]MIL (19). In the last few years functional and/or biochemical correlates have been reported to 5-HT_1 (27–36) and 5-HT_2 binding (37–41). It is now established that 5-HT_2 receptors are identical to the 5-HT D receptor of Gaddum and Picarelli (1, 37, 40). Recently, Bradley *et al.* (42) proposed to reconcile the two main nomenclatures on 5-HT receptors: 5-HT_1 receptors correspond to 5-HT_1 binding, 5-HT_2 receptors (initially 5-HT D) correspond to 5-HT_2-binding sites, and 5-HT_3 receptors correspond to the 5-HT M receptor.

Much progress has been achieved in the 5-HT_3 receptor field, due to the recent availability of potent and selective 5-HT_3 receptor antagonists: MDL 72222 (43), ICS 205-930 (44), BRL 43694 (45), and GR 38032 F (46). 5-HT_3 receptors have been identified in the peripheral nervous system (1, 42–44, 47, 48) and in a neuroblastoma cell line (49–52). Their presence in the central nervous system has been suggested recently (53–55). In contrast to 5-HT_1 and 5-HT_2 receptors, 5-HT_3 receptors had not, until recently, been identified using the radioligand binding technique (55, 56). Neijt *et al.* (51, 52) have pharmacologically characterized the 5-HT-induced activation of ion channels on N1E-115 neuroblastoma cells. In these cells, 5-HT and 2-methyl-5-HT induce membrane depolarization which can be blocked by nM concentrations of ICS 205-930 and MDL 72222, indicating that these effects are mediated via 5-HT_3 receptors.

The aim of this study was to document the presence of 5-HT_3 receptors in N1E-115 cells using the radioligand [^{3}H]ICS 205-930, a potent and selective 5-HT_3 receptor antagonist.

Materials and Methods

Cell culture. Mouse neuroblastoma cells of the clone N1E-115 (57) were grown in Dulbecco's modified Eagle's medium with Hepes (7.6 mM) and sodium bicarbonate (30 mM). The antibiotics penicillin (100 IU/ml) and streptomycin (100 μg/ml) were supplemented, as well as 7.5% fetal calf serum (Gibco) and the following amino acids (in mM concentrations): cysteine-hydrochloride, 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° in closed tissue culture roller bottles (Falcon, 850 cm^2, 0.75 rpm), gassed with CO_2, fed every second day, and subcultured every 5 days. The cells were grown to a density of 8–15 $\times$ 10^7 cells/bottle (log phase sparse culture) and harvested by vigorous shaking.

Membrane preparation. Harvested cells were centrifuged at 4° at 900 $\times$ *g* for 5 min. The supernatant was discarded and the cell pellet was resuspended in Tris buffer (20 mM, pH 7.5) containing 154 mM NaCl and homogenized with a Brinkmann Polytron (position 9, 2 $\times$ 15 sec). 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° until used.

Radioligand binding studies. Receptor binding assays were performed as described (5, 7). Briefly, fresh or frozen membranes were diluted to approximately 2 $\times$ 10^6 cells/ml in Tris-NaCl buffer. Binding assays consisted of 50 μl of radioligand, 50 μl of buffer or drug, and 150 μl of membrane suspension. Experiments carried out with a final volume of 1000 μl (50 μl of radioligand, 200 μl of buffer or drug, and 750 μl of membranes) produced similar results. The experiments were started by the addition of membranes (200–500 μg of protein/assay) to polystyrene tubes containing radioligand and drug; tubes were then incubated at 37° for 60 min. The incubation was stopped by rapid filtration and washing with ice-cold Tris-NaCl buffer (twice at 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, Switzerland). After incubation at 56° for 30 min, radioactivity was counted in a Packard 4600 Tricarb β-counter, at 67% counting efficiency. Nonspecific binding was defined in the presence of 10 μM MDL 72222. Competition (displacement) experiments were carried out with 8–12 concentrations of drug and 2–4 nM [^{3}H]ICS 205-930. Saturation experiments were performed with 12 concentrations of radioligand ranging from 0.1 to 20 nM. Experiments were carried out in triplicate determinations. Results are expressed as pK_D values (−log mol/liter) ± standard error of *n* independent experiments. B_{max} values are expressed as fmol/mg of protein. Protein concentrations were determined according to the method of Bradford (58).

Data analysis. Competition data were analyzed using the nonlinear regression computer program, SCTFIT, developed by De Lean (59). Competition curves were first analyzed according to a one-site model and then for the two-site model. The statistical analysis was based on the "extra sum of square principle," according to the method of Rodbard (60) and *F* test analysis. A two-site model was considered to be acceptable only for $p < 0.001$. Saturation experiments were analyzed with SCTFIT and according to the method of Scatchard (61).

Drugs. Drugs were obtained from the following sources: quipazine, Miles Laboratories, Elkhart, IN; methiothepin, Hoffmann-LaRoche, Basel, Switzerland; phentolamine, Ciba-Geigy, Basel, Switzerland; cimetidine, Smith, Kline & French, Philadelphia, PA; SCH 23390, Schering Corp., Bloomfield, NJ; metoclopramide, Delagrange, Chilly-Mazarin, France; ketanserin and spiperone, Janssen, Beerse, Belgium sulpiride, Ravizza SpA, Muggio, Italy; propranolol and phenylbiguanide, ICI, Macclesfield, Cheshire, England; 5-HT and tetraethylammonium, Sigma Chemical Co., St. Louis, MO; BRL 24924, Beecham, Harlow, Essex, England; cyproheptadine, Merck, Sharp & Dohme, Princeton, NJ; mianserin, Organon, Oss, The Netherlands; chlorpromazine, Rhône Poulenc Santé, Vitry, France; MDL 72222, Merrel-Dow, Strasbourg, France. The following compounds were synthesized at SANDOZ: 2-methyl-5-HT, methysergide, pindolol, 8-OH-DPAT, 5-CT, 5-MeOT, mCPP, ICS 205-930, GR 38032 F, BRL 43694, SDZ 210-204, SDZ 210-205, SDZ 206-792, SDZ 206-830, and [^{3}H]ICS 205-930 (specific activity, 33.9 Ci/mmol). SDZ 206-830 and SDZ 206-792 refer to compounds 1 and 2 described by Richardson *et al.* (44).

Results

Saturation experiments. [^{3}H]ICS 205-930 bound with high affinity (pK_D = 9.20 ± 0.05, B_{max} = 40.3 ± 4.7 fmol/mg of protein, n = 11) to an apparently homogeneous population of binding sites. Nonlinear regression analysis of the data was best fit for a one-site model, and Scatchard transforms of saturation curves were linear (Fig. 1). Nonspecific binding determined in the presence of 10 μM MDL 72222 was low and increased linearly with the free radioligand concentration.

Kinetic experiments. [^{3}H]ICS 205-930 binding to membranes of N1E-115 cells was rapid and fully reversible (Fig. 2). Both the association and the dissociation reactions (induced by an excess of MDL 72222) were monophasic. Maximal binding was reached within 10 min and remained stable up to 150 min. The association rate constant k_{on} was estimated to 2.77 $\times$ 10^8

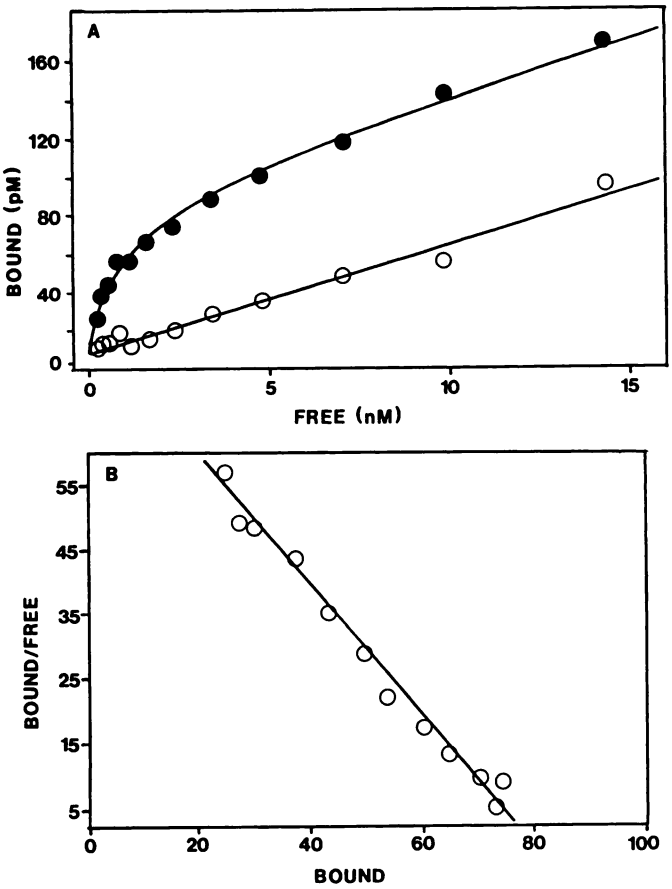

Fig. 1. Saturation experiment with [^{3}H]ICS 205-930 in membranes of N1E-115 cells. Membranes (200–500 μg/assay) were incubated with varying concentrations of [^{3}H]ICS 205-930 in the absence (●) (total binding) and presence (○) (nonspecific binding) of 10 μM MDL 72222 at a final volume of 250 μl. A represents bound (pM) versus free ligand (nM). Points are means of triplicate determinations. The data were best fit for a one-site model. B represents the transformation of the saturation data according to the method of Scatchard (61), bound/free versus bound (pM). This is representative of 11 similar experiments.

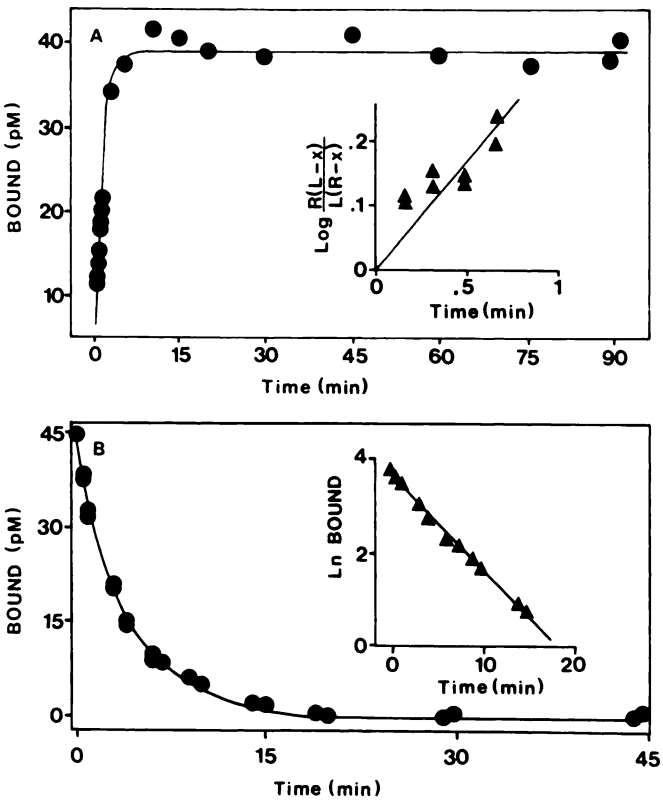

Fig. 2. Time course of association and dissociation of [^{3}H]ICS 205-930 to N1E-115 membranes. N1E-115 membranes were incubated in the absence and presence of 10 μM MDL 72222 in a volume of 1000 μl. The data are expressed as specific binding (pM) versus incubation time (min). The data points are means 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 μM MDL 72222. 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} is the association rate constant. k_{on} was estimated to 4.7×10^8 M^{-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} is the dissociation rate constant. k_{off} was estimated to 0.23 min^{-1}. The *inset* in B is a linearization of the dissociation reaction. This is representative of four similar experiments.

M^{-1}·min^{-1}, and the dissociation rate constant k_{off} was estimated to 0.27 min^{-1}. The ratio k_{off}/k_{on} gave a K_D of 0.97 nM, which is in good agreement with the K_D (0.63 nM) obtained in saturation experiments.

Competition experiments. To further characterize the binding sites labeled by [^{3}H]ICS 205-930, a large variety of drugs was analyzed in competition experiments with [^{3}H]ICS 205-930. Fig. 3 shows representative competition curves obtained with potent 5-HT$_3$ receptor agonists (5-HT and 2-methyl-5-HT) and antagonists (SDZ 206-830, ICS 205-930, BRL 24924, and MDL 72222). The affinity values of the tested compounds are listed in Table 1. All compounds that competed with [^{3}H]ICS 205-930 binding displayed monophasic and steep competition curves. They all reached, at higher concentrations, the same plateau as did MDL 72222, which was used to define nonspecific binding. The pseudo-Hill coefficient of the tested drugs was not different from unity, except for 5-HT and 2-methyl-5-HT which had slope coefficients of 1.2–1.4. These data again suggest that [^{3}H]ICS 205–930 labels a homogeneous class of recognition sites. The binding of [^{3}H]ICS 205-930 was stereoselective as SDZ 210-204 and 210-205, two stereoisomers, showed a 7-fold difference in affinity (Fig. 4). Table 1 shows that 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 significant affinities. In contrast, 5-HT$_1$ and 5-HT$_2$ ligands were weak inhibitors (except for quipazine and methiothepine). Most of the compounds active at other receptors were essentially inactive at the site labeled by [^{3}H]ICS 205-930. Together, these data are compatible with [^{3}H]ICS 205-930 binding to a 5-HT$_3$ recognition site.

Competitive interaction between [^{3}H]ICS 205-930, 5-HT and SDZ 206-830. The competitive interaction between [^{3}H]ICS 205-930 and unlabeled 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 10^{-6} M 5-HT or 2×10^{-9}M SDZ 206-830 (Fig. 5). B_{max} and K_D values were determined. B_{max} values obtained in the presence of 5-HT and SDZ 206-830 were not significantly different from those obtained in the absence of these compounds. In contrast, apparent K_D values were increased, suggesting a competitive interaction between the radioligand and the unlabeled com-

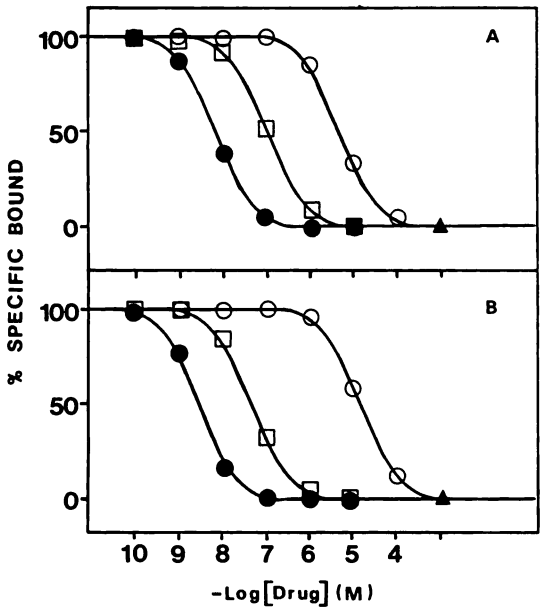

Fig. 3. Inhibition of [^{3}H]ICS 205-930 binding by various agonists and antagonists. N1E-115 membranes were incubated as described in the presence of [^{3}H]ICS 205-930 and varying concentrations of the indicated compounds. A. ●, ICS 205-930; □, MDL 72222; ○, 5-HT. B. ●, SDZ 206-830; □, BRL 24924; ○, 2-methyl-5-HT. The data points are means of triplicate determinations. The results are expressed as per cent specific binding versus the drug concentrations (−log *M*). ▲, nonspecific binding. All curves were best fit for a one-site model; representative of 3–14 independent experiments.

pounds at the binding site. Apparent pK_B values were estimated for 5-HT and SDZ 206-830, according to the equation $\log(DR - 1) = \log B - \log K_B$, where DR is the dose ratio (the factor by which the concentration of the radioligand has to be increased in the presence of the unlabeled compound to obtain identical binding to that observed in the absence of the unlabeled compound), B is the concentration of the unlabeled drug, and K_B its apparent dissociation constant. Using this method, the apparent pK_B values were 6.26 ± 0.11 for 5-HT ($n = 4$) and 9.23 ± 0.23 for SDZ 206-830 ($n = 4$). These values are in good agreement with the pK_D values estimated from competition (displacement) curves (see Table 1).

Effects of nucleotides, Ca^{2+} ions and temperature on [^{3}H]ICS 205-930 binding. The effects of nucleotides (GTP, GppNHp and ATP, 1 μM–1 mM) on [^{3}H]ICS 205-930 binding were studied in N1E-115 membranes. None of the nucleotides, even at 1 mM, 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 absence of 0.1–1 mM GppNHp. The competition curves of the two agonists were not affected by the presence of GppNHp, even in the presence of 10 mM Mg^{2+}. pK_D values for 5-HT and 2-methyl-5-HT determined in the presence of GppNHp (6.32 ± 0.22 and 5.90 ± 0.17, respectively) were similar to those obtained in parallel controls (6.19 ± 0.15 and 5.79 ± 0.18, n = 6–7).

The incubation buffer contained usually 20 mM Tris and 154 mM NaCl. Some experiments were carried out with the buffer used for 5-HT_1 and 5-HT_2 receptor binding (50 mM Tris, 4 mM $CaCl_2$). The addition of Ca^{2+} ions did not affect [^{3}H]ICS 205-930 binding (data not shown).

The usual incubation temperature was 37°. Experiments were

TABLE 1

Affinity values of various drugs to [^{3}H]ICS 205-930 recognition sites on N1E-115 membranes

Affinity values are expressed as pK_D (mean ± standard error of n experiments) obtained in competition experiments as described under Materials and Methods.

Drug	pK_D	SE	n
SDZ 206-830	9.80	0.10	5
SDZ 206-792	9.14	0.18	5
ICS 205-930	9.09	0.07	11
BRL 43694	8.85	0.05	6
Quipazine	8.69	0.11	7
BRL 24924	8.50	0.06	3
SDZ 210-204	8.30	0.06	4
MDL 72222	8.21	0.08	8
GR 38032F	7.87	0.19	4
SDZ 210-205	7.48	0.05	4
Methiothepin	7.41	0.13	6
Mianserin	7.19	0.09	5
mCPP	6.99	0.11	3
Metoclopramide	6.65	0.05	3
Cyproheptadine	6.58	0.06	6
5-HT	6.42	0.12	14
Phenylbiguanide	6.14	0.09	11
Chlorpromazine	6.01	0.18	3
2-Methyl-5-HT	5.89	0.13	11
Phentolamine	4.89	0.28	3
(−)-Sulpiride	4.82	0.45	3
SCH 23390	4.76	0.05	4
5-CT	4.71	0.23	4
8-OH-DPAT	4.58	0.12	3
5-MeOT	4.48	0.11	4
Cimetidine	4.47	0.41	4
Methysergide	4.46	0.14	4
(−)-Propranolol	4.46	0.11	3
5-HTP dipeptide*	4.38	0.31	4
Pindolol	3.94	0.12	3
Ketanserin	3.64	0.22	6
Spiperone	3.64	0.32	3
Tetraethylammonium	2.36	0.29	3

* 5-HTP dipeptide, *N*-acetyl-5-hydroxytrytophyl-5-hydroxytryptophan amide.

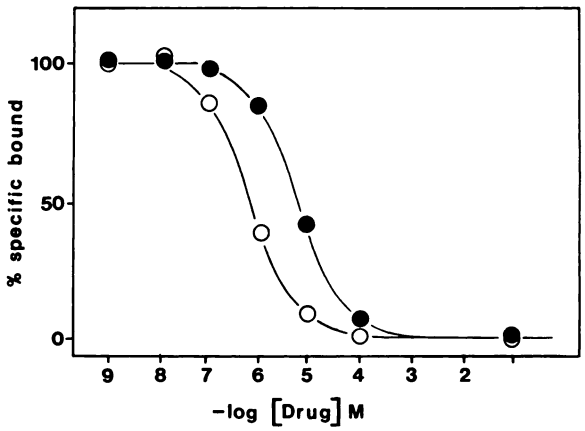

Fig. 4. Stereoselectivity of [^{3}H]ICS 205-930 binding to N1E-115 membranes. Membranes were incubated as described in the legend to Fig. 2 with [^{3}H]ICS 205-930 and varying concentrations of SDZ 210-204 (○) and SDZ 210-205 (●). The competition curves were best fit for a one-site model. The data are representative of four similar experiments.

also carried out at 20°. The apparent affinity of agonists was slightly reduced under those conditions which are used in electrophysiological experiments (51, 52) (see Table 2). The affinity of [^{3}H]ICS 205-930 and antagonists determined at 20° remained essentially unchanged or was very slightly increased (except for quipazine) when compared to the affinity obtained in parallel control experiments carried out at 37°. In three

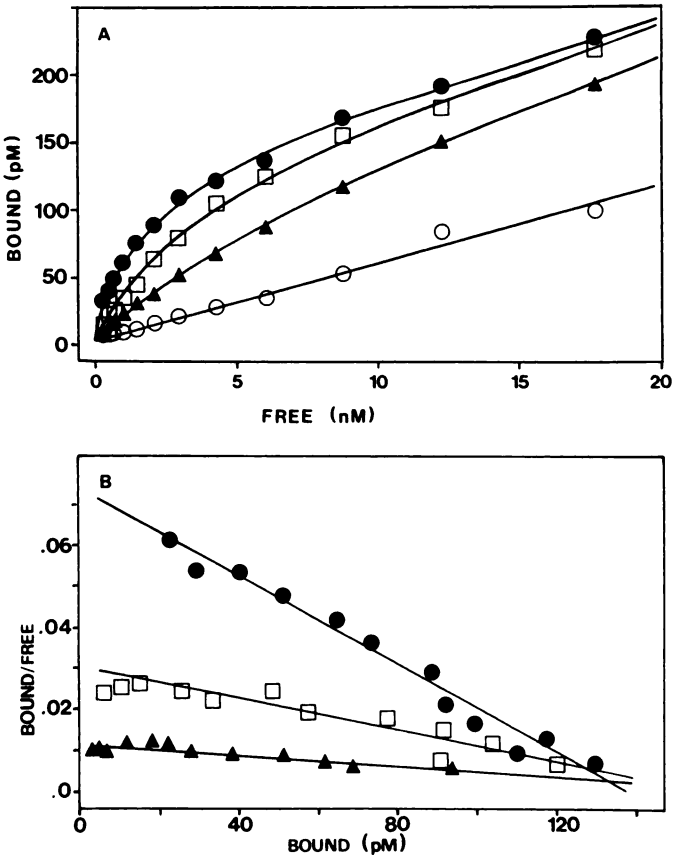

Fig. 5. Saturation experiments with [^{3}H]ICS 205-930 in the absence and presence of 5-HT and SDZ 206-830. Saturation experiments were performed as described with [^{3}H]ICS 205-930 in the absence (●) and presence of: 1 μM 5-HT (□), 2 nM SDZ 206-830 (▲) (total binding), and 10 μM MDL 72222 (○) (nonspecific binding) in 250 μl. A represents bound (pM) versus free radioligand (nM). B_{max} values were similar in the absence and presence of 5-HT or SDZ 206-830, whereas K_D values were increased 2.2- and 6-fold in the presence of 5-HT and SDZ 206-830 when compared to controls. B results from the transformation of the data according to the method of Scatchard (61). The data are representative of four similar experiments.

TABLE 2

Affinity values of agonists and antagonists for [^{3}H]ICS 205-930 recognition sites at 37° and 20°

Affinity values were determined in competition or saturation experiments performed in parallel at the indicated temperature. The data are expressed as in Table 1.

Drug	pK_D, 37°	n	pK_D, 20°	n
5-HT	6.42 ± 0.04	(4)	6.14 ± 0.18	(4)
2-Methyl-5-HT	6.02 ± 0.12	(4)	5.48 ± 0.22	(4)
Phenylbiguanide	6.11 ± 0.12	(4)	5.38 ± 0.21	(4)
ICS 205-930	8.99 ± 0.01	(3)	8.93 ± 0.07	(3)
BRL 43694	8.81 ± 0.05	(3)	8.72 ± 0.06	(3)
Quipazine	8.70 ± 0.05	(3)	8.11 ± 0.13	(3)
[^{3}H]ICS 205-930	9.22 ± 0.13	(4)	9.52 ± 0.06	(4)

parallel experiments, pK_D values were 9.22 ± 0.13 at 37° and 9.52 ± 0.06 at 20°. B_{max} values were not significantly affected by the change in temperature.

Discussion

The major finding of the present study is that [^{3}H]ICS 205-930 labels a population of binding sites in membranes of murine neuroblastoma N1E-115 cells which displays the pharmacological profile of a 5-HT$_3$ receptor. The binding of [^{3}H]ICS 205-930 was rapid, reversible, saturable, stereospecific, and of high affinity (K_D = 630 pM). [^{3}H]ICS 205-930 showed rather low nonspecific binding. Saturation experiments indicated the presence of a homogeneous class of [^{3}H]ICS 205-930 recognition sites on N1E-115 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 or only slightly higher than 1 for 5-HT and 2-methyl-5-HT), which again suggests that [^{3}H]ICS 205-930 labels a single population of recognition sites. The affinity values determined for ICS 205-930 in saturation, kinetic, and competition experiments were in good agreement. The pharmacological nature of the sites labeled by [^{3}H]ICS 205-930 is consistent with that of a 5-HT$_3$ receptor. Besides 5-HT, two 5-HT$_3$ receptor agonists, 2-methyl-5-HT and phenylbiguanide, displayed the highest affinity to these sites, whereas 5-CT and 5-MeOT showed very low affinity. Potent 5-HT$_3$ receptor antagonists such as SDZ 206-830, SDZ 206-792, ICS 205-930 (44), BRL 43694 and BRL 24924 (46), MDL 72222 (43), GR 38032 F (45), and quipazine (62, 63) displayed nM or sub-nM affinities for [^{3}H]ICS 205-930 recognition sites. Metoclopramide and mCPP, which act also as antagonists at 5-HT$_3$ receptors (62, 63), showed sub-μM affinity. In contrast, drugs with high affinity for 5-HT$_1$ and 5-HT$_2$, α- and β-adrenergic, D$_1$ and D$_2$ dopaminergic, and histaminergic receptors (except for methiothepin) revealed only very low affinity for [^{3}H]ICS 205-930-binding sites.

The interaction of agonists and antagonists with the sites labeled by [^{3}H]ICS 205-930 was of competitive nature and mutually exclusive. The binding of agonists and antagonists with these sites is not modulated by guanine nucleotides or Ca^{2+} ions. There is at present no evidence for the existence of different affinity states of the agonist 5-HT$_3$ receptor complex, in contrast to what is found with 5-HT$_1$ and 5-HT$_2$ and other receptors linked to G (guanine nucleotide regulatory)-proteins. However, the slope of agonist competition curves was slightly higher than 1, suggesting the possibility of an allosteric modulation by agonists. Taken together, these experiments suggest that 5-HT$_3$ recognition sites do not interact with G-proteins.

It has been known for some time that 5-HT receptors are present on N1E-115 cells (49, 50, 57), and their 5-HT$_3$ nature was established recently (51, 52). Indeed, 5-HT and 2-methyl-5-HT are potent agonists and produce depolarization on N1E-115 cells, which can be inhibited by nM concentrations of ICS 205-930 and MDL 72222. The pharmacological profile of [^{3}H] ICS 205-930 recognition sites is similar to that of 5-HT$_3$ receptors identified in various models of the peripheral nervous system (44), e.g., in the superior cervical and nodose ganglion of the rabbit (62, 64) and the rat vagus nerve (63). 5-HT, 2-methyl-5-HT, and phenylbiguanide act as potent agonists in these models and have a depolarizing effect. This effect is inhibited by ICS 205-930, MDL 72222, quipazine, mCPP, and metoclopramide (62–64). A similar rank order of affinity is found in [^{3}H]ICS 205-930-binding studies on N1E-115 cells. The affinity values found in binding experiments were similar to apparent pA_2 or pD_2 values found in electrophysiological studies. When the binding was performed at 20°, at which functional experiments were carried out, a slight reduction was observed for agonist affinity, but these values are still quite comparable to those found in functional experiments. Electro-

physiological studies on N1E-115 cells were performed on differentiated cells (51, 52), whereas binding studies were carried out in membranes from nondifferentiated cells. However, the electrophysiological data could be reproduced on nondifferentiated cells as well.[1]

The existence of subtypes of 5-HT_3 receptors was recently proposed (43, 44, 48), based on the potency of a variety of 5-HT_3 receptor antagonists in different 5-HT_3 receptor models (48). For instance, ICS 205-930 is a very potent 5-HT_3 receptor antagonist in sensory nerves, sympathetic and parasympathetic nerves, and enteric nerves. In contrast, MDL 72222, which is a potent antagonist in the three first systems, is almost inactive on enteric nerves (43, 44, 62-64). Also, close congeners of ICS 205-930 show different pA_2 values, depending on the system (44). At present, it seems that the site labeled by [^{3}H]ICS 205-930 on N1E-115 cells is similar to the 5-HT_3 receptor found on sensory neurones and on the vagus nerve (62–64) (see Fig. 6), which was tentatively termed 5-HT_{3A} (48). Due to the high affinity of MDL 72222, [^{3}H]ICS 205-930 recognition sites on N1E-115 cells are probably different from the 5-HT_3 receptor (5-HT_{3C}) found on enteric nerves (43, 48). However, before the nature of the 5-HT_3 receptor of N1E-115 cells can be established precisely, more subtype-selective 5-HT_3 antagonists will be needed.

Previous attempts to label 5-HT_3 receptors with [^{3}H]5-HT have been reported by Gershon and collaborators (65, 66). A high affinity [^{3}H]5-HT site was identified in the enteric plexus of the guinea pig, which was termed 5-HT_{1p}. This site showed nM affinity for [^{3}H]5-HT and μM affinity to the 5-HTP dipeptide. It displayed only little affinity to ICS 205-930 and MDL 722222, and it is clear that the sites labeled by [^{3}H]ICS 205-930 on N1E-115 cells are different from the 5-HT_{1p} site. After completion of this work, we were informed that Kilpatrick *et al.* (55) succeeded in labeling 5-HT_3 sites in rat brain membranes with a ^{3}H-derivative of GR 38032 F.

In conclusion, this report identifies a 5-HT_3 recognition site in N1E-115 cells using the radioligand binding technique. This was made possible by the high affinity of [^{3}H]ICS 205-930 for 5-HT_3 receptors, by the fact 5-HT_3 receptors had been functionally and pharmacologically identified on neuroblastoma N1E-115 cells, and because cell lines in general provide a convenient model to perform binding studies. Recently, we were able to label 5-HT_3 recognition sites with [^{3}H]ICS 205-930 in neuroblastoma-glioma NG108-15 cells (56). By using [^{3}H]ICS 205-930, it should be possible to identify 5-HT_3 recognition sites in various tissues, by radioligand binding and autoradiography, since ICS 205-930 has high affinity for the presumed 5-HT_3 receptor subtypes.

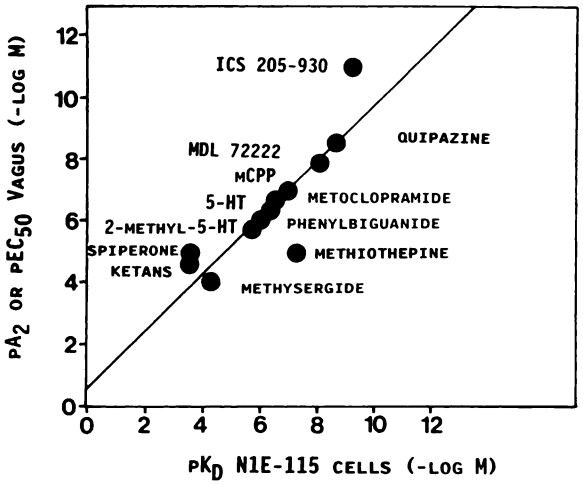

Fig. 6. Correlation of binding affinities of drugs to 5-HT_3 sites on N1E-115 cells and their effects on 5-HT-induced depolarization on the rat isolated vagus nerve. The figure represents pK_D values (Table 1) and pEC_{50}, pA_2, or pK_B values obtained with agonists and antagonists on 5-HT-induced depolarization of the rat vagus nerve [taken from Ireland and Tyers (63)]. The slope was 0.91 and the correlation coefficient $r = 0.85$, $p = 0.001$.

[1] H. C. Neijt, unpublished results.

References

1. Gaddum, J. H., and Z. P. Picarelli. Two kinds of tryptamine receptor. *Br. J. Pharmacol. Chemother.* **12**:323–328 (1957).
2. Peroutka, S. J., and S. H. Snyder. Multiple serotonin receptors: differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H] spiroperidol. *Mol. Pharmacol.* **16**:687–699 (1979).
3. Pedigo, N. W., H. I. Yamamura, and D. L. Nelson. Discrimination of multiple [^{3}H]5-hydroxytryptamine-binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* **36**:220–226 (1981).
4. Schnellman, R. G., S. J. Waters, and D. L. Nelson. [^{3}H]5-Hydroxytryptamine binding sites: species and tissue variation. *J. Neurochem.* **42**:65–70 (1984).
5. Pazos, A., D. Hoyer, and J. M. Palacios. The binding of serotonergic ligand to the porcine choroid plexus: characterization of a new type of serotonin recognition site. *Eur. J. Pharmacol.* **106**:539–546 (1984).
6. Hoyer, D., G. Engel, and H. O. Kalkman. Characterization of the 5-HT_{1B} recognition site in rat brain: binding studies with [^{125}I]iodocyanopindolol. *Eur. J. Pharmacol.* **118**:1–12 (1985).
7. Hoyer, D., G. Engel, and H. O. Kalkman. Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (−)[^{125}I]iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin. *Eur. J. Pharmacol.* **118**:13–23 (1985).
8. Asarch, K. B., R. W. Ransom, and J. C. Shih. 5-HT_{1A} and 5-HT_{1B} selectivity of two phenylpiperazine derivatives: evidence for 5-HT_{1B} heterogeneity. *Life Sci* **36**:1265–1273 (1985).
9. Heuring, R. A., J. R. Schlegel, and S. J. Peroutka. Species variations in RU 24969 interactions with non-5-HT_{1A} binding sites. *Eur. J. Pharmacol.* **122**:279–282 (1986).
10. Peroutka, S. J. Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} binding sites in rat frontal cortex. *J. Neurochem.* **47**:529–540 (1986).
11. Heuring, R. E., and S. J. Peroutka. Characterization of a novel ^{3}H-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J. Neurosci.* **7**:894–903 (1987).
12. Hoyer, D., C. Waeber, and J. M. Palacios. Identification of a 5-HT_1 recognition site in pig and human brain different from 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C}. *Br. J. Pharmacol.* **92**:561P (1987).
13. Gozlan, H., S. El Mestikawy, L. Pichat, J. Glowinski, and M. Hamon. Identification of presynaptic serotonin autoreceptors by a new ligand: ^{3}H-PAT. *Nature (Lond.)* **305**:140–142 (1983).
14. Peroutka, S. J. Selective labelling of 5-HT_{1A} and 5-HT_{1B} binding sites in bovine brain. *Brain Res.* **344**:167–171 (1985).
15. Dompert, W. U., T. Glaser, and J. Traber. ^{3}H-TVX Q 7821: identification of 5-HT_1 binding sites as target for a novel putative anxiolytic. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **328**:-467–470 (1985).
16. Ransom, R. W., K. B. Asarch, and J. C. Shih. [^{3}H]1-[2-(4-Aminophenyl)ethyl] -4-(3-trifluoromethylphenyl)piperazine: a selective radioligand for 5-HT_{1A} receptors in rat brain. *J. Neurochem.* **46**:68–75 (1986).
17. Pazos, A., G. Engel, and J. M. Palacios. β-Adrenoreceptor blocking agents recognize a subpopulation of serotonin receptors in brain. *Brain Res.* **343**:403–408 (1985).
18. Hoyer, D., S. Srivatsa, A. Pazos, G. Engel, and J. M. Palacios. [^{125}I]LSD labels 5-HT_{1C} recognition sites in pig choroid plexus membranes: comparison with [^{3}H]mesulergine and [^{3}H]5-HT binding. *Neurosci. Lett.* **69**:269–274 (1986).
19. Yagaloff, K. A., and P. R. Hartig. [^{125}I]Iodo-LSD binds to a novel 5-HT site on rat choroid plexus epithelial cells. *J. Neurosci.* **5**:3178–3183 (1985).
20. Yagaloff, K. A., and P. R. Hartig. Solubilization and characterization of the serotonin 5-HT_{1C} site from pig choroid plexus. *Mol. Pharmacol.* **29**:120–125 (1986).
21. Hoyer, D., A. Pazos, A. Probst, and J. M. Palacios. Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites. Apparent absence of 5-HT_{1B} recognition sites. *Brain Res.* **376**:85–96 (1986).
22. Leysen, J. E., C. J. E. Niemegeers, J. P. Tollenaere, and P. M. Laduron. Serotonergic component of neuroleptic receptors. *Nature (Lond.)* **272**:168–171 (1978).

23. Leysen, J. E., C. J. E. Niemegeers, J. M. Van Nueten, and P. M. Laduron. [^{3}H]Ketanserin (R41 468), a selective ^{3}H ligand for serotonin 2 receptor binding sites. *Mol. Pharmacol.* **21**:301–314 (1982).
24. Closse, A. [^{3}H]Mesulergine, a selective ligand for serotonin-2 receptors. *Life Sci.* **32**:2485–2495 (1983).
25. Hartig, P. R., M. J. Kadan, M. J. Evans, and A. M. Krohn. [^{125}I]LSD: a high selectivity ligand for serotonin receptors. *Eur. J. Pharmacol.* **89**:321–323 (1983).
26. Engel, G., E. Müller-Schweinitzer, and J. M. Palacios. 2-[^{125}Iodo]LSD, a new ligand for the characterization and localization of 5-HT$_2$ receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **325**:328–336 (1984).
27. De Vivo, M., and S. Maayani. Characterization of 5-hydroxytryptamine$_{1A}$-receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea-pig and rat hippocampal membranes. *J. Pharmacol. Exp. Ther.* **238**:248–253 (1986).
28. Markstein, R., D. Hoyer, and G. Engel. 5-HT$_{1A}$-receptors mediate stimulation of adenylate cyclase in rat hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **333**:335–341 (1986).
29. Shenker, A., S. Maayani, H. Weinstein, and J. P. Green. Pharmacological characterization of two 5-hydroxytryptamine receptors coupled to adenylate cyclase in guinea pig hippocampal membranes. *Mol. Pharmacol.* **31**:357–367 (1987).
30. Bockaert, J., A. Dumuis, R. Bouhelal, M. Sebben, and R. N. Cory. Piperazine derivatives including the putative anxiolytic drugs, buspirone and ipsapirone, are agonists at 5-HT$_{1A}$ receptors negatively coupled with adenylate cyclase in hippocampal neurons. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **335**:588–592 (1987).
31. Engel, G., M. Göthert, D. Hoyer, E. Schlicker, and K. Hillenbrand. Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT$_{1B}$ binding sites. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **332**:1–7 (1986).
32. Bouhelal, R., L. Smounya, and J. Bockaert. 5-HT$_{1B}$ receptors are negatively coupled with adenylate cyclase in rat substantia nigra. *Eur. J. Pharmacol.*, in press.
33. Raiteri, M., G. Maura, G. Bonanno, and A. Pittaluga. Differential pharmacology and function of two 5-HT$_1$ receptors modulating transmitter release in rat cerebellum. *J. Pharmacol. Exp. Ther.* **237**:644–648 (1986).
34. Molderings, G. J., K. Fink, E. Schlicker and M. Göthert. Inhibition of noradrenaline release in the rat vena cava via presynaptic 5-HT$_{1B}$ receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **336**:245–250 1987).
35. Conn, P. J., and E. Sanders-Bush. Agonist-induced phosphoinositide hydrolysis in choroid plexus. *J. Neurochem.* **47**:1754–1760 (1986).
36. Conn, P. J., E. Sanders-Bush, B. J. Hoffman, and P. R. Hartig. A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. *Proc. Natl. Acad. Sci. USA* **83**:4086–4088 (1986).
37. Engel, G., D. Hoyer, H. O. Kalkman, and M. B. Wick. Pharmacological similarity between the 5-HT-D receptor on the guinea pig ileum and the 5-HT$_2$ binding site. *Br. J. Pharmacol.* **84**:106P (1985).
38. De Chaffoy de Courcelles, D., J. E. Leysen, F. de Clerck, H. Van Belle, and P. A. J. Janssen. Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-S$_2$ receptor sites. *J. Biol. Chem.* **260**:7603–7608 (1985).
39. Doyle, V. M., J. A. Creba, U. T. Rüegg, and D. Hoyer. Serotonin increases the production of inositol phosphates and mobilises calcium via the 5-HT$_2$ receptor in A$_7$r$_5$ smooth muscle cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **333**:98–103 (1986).
40. Leysen, J. E., D. De Chaffoy De Courcelles, F. De Clerck, C. J. E. Niemegeers, and J. M. Van Nueten. Serotonin-S$_2$ receptor binding sites and functional correlates. *Neuropharmacology* **23**:1493–1501 (1984).
41. Cory, R. N., B. Rouot, G. Guillon, F. Sladeczek, M.-N. Balestre, and J. Bockaert. The 5-hydroxytryptamine (5-HT$_2$) receptor stimulates inositol phosphate formation in intact and broken WRK1 cells: determination of occupancy-response relationships for 5-HT agonists. *J. Pharmacol. Exp. Ther.* **241**:258–267 (1986).
42. Bradley, P. B., G. Engel, W. Feniuk, J. R. Fozard, P. P. A. Humphrey, D. N. Middlemiss, E. J. Mylecharane, B. P. Richardson, and P. R. Saxena. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology* **25**:563–576 (1986).
43. Fozard, J. R. MDL 72222, a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **326**:36–44 (1984).
44. Richardson, B. P., G. Engel, P. Donatsch, and P. A. Stadler. Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature (Lond.)* **316**:126–131 (1985).
45. Fake, C. S., F. D. King, and G. J. Sanger. BRL 43694: a potent and novel 5-HT$_3$ receptor antagonist. *Br. J. Pharmacol.* **91**:335P (1987).
46. Brittain, R. T., A. Butler, I. H. Coates, D. H. Fortune, R. Hagan, J. M. Hill, D. C. Humber, P. P. A. Humphrey, S. J. Ireland, D. Jack, C. C. Jordan, A. Oxford, D. W. Straughan, and M. B. Tyers. GR38032F, a novel selective 5HT$_3$ receptor antagonist. *Br. J. Pharmacol.* **90**:87P (1987).
47. Fozard, J. R. Neuronal 5-HT receptors in the periphery. *Neuropharmacology* **23**:1473–1486 (1984).
48. Richardson, B. P., and G. Engel. The pharmacology and function of the 5-HT$_3$ receptors. *Trends Neurosci.* **9**:424–428 (1986).
49. Guharay, F., R. L. Ramsey, and P. N. R. Usherwood. 5-Hydroxytryptamine-activated single-channel currents recorded from murine neuroblastoma cells. *Brain Res.* **340**:325–332 (1985).
50. Peters, J. A., and J. N. R. Usherwood. 5-Hydroxytryptamine responses of murine neuroblastoma cells: ions and putative antagonists. *Br. J. Pharmacol.* **80**:523P (1983).
51. Neijt, H. C., H. P. M. Vijverberg, and J. Van den Berken. The dopamine response in mouse neuroblastoma cells is mediated by serotonin 5HT receptors. *Eur. J. Pharmacol.* **127**:271–274 (1986).
52. Neijt, H. C., I. J. te Duits, and H. P. M. Vijverberg. Pharmacological characterization of serotonin 5-HT$_3$ receptor-mediated electrical response in cultured mouse neuroblastoma cells. *Neuropharmacology*, in press.
53. Costall, B., A. M. Domeney, M. E. Kelly, R. J. Naylor, and M. B. Tyers. The antipsychotic potential of GR38032F, a selective antagonist of 5HT$_3$ receptors in the central nervous system. *Br. J. Pharmacol.* **90**:89P (1987).
54. Hagan, R. M., A. Butler, J. M. Hill, C. C. Jordan, S. J. Ireland, and M. B. Tyers. Effect of 5-HT$_3$ receptor antagonist, GR38032F, on responses to injection of a neurokinin agonist into the ventral tegmental area of the rat brain. *Eur. J. Pharmacol.* **138**:303–305 (1987).
55. Kilpatrick, G. J., B. J. Jones, and M. B. Tyers. The identification and distribution of 5-HT$_3$ receptors in rat brain using radioligand binding. *Nature (Lond.)* **330**: 746-748 (1987).
56. Hoyer, D., and H. C. Neijt. Identification of serotonin 5-HT$_3$ recognition sites by radioligand binding in NG 108-15 neuroblastoma-glioma cells. *Eur. J. Pharmacol.* **143**:291–292 (1987).
57. MacDermot, J., H. Higashida, S. P. Wilson, H. Matsuzawa, J. Minna, and M. Nirenberg. Adenylate cyclase and acetylcholine release regulated by separate serotonin receptors of somatic cell hybrids. *Proc. Natl. Acad. Sci. USA* **76**:1135–1139 (1979).
58. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
59. De Lean, A. SCTFIT, a computer program for simultaneous analysis of saturation and competition curves. Howard Hughes Medical Institute, Duke University, Medical Center, Durham, NC (1979).
60. Rodbard, D. Statistical quality control and routine data processing for radioimmunoassay and immunoradiometric assays. *Clin. Chem.* **20**:1255–1270 (1974).
61. Scatchard, G. Attraction of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–679 (1949).
62. Round, A., and D. I. Wallis. Further studies on the blockade of 5-HT depolarizations of rabbit vagal afferent and sympathetic ganglion cells by MDL 72222 and other antagonists. *Neuropharmacology* **26**:39–48 (1987).
63. Ireland, S. J., and M. B. Tyers. Pharmacological characterization of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve. *Br. J. Pharmacol.* **90**:229–238 (1987).
64. Round, A., and D. I. Wallis. The depolarizing action of 5-hydroxytryptamine on rabbit vagal afferent and sympathetic neurones *in vitro* and its selective blockade by ICS 205-930. *Br. J. Pharmacol.* **88**:485–494 (1986).
65. Branchek, T., M. Kates, and M. D. Gershon. Enteric receptors for 5-hydroxytryptamine. *Brain Res.* **324**:107–118 (1984).
66. Mawe, G. M., T. A. Branchek, and M. D. Gershon. Peripheral neural serotonin receptors: identification and characterization with specific antagonists and agonists. *Proc. Natl. Acad. Sci. USA* 83:9799–9803 (1986).
67. Deleted in proof.

Send reprint requests to: Dr. Daniel Hoyer, Preclinical Research 386/525, SANDOZ Ltd., CH 4002 Basel, Switzerland.