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Characteristics of 5-HT_3 binding sites in NG108-15, NCB-20 neuroblastoma cells and rat cerebral cortex using [^{3}H]-quipazine and [^{3}H]-GR65630 binding

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1 The biochemical and pharmacological properties of 5-HT_3 receptors in homogenates of NG108-15 and NCB-20 neuroblastoma cells and rat cerebral cortex have been ascertained by the use of [^{3}H]-quipazine and [^{3}H]-GR65630 binding.

2 In NG108-15 and NCB-20 cell homogenates, [^{3}H]-quipazine bound to a single class of high affinity (NG108-15: $K_d = 6.2 \pm 1.1$ nM, $n = 4$; NCB-20: $K_d = 3.0 \pm 0.9$ nM, $n = 4$; means ± s.e.means) saturable (NG108-15: $B_{max} = 1340 \pm 220$ fmol mg^{-1} protein; NCB-20: $B_{max} = 2300 \pm 200$ fmol mg^{-1} protein) binding sites. In rat cortical homogenates, [^{3}H]-quipazine bound to two populations of binding sites in the absence of the 5-hydroxytryptamine (5-HT) uptake inhibitor, paroxetine ($K_{d1} = 1.6 \pm 0.5$ nM, $B_{max1} = 75 \pm 14$ fmol mg^{-1} protein; $K_{d2} = 500 \pm 300$ nM, $B_{max2} = 1840 \pm 1040$ fmol mg^{-1} protein, $n = 3$), and to a single class of high affinity binding sites ($K_d = 2.0 \pm 0.5$ nM, $n = 3$; $B_{max} = 73 \pm 6$ fmol mg^{-1} protein) in the presence of paroxetine. The high affinity (nanomolar) component probably represented 5-HT_3 binding sites and the low affinity component represented 5-HT uptake sites.

3 [^{3}H]-paroxetine bound with high affinity ($K_d = 0.02 \pm 0.003$ nM, $n = 3$) to a site in rat cortical homogenates in a saturable ($B_{max} = 323 \pm 45$ fmol mg^{-1} protein, $n = 3$) and reversible manner. Binding to this site was potently inhibited by 5-HT uptake blockers such as paroxetine and fluoxetine (pK_is = 8.6–9.9), while 5-HT_3 receptor ligands exhibited only low affinity ($pK_i < 7$). No detectable specific [^{3}H]-paroxetine binding was observed in NG108-15 or NCB-20 cell homogenates.

4 [^{3}H]-quipazine binding to homogenates of NG108-15, NCB-20 cells and rat cortex (in the presence of 0.1 μM paroxetine) exhibited similar pharmacological characteristics. 5-HT_3 receptor antagonists competed for [^{3}H]-quipazine binding with high nanomolar affinities in the three preparations and the rank order of affinity was: (S)-zacopride > quarternized ICS 205-930 ≥ granisetron > ondansetron > ICS 205-209 ≥ (R)-zacopride > quipazine > renzapride > MDL-72222 > butanopride > metoclopramide.

5 [^{3}H]-GR65630 labelled a site in NCB-20 cell homogenates with an affinity ($K_d = 0.7 \pm 0.1$ nM, $n = 4$) and density ($B_{max} = 1800 \pm 1000$ fmol mg^{-1} protein) comparable to that observed with [^{3}H]-quipazine. Competition studies also indicated a good correlation between the pharmacology of 5-HT_3 binding sites when [^{3}H]-GR65630 and [^{3}H]-quipazine were used in these cells.

6 In conclusion, [^{3}H]-quipazine labelled 5-HT_3 receptor sites in homogenates of NG108-15 cells, NCB-20 cells and rat cerebral cortex. In rat cortical homogenates, [^{3}H]-quipazine also bound to 5-HT uptake sites, which could be blocked by 0.1 μM paroxetine. The pharmacological specificity of the 5-HT_3 receptor labelled by [^{3}H]-quipazine was similar in the neuroblastoma cells and rat cortex and was substantiated in NCB-20 cells by the binding profile of the selective 5-HT_3 receptor antagonist, [^{3}H]-GR65630.

Keywords: 5-HT_3 receptors; NCB-20 cells; NG108-15 cells; [^{3}H]-quipazine; [^{3}H]-GR65630

Introduction

Pharmacological studies have led to the differentiation and classification of 5-hydroxytryptamine (5-HT) receptors into at least three major classes, namely 5-HT_1, 5-HT_2, and 5-HT_3 (see Bradley *et al.*, 1986). 5-HT_1 receptors have been further subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} subtypes (Bradley *et al.*, 1986). Functional data obtained with 5-HT_3 antagonists (Round & Wallis, 1986; Fozard, 1984) have indicated the possible existence of multiple 5-HT_3 receptors although these may be attributed, in part, to species differences (Bradley *et al.*, 1986; Richardson & Engel, 1986; Butler *et al.*, 1990). *In vitro* or *in vivo* stimulation of 5-HT_3 receptors evokes depolarization (Neijt *et al.*, 1988a; Lambert *et al.*, 1989; Higgins *et al.*, 1989) and release of acetylcholine (Barnes *et al.*, 1989a) and dopamine (Blandina *et al.*, 1988). 5-HT_3 receptor antagonists act as potent antiemetics in animals and man (Cunningham *et al.*, 1987; Smith *et al.*, 1988a,b; Butler *et al.*, 1988; Higgins *et al.*, 1989), and as anxiolytics (Jones *et al.*, 1988), antipsychotics (Costall *et al.*, 1987) and inhibitors of withdrawal from drugs of abuse (Costall *et al.*, 1990) in animal models.

Radioligands such as [^{3}H]-ICS 205-930 (Hoyer & Neijt, 1988), [^{3}H]-quaternized ICS 205-930 (Watling *et al.*, 1988), [^{3}H]-GR65630 (Kilpatrick *et al.*, 1987), [^{3}H]-quipazine (Schmidt *et al.*, 1989; Milburn & Peroutka, 1989), [^{3}H]-zacopride (Barnes *et al.*, 1988; 1989b; Pinkus *et al.*, 1990), [^{3}H]-BRL43694 (Nelson & Thomas, 1989) and [^{3}H]-LY278584 (Wong *et al.*, 1989) have been used to label 5-HT_3 receptors in tissue homogenates (see above) and on tissue sections in autoradiographic studies (Waeber *et al.*, 1988; 1989; Higgins *et al.*, 1989; Kilpatrick *et al.*, 1989). While [^{3}H]-quipazine binds with high affinity to 5-HT_3 receptors and 5-HT uptake sites in the rat cerebral cortex (Peroutka, 1988; Milburn & Peroutka, 1989), [^{3}H]-GR65630 has been reported to exhibit selective binding to 5-HT_3 receptors (Kilpatrick *et al.*, 1987).

5-HT_3 binding sites in NG108-15 and N1E-115 neuroblastoma cells have been labelled with [^{3}H]-ICS 205-930 (Hoyer & Neijt, 1988; Neijt *et al.*, 1988a,b) and identified as functional 5-HT_3 receptors (Neijt *et al.*, 1988a,b; Lambert *et al.*, 1989). However, there is relative little information on the use of other

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radioligands for the study of 5-HT_3 receptors in these and other cells. In addition, the binding properties of 5-HT_3 receptors in the NCB-20 neuroblastoma cell-line have not been investigated to-date even though functional 5-HT_3 receptors have been characterized in these cells by electrophysiological techniques (Lambert *et al.*, 1989).

In the present study, the pharmacological properties of [^{3}H]-quipazine binding to 5-HT_3 receptor binding sites in the rat cerebral cortex have been examined in the presence or absence of the 5-HT uptake inhibitor, paroxetine. Furthermore, 5-HT_3 receptors on NG108-15 and NCB-20 neuroblastoma cells have been characterized by use of [^{3}H]-quipazine and [^{3}H]-GR65630. These studies have confirmed the previously known specificity of [^{3}H]-GR65630, and shown [^{3}H]-quipazine to label 5-HT_3 receptors and 5-HT uptake sites in the rat cortex, but only 5-HT_3 sites in neuroblastoma cells. These studies have also highlighted the high density of 5-HT_3 receptors on NG108-15 and NCB-20.

A preliminary account of the present studies has been published (Sharif *et al.*, 1990).

Methods

Cell culture

Mouse neuroblastoma—rat glioma hybridoma cells of the clone NG108-15 were grown in Dulbecco's modified Eagle's medium (DMEM). The media were supplemented with 10% foetal calf serum (Cellect) and HAT (hypoxanthine 0.1 mM, aminoterin 0.4 μM, and thymidine 16 μM). Cells were cultured in 150 cm^2 flasks at 37°C in 10% CO_2, fed every third day and subcultured every 7 days. The cells were grown to a density of 6×10^6 cells/flask and harvested by incubating with 0.25% trypsin for 1 min at 25°C.

Mouse neuroblastoma-hamster brain hybridoma cells of the clone NCB-20 cells were grown in high glucose DMEM medium containing 10% newborn calf serum and HAT supplement. Cells were cultured in 150 cm^2 flasks at 37°C in 10% CO_2, fed every third day and subcultured every 7 days. For experimental use the cells were grown to a density of 10^6 cells/flask and detached by incubation with 0.05% EDTA in phosphate buffered saline (PBS) for 1 min at 25°C.

Cell homogenate preparation

Harvested cells were centrifuged at 500 *g* for 6 min. The supernatant was discarded and the cell pellet was homogenized in 50 mM Tris HCl, 5 mM Na_2EDTA buffer (pH 7.4 at 4°C) in a polytron P10 tissue disrupter (setting 6; 2×10 s bursts). The homogenate was centrifuged at 48,000 *g* for 12 min. The pellets were washed, by resuspension and centrifugation, once in homogenizing buffer and twice in 50 mM Tris HCl, 0.5 mM EDTA buffer (pH 7.4 at 4°C). The homogenates were stored under liquid nitrogen (−70°C) until required.

Rat cerebral cortex homogenate preparation

Homogenates were prepared from the cerebral cortices of rat brains obtained from Pel-Freez. Tissues were homogenized in 50 mM Tris HCl, 5 mM Na_2EDTA (pH 7.4 at 4°C) in a polytron P10 tissue disrupter (setting 10, 2×10 s bursts). The homogenate was centrifuged at 48,000 *g* for 12 min and the pellet obtained was washed by resuspension and centrifugation, once in homogenizing buffer, and twice in resuspension buffer (50 mM Tris HCl, 0.5 mM EDTA, pH 7.4 at 4°C). Membranes were stored under liquid nitrogen until required.

Ligand binding studies

Binding assays for [^{3}H]-quipazine and [^{3}H]-GR65630 were conducted in Tris-Krebs (pH 7.4) assay buffer of the following composition (mM): NaCl 154, KCl 5.4, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgCl_2$ 1.0, D-glucose 11, Tris 10. In all studies (S)-zacopride (1 μM) was used to define non-specific radioligand binding (NSB). For competition studies 5-HT_3 receptors present in rat cortical and cell homogenates were labelled with 0.7–1.5 nM [^{3}H]-GR65630 or [^{3}H]-quipazine. For [^{3}H]-quipazine binding to rat cortical homogenates, 0.1 μM paroxetine was added to the assay mixture to prevent [^{3}H]-quipazine binding to 5-HT uptake sites. Saturation studies on the three preparations were conducted with [^{3}H]-quipazine (0.08–72 nM) and [^{3}H]-GR65630 (0.04–10 nM). In rat cortex, paroxetine was included (19 fold greater than radioligand concentration to block binding to 5-HT uptake sites). Incubations (0.25 ml total vol.) were carried out for 45 min at 25°C and were terminated by vacuum filtration over Whatman GF/B glass fibre filters using a Brandel 48 well cell harvester. The filters were pretreated with 0.3% polyethyleneimine in order to reduce filter binding of the radioligand. After filtration the filters were washed for 8 s with ice-cold 0.1 M NaCl. Radioactivity retained on the filters was determined by liquid scintillation spectrometry.

Binding assays for [^{3}H]-paroxetine were conducted in 50 mM Tris-HCl containing 120 mM NaCl and 5 mM KCl (pH 7.4). Tissue homogenates were incubated at 23°C for 60 min with [^{3}H]-paroxetine (0.02–15 nM) and competing drugs in a total volume of 1.25 ml. Nonspecific binding was defined by use of 100 μM 5-HT (Marcusson *et al.*, 1988). The incubations were terminated and the filter bound radioactivity counted as described above for [^{3}H]-quipazine binding.

Protein analysis were performed with the Biorad colorimetrical method with bovine γ-globulin as the standard (Bradford, 1976).

Data analysis

All competition data were analyzed by iterative curve fitting procedures as described previously (Michel & Whiting, 1984). The apparent dissociation constant (K_i) of competing ligands was calculated from IC_{50} values by the Cheng-Prusoff equation (Cheng & Prusoff, 1973). Analyses of saturation data were performed with the 'LIGAND' computer programme (Munson & Rodbard, 1980). Statistical analyses were performed with an unpaired Student's *t* test.

Materials

NG108-15 cells were obtained from Dr R. Heller, Institute of Cancer and Developmental Biology, Syntex Research, Palo Alto, CA, U.S.A. NCB-20 cells were a generous gift from Dr T. Mansour, Stanford University, Palo Alto, CA, U.S.A. Frozen rat brains were purchased from Pel-Freez (Arkansas). [^{3}H]-quipazine (specific activity 55 Ci mmol^{-1}, Lot. No. 2389-249; 66 Ci mmol^{-1}, Lot No. 2604-123) and [^{3}H]-paroxetine (28.6 Ci mmol^{-1}) were purchased from DuPont NEN/Boston. The purity of [^{3}H]-quipazine on receipt from NEN was 98% and 94% for the two batches respectively (Dr S. Hurt, personal communication). [^{3}H]-GR65630 was a gift from Dr S. Hurt of DuPont NEN/Boston. Zacopride and its isomers, BRL 43684 (granisetron), BRL 24682 (renzapride), GR38032F (ondansetron), ICS 205-930, MDL 72222 and N-methyl-quipazine were synthesized by Dr R. Clark and co-workers, Institute of Organic Chemistry, Syntex Research, Palo Alto, CA, U.S.A. Paroxetine and fluoxetine were generous gifts from Beechams Pharmaceuticals (Harlow, UK) and Eli Lilly and Company (Indianapolis, USA) respectively. All other chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.) and Aldrich (U.S.A.).

Results

Saturation analysis

[^{3}H]-quipazine bound to homogenates of neuroblastoma cells (NG108-15 and NCB-20) and rat cortex in a saturable and reversible manner (Figures 1 and 2). [^{3}H]-quipazine interacted

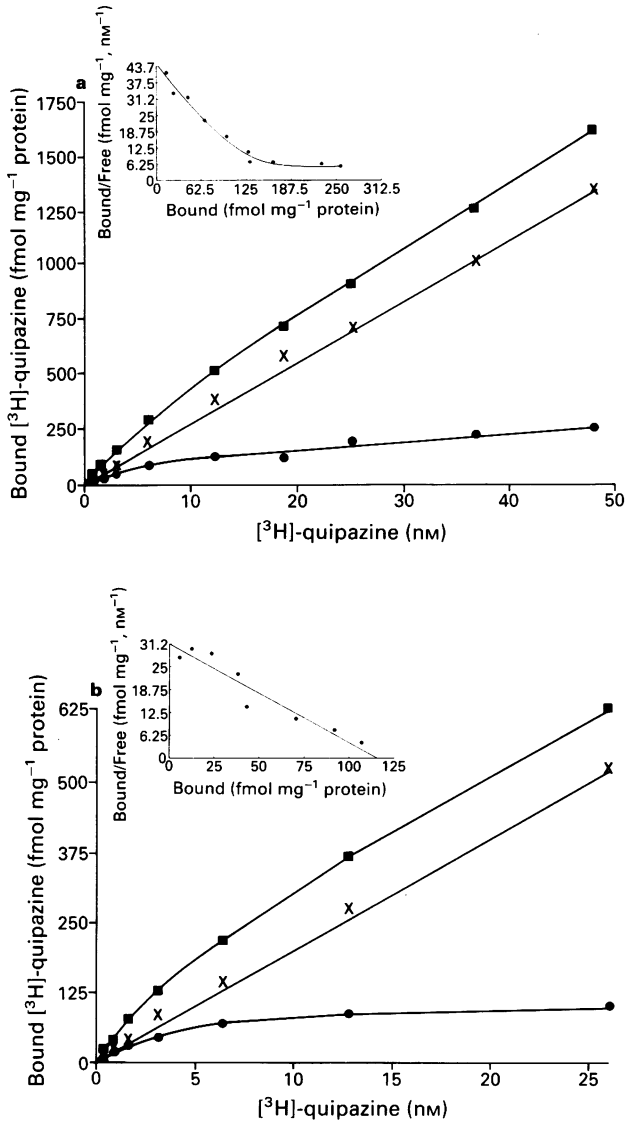

Figure 1 (a) Saturation analysis of [^{3}H]-quipazine binding to rat cortex homogenates in absence of paroxetine. Total (■), non-specific (×) and specific (●) binding from a representative experiment is shown. Inset: Scatchard analysis of the specific [^{3}H]-quipazine binding component. (b) Saturation analysis of [^{3}H]-quipazine binding to rat cortex homogenates in presence of paroxetine. Paroxetine was added to the [^{3}H]-quipazine and total (■), non-specific (×) and specific (●) binding determined as described in the Methods section. Inset: Scatchard analysis of the specific [^{3}H]-quipazine binding component.

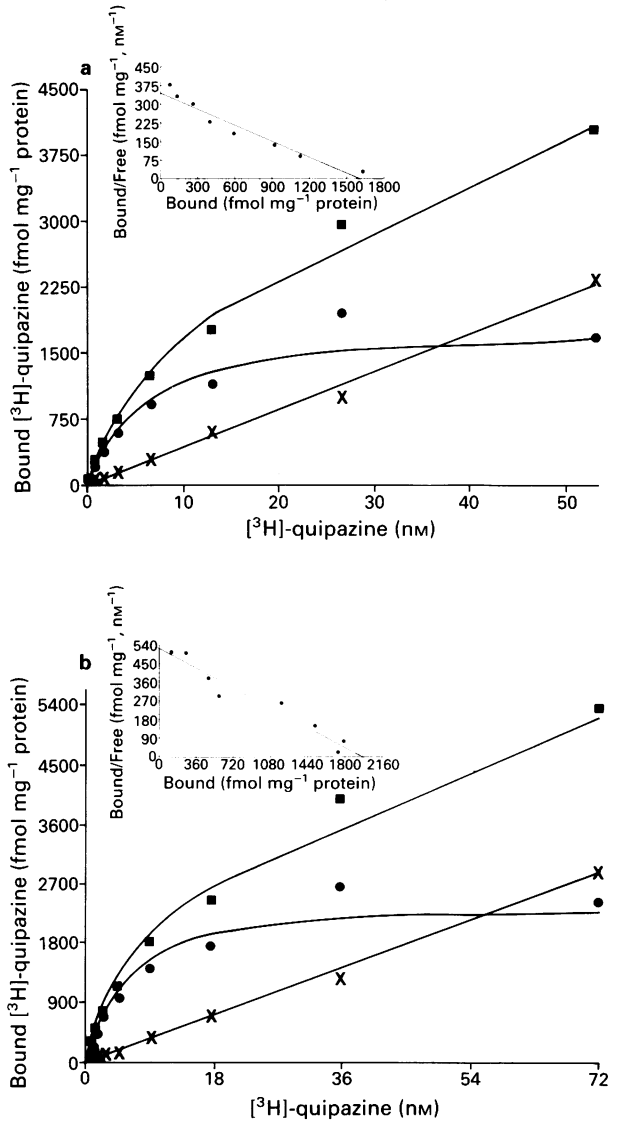

Figure 2 (a) Saturation analysis of [^{3}H]-quipazine binding to NG108-15 cell homogenates. Total (■), non-specific (×) and specific (●) binding is depicted from a sample experiment. Assays were conducted in the absence of any exogenous paroxetine. Inset: Scatchard analysis of specific [^{3}H]-quipazine binding to NG108-15 cell homogenates. (b) Example of saturation analysis of [^{3}H]-quipazine binding to NCB-20 cell homogenates in the absence of any exogenous paroxetine. Total (■), non-specific (×) and specific (●) binding from a sample assay is shown. Inset: Scatchard analysis of specific [^{3}H]-quipazine binding to NCB-20 cell homogenates.

with a single population of sites in the cultured cell homogenates but with two sites in the rat cortical homogenates (Figures 1 and 2; Table 1). In the presence of paroxetine, the biphasic Scatchard plot of [^{3}H]-quipazine binding in the rat cortex was rendered monophasic thereby indicating the presence of a single population of sites (Figure 1b).

[^{3}H]-quipazine binding to 5-HT$_3$ sites in the neuroblastoma cells and rat cortex exhibited a high affinity (2–6 nM) which was similar in all three preparations. The neuroblastoma cells have a greater B_{max} than the rat cortical homogenates (Table 1). The use of [^{3}H]-GR65630 to label 5-HT$_3$ receptors in NCB-20 cell membranes confirmed the identity and the density of 5-HT$_3$ sites in this cell-line (Table 1).

[^{3}H]-paroxetine bound with high affinity (K_d = 0.02 ± 0.003 nM, n = 3; means ± s.e.means) to 5-HT uptake sites in rat cortical homogenates in a saturable (B_{max} = 323 ± 45 fmol mg^{-1} protein) and reversible manner. No specific [^{3}H]-paroxetine binding was detected in either of the neuroblastoma cell-lines.

Competition studies

Specific [^{3}H]-quipazine (0.8–1.2 nM) binding in the rat cortex comprised 35 ± 1% (n = 10) of the total binding in the absence of 0.1 μM paroxetine. In the presence of this agent the specific binding was significantly ($P < 0.001$) increased to 44 ± 2% (n = 21). In contrast, specific [^{3}H]-quipazine binding, in the absence of paroxetine, was 95 ± 0.2% (n = 18) and 85 ± 1% (n = 15) of total binding in the NG108-15 and NCB-20 cells respectively. Specific [^{3}H]-GR65630 binding represented 88 ± 1% (n = 7) of the total binding in NCB-20 cell homogenates.

In rat cerebral cortical homogenates, 5-HT$_3$ receptor antagonists inhibited [^{3}H]-quipazine binding with shallow inhibition curves (Hill numbers < 1). The inclusion of 0.1 μM paroxetine made these curves steeper (Hill coefficient ≃ 1). (S)-zacopride exhibited a greater affinity than (R)-zacopride (Figure 3a, Table 3). The inclusion of 0.1 μM of (S)-zacopride but not paroxetine gave rise to a single high affinity [^{3}H]-

Table 1 Labelling of 5-HT_3 binding sites in rat cortex, NG108-15 and NCB-20 cell homogenates by [^{3}H]-quipazine and [^{3}H]-GR65630

Tissue	K_{d1} (nM)	K_{d2} (nM)	B_{max1} (fmol mg^{-1} protein)	B_{max2} (fmol mg^{-1} protein)
(a) [^{3}H]-quipazine				
Rat cortex (no paroxetine)	1.6 ± 0.5	500 ± 300	75 ± 14	1840 ± 1040
Rat cortex (+ paroxetine)	2.0 ± 0.5	—	73 ± 6	—
NG108-15 (no paroxetine)	6.2 ± 1.1	—	1340 ± 220	—
NCB-20 (no paroxetine)	3.0 ± 0.9	—	2300 ± 200	—
(b) [^{3}H]-GR65630				
NCB-20 (no paroxetine)	0.7 ± 0.1	—	1800 ± 1000	—

Data are means ± s.e.means from 3–4 experiments in each preparation using 0.4–72 nM [^{3}H]-quipazine and 0.04–10 nM [^{3}H]-GR65630.

quipazine binding site which showed high affinities for 5-HT uptake blockers (Table 2). These latter sites could also be labelled with [^{3}H]-paroxetine and represented labelling of 5-HT uptake sites. Conversely, the 5-HT_3 receptor antagonists tested exhibited low affinity for the [^{3}H]-paroxetine binding site (Table 2).

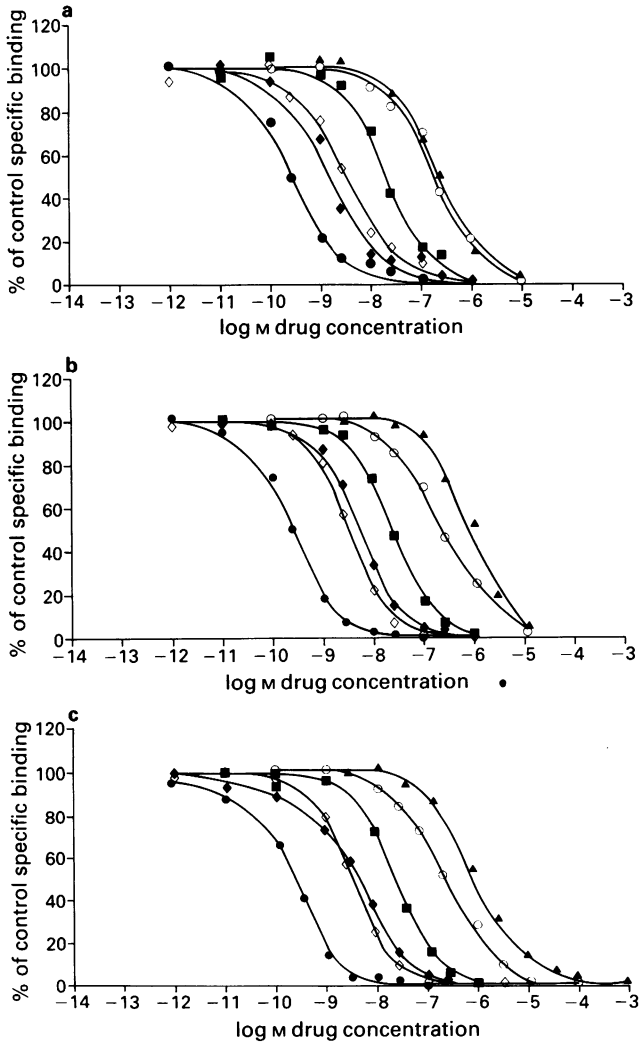

Figure 3 Inhibition [^{3}H]-quipazine binding to 5-HT_3 receptor binding sites in (a) homogenates from rat cortex, (b) NG108-15 cells and (c) NCB-20 cells, by (S)-zacopride (●), ondansetron (◆), (R)-zacopride (◇), MDL-72222 (■), metoclopramide (○), and 2-methyl-5-HT (▲).

In contrast to the situation in rat cortex, specific [^{3}H]-paroxetine binding was not detectable in the cell lines tested. [^{3}H]-quipazine binding in the cell membranes yielded good specific binding (see above) and was displaceable by 5-HT_3 receptor antagonists with high affinity and mass action profiles. Ligands which lack 5-HT_3 receptor affinity exhibited low affinities at these 5-HT_3 sites (Tables 3 and 4). [^{3}H]-GR65630 was shown to label a site in the NCB-20 cells with a pharmacological specificity very similar to that observed with [^{3}H]-quipazine (Table 4).

Comparisons were made between the rank order of potency of displacing ligands against [^{3}H]-quipazine binding in rat cortical homogenates (plus paroxetine) and [^{3}H]-quipazine binding to homogenates of NG108-15 and NCB-20 cells, and to [^{3}H]-GR65630 binding to NCB-20 cell homogenates. Significant correlations were observed for the pharmacology of [^{3}H]-quipazine binding in rat cortex and NG108-15 cells ($r = 0.98$, $P < 0.0001$), NCB-20 cells and NG108-15 cells ($r = 0.98$, $P < 0.001$), NCB-20 cells and rat cortex ($r = 0.97$, $P < 0.001$), and between [^{3}H]-quipazine and [^{3}H]-GR65630 binding in NCB-20 cells ($r = 0.97$, $P < 0.001$).

Discussion

The present studies demonstrated that [^{3}H]-quipazine binding to 5-HT_3 sites in homogenates of neuroblastoma cells (NG108-15, NCB-20) and rat cortex was saturable, reversible and of high affinity. Scatchard analysis revealed that [^{3}H]-quipazine also bound to low affinity sites in the rat cortex in contrast to the single high affinity population of sites observed in neuroblastoma cell homogenates. The present data are explicable in terms of the ability of [^{3}H]-quipazine to label the 5-HT uptake site in addition to the 5-HT_3 binding site. Labelling of the 5-HT uptake site was substantiated further by the high affinity of standard 5-HT uptake blockers for [^{3}H]-quipazine and [^{3}H]-paroxetine binding in rat cortex (this study and Milburn & Peroutka, 1989). The high density of [^{3}H]-paroxetine binding sites (B_{max} = 323 ± 45 fmol mg^{-1} protein) in the rat cortex is consistent with the existence of a high density of 5-hydroxytryptaminergic terminals in this tissue (Marcusson *et al.*, 1988). The high proportion of these uptake sites relative to the low number of 5-HT_3 receptors in this tissue gave rise to the resultant curvilinear Scatchard plots. Inclusion of 100 nM paroxetine to exclude the uptake component allowed the selective labelling of 5-HT_3 receptor binding sites. The superior profile of [^{3}H]-quipazine and [^{3}H]-GR65630 in neuroblastoma cell lines compared to that found in rat cortex can be explained by the greater density of 5-HT_3 binding sites and the apparent lack of 5-HT uptake sites as shown by the absence of [^{3}H]-paroxetine binding.

A limited number of studies have been performed to identify and characterize 5-HT_3 receptors on neuroblastoma cell lines

Table 2 Affinity of 5-HT uptake blockers and 5-HT$_3$ receptor antagonists for 5-HT uptake sites

	[^{3}H]-paroxetine		[^{3}H]-quipazine	
Compound	pK$_i$	nH	pK$_i$	nH
5-HT uptake blockers				
Paroxetine	9.9 ± 0.1	1.2 ± 0.1	8.5 ± 0.2	1.2 ± 0.3
Sertraline	9.2 ± 0.1	1.0 ± 0.1	8.6 ± 0.2	1.2 ± 0.1
Chlorimipramine	9.1 ± 0.1	1.0 ± 0.1	8.2 ± 0.1	0.9 ± 0.1
Fluoxetine	8.6 ± 0.1	1.0 ± 0.1	8.2 ± 0.2	1.0 ± 0.1
5-HT$_3$ receptor ligands				
Quipazine	7.6 ± 0.1	1.1 ± 0.1		
Bufotenine	6.6 ± 0.1	1.1 ± 0.2		
ICS 205-930	6.4 ± 0.1	1.1 ± 0.1		
MDL 72222	6.0 ± 0.1	1.1 ± 0.1		
2-methyl-5-HT	5.1 ± 0.1	1.5 ± 0.2		
Granisetron	5.2 ± 0.1	1.2 ± 0.1		
(S)-zacopride	4.9 ± 0.1	1.3 ± 0.1		
Ondansetron	4.8 ± 0.1	1.3 ± 0.2		
Phenylbiguanide	<4	—		

Data are means ± s.e.means from 3–4 experiments. The [^{3}H]-quipazine binding experiments were conducted in the presence of 0.1 μM zacopride to prevent [^{3}H]-quipazine binding to 5-HT$_3$ sites. The Hill coefficients were not statistically significantly different from unity.

(Hoyer & Neijt, 1988; Neijt *et al.*, 1988a,b). The present study has highlighted the importance of these cells, using two different radioligands, as a model system for 5-HT$_3$ receptor research. The problem of a low density of 5-HT$_3$ receptors in the mammalian brain (Kilpatrick *et al.*, 1987; 1989; Barnes *et al.*, 1989b; Wong *et al.*, 1989; Nelson & Thomas, 1989; Milburn & Peroutka, 1989; this study) has hampered pharmacological and biochemical investigations into issues of receptor regulation, receptor-effector mechanisms, biophysical characterization (Lummis *et al.*, 1990) and receptor purification. Hence the neuroblastoma cells represent a very good source of 5-HT$_3$ receptors for studies of this nature.

A question of the specificity of [^{3}H]-quipazine as a 5-HT$_3$ receptor radioligand was raised as a result of a report by Schmidt *et al.* (1989) on a [^{3}H]-quipazine-degradation-product with high affinity for 5-HT uptake sites. We believe the data obtained in the present study are not explicable in terms of the presence of such an impurity in our radioligand

Table 3 Dissociation constants of different pharmacological agents at the 5-HT$_3$ binding sites in the rat cortex and NG108-15 cell homogenates

	Receptor affinities					
	Cortex 5-HT$_3$ sites			*NG108-15 5-HT$_3$ sites*		
Compound	pK$_i$	nH	*n*	pK$_i$	nH	*n*
5-HT$_3$ antagonists						
(S)-zacopride	9.6 ± 0.1	0.9 ± 0.2	3	9.5 ± 0.1	1.1 ± 0.2	3
Q-ICS 205-930	9.1 ± 0.2	0.6 ± 0.1*	3	8.8 ± 0.2	1.0 ± 0.1	3
Granisetron	9.1 ± 0.1	1.2 ± 0.2	5	8.8 ± 0.1	1.3 ± 0.2	3
N-methyl quipazine	9.1 ± 0.1	0.9 ± 0.1	3	8.9 ± 0.1	1.0 ± 0.1	3
(R/S)-zacopride	8.9 ± 0.1	0.8 ± 0.1	8	9.4 ± 0.2	0.9 ± 0.2	4
Ondansetron	8.9 ± 0.1	0.8 ± 0.1	6	8.4 ± 0.1	1.0 ± 0.1	3
BRL 24682	8.8 ± 0.1	1.0 ± 0.1	3	8.8 ± 0.1	1.1 ± 0.1	3
ICS 205-930	8.7 ± 0.2	1.1 ± 0.1	5	8.3 ± 0.4	0.9 ± 0.2	3
Quipazine	8.7 ± 0.2	1.3 ± 0.3	6	8.1 ± 0.2	0.8 ± 0.2	4
(R)-zacopride	8.7 ± 0.2	1.2 ± 0.3	3	8.5 ± 0.1	1.3 ± 0.1	3
Renzapride	8.3 ± 0.1	1.1 ± 0.1	3	8.4 ± 0.1	1.0 ± 0.1	3
MDL-72222	7.4 ± 0.1	1.3 ± 0.2	9	7.6 ± 0.1	1.1 ± 0.1	3
Butanopride	7.1 ± 0.1	1.1 ± 0.1	3	7.2 ± 0.1	1.2 ± 0.1	3
Metoclopramide	6.7 ± 0.1	1.2 ± 0.1	4	6.7 ± 0.1	1.0 ± 0.1	3
5-HT$_3$ agonists						
Bufotenine	7.3 ± 0.1	1.2 ± 0.1	3	7.2 ± 0.1	0.8 ± 0.1	3
Phenylbiguanide	7.2 ± 0.1	1.4 ± 0.1	3	6.4 ± 0.1	1.2 ± 0.2	3
2-methyl-5-HT	6.8 ± 0.1	1.2 ± 0.1	3	6.3 ± 0.1	0.9 ± 0.1	3
5-HT	6.3 ± 0.1	1.1 ± 0.1	3	6.3 ± 0.3	1.3 ± 0.2	4
5-Methoxytryptamine	5.4 ± 0.2	1.6 ± 0.1*	3	4.8 ± 0.1	1.0 ± 0.1	3
Other compounds						
RU 24969	5.9 ± 0.1	1.1 ± 0.2	3	5.1 ± 0.5	1.3 ± 0.2	3
Ritanserin	5.9 ± 0.1	1.2 ± 0.1	3	5.0 ± 0.3	1.4 ± 0.3	3
SCH 23390	5.8 ± 0.1	1.0 ± 0.1	3	5.3 ± 0.1	1.2 ± 0.1	3
Ketanserin	5.7 ± 0.4	1.1 ± 0.2	6	5.4 ± 0.4	0.9 ± 0.1	3
5-methyl-Tryptamine	5.6 ± 0.3	1.2 ± 0.1	3	5.1 ± 0.1	1.2 ± 0.1	3
(−)-Propanolol	5.5 ± 0.1	0.8 ± 0.1	3	4.9 ± 0.1	1.2 ± 0.1	3
8-OH-DPAT	5.4 ± 0.1	0.9 ± 0.1	3	5.3 ± 0.1	1.0 ± 0.2	4
(S)-Sulpiride	5.4 ± 0.4	1.2 ± 0.2	3	5.2 ± 0.4	1.0 ± 0.1	3
Methiothepin	5.4 ± 0.2	1.1 ± 0.1	6	5.9 ± 0.1	1.4 ± 0.2	3
Sumatriptan	5.0 ± 0.1	1.1 ± 0.2	3	4.3 ± 0.3	0.7 ± 0.1	4

Data are means ± s.e.means from the indicated number (*n*) of experiments for each tissue.
Q-ICS 205-930 = quaternized ICS 205-930.
* Statistically significantly different from unity, $P < 0.05$, by Student's unpaired *t* test.

Table 4 Inhibition of [^{3}H]-quipazine and [^{3}H]-GR65630 binding in NCB-20 cell homogenates

Compound	[^{3}H]-quipazine		[^{3}H]-GR65630	
	pK_i	nH	pK_i	nH
5-HT$_3$ receptor antagonists				
(S)-zacopride	10.0 ± 0.1	1.4 ± 0.2*	9.9 ± 0.1	1.1 ± 0.1
ICS 205-930	9.1 ± 0.1	0.9 ± 0.1	9.2 ± 0.1	1.2 ± 0.1
Granisetron	9.0 ± 1.2	1.2 ± 0.1	8.9 ± 0.1	1.0 ± 0.1
(R)-zacopride	8.4 ± 0.1	1.0 ± 0.1	8.6 ± 0.1	1.1 ± 0.1
Renzapride	8.4 ± 0.1	0.7 ± 0.1	8.3 ± 0.1	0.9 ± 0.1
Ondansetron	8.3 ± 0.1	0.9 ± 0.1	8.5 ± 0.1	1.0 ± 0.1
MDL 72222	7.6 ± 0.2	1.3 ± 0.3	8.1 ± 0.1	0.9 ± 0.1
Butanopride	7.2 ± 0.1	1.0 ± 0.1	7.2 ± 0.1	1.1 ± 0.1
(+)-Tubocurarine	7.0 ± 0.1	0.9 ± 0.1	7.4 ± 0.1	0.8 ± 0.1
Metoclopramide	6.4 ± 0.2	0.9 ± 0.1	6.9 ± 0.1	1.0 ± 0.1
5-HT$_3$ receptor agonists				
Bufotenine	7.2 ± 0.1	0.8 ± 0.1	7.3 ± 0.1	1.0 ± 0.1
5-HT	7.1 ± 0.1	1.1 ± 0.1	6.8 ± 0.1	1.2 ± 0.1
2-methyl-5-HT	6.6 ± 0.1	1.0 ± 0.1	6.1 ± 0.1	0.9 ± 0.1
Phenylbiguanide	6.4 ± 0.1	0.9 ± 0.1	6.3 ± 0.1	0.9 ± 0.1

Data are means ± s.e.means for the inhibition constants (pK_i) and Hill coefficients (nH) from at least three separate experiments.
* Statistically significantly different from unity, $P < 0.05$ by Student's unpaired t test.

because of the following reasons: (1) the batches of radioligands employed in the present study were different from that used by Schmidt *et al.* (1989) (Lot No. 2389-205); (2) the batches of [^{3}H]-quipazine used here were 94–98% pure (see Materials section); (3) contrary to previous reports (Milburn & Peroutka, 1989; Schmidt *et al.*, 1989; Perry, 1990) any possibility of labelling the 5-HT uptake sites has been eliminated by the inclusion of paroxetine in the assay buffer; (4) (S)-zacopride was used to define the nonspecific binding to ensure exclusive labelling of 5-HT$_3$ receptor sites under our experimental conditions. These data therefore indicated that the nanomolar affinity of [^{3}H]-quipazine binding in rat cortical homogenates reflected radiolabelling of 5-HT$_3$ binding sites.

The hypothesis that [^{3}H]-quipazine labelled 5-HT$_3$ binding sites with characteristics of 5-HT$_3$ receptors in both the rat cortex and neuroblastoma cells was confirmed by the pharmacological characteristics of [^{3}H]-quipazine and [^{3}H]-GR65630 binding. Thus, potent 5-HT$_3$ receptor antagonists such as zacopride (Smith *et al.*, 1988a,b), ICS 205-930 (Richardson *et al.*, 1985), granisetron, renzapride and BRL 24682 (Sanger, 1987; Fake *et al.*, 1987), ondansetron (Ireland & Tyers, 1987; Butler *et al.*, 1988) and quipazine (Ireland & Tyers, 1987) exhibited high affinity for sites in rat cortical and NG108-15 and NCB-20 cell homogenates. Other 5-HT$_3$ antagonists such as metoclopramide (Ireland & Tyers, 1987) and MDL 72222 (Fozard, 1984) also inhibited [^{3}H]-quipazine binding in these preparations but with lower affinity than the above-mentioned antagonists. 5-HT$_3$ receptor agonists such as 5-HT, 2-methyl-5-HT, bufotenine and phenylbiguanide (Richardson *et al.*, 1985; Ireland & Tyers, 1987; Fozard, 1984) competed for [^{3}H]-quipazine binding in rat cortical and NG108-15 and NCB-20 cell homogenates with a relatively low affinity. Although the functional significance of the potent displacement of [^{3}H]-quipazine and [^{3}H]-GR65630 binding in NCB-20 cell homogenates by (+)-tubocurarine remains to be determined, the high affinity of this nicotinic antagonist for 5-HT$_3$ binding sites correlated well with its ability to block 5-HT-induced inward currents in N1E-115 (Peters *et al.*, 1990) and NG108-15 (Yakel & Jackson, 1988) cells. However, the effect of (+)-tubocurarine on 5-HT$_3$ receptor function in NCB-20 cells has not been investigated so far.

In general, the inhibition of [^{3}H]-quipazine binding to rat cortical and neuroblastoma cell homogenates by 5-HT$_3$ antagonists was consistent with the recognition of a single population of sites, as indicated by the Hill coefficients being close to unity. However, it is of interest that quaternized-ICS 205-930 apparently inhibited cortical 5-HT$_3$ binding with a Hill coefficient less than unity suggesting interaction with more than one population of sites. In contrast, quaternized ICS 205-930 gave mass action inhibition curves in NG108-15 cells. It is not clear at present whether the compound was recognizing different affinity states or subtypes of 5-HT$_3$ receptors in the rat cortex. The pharmacological relevance of this observation requires further study. Displacement of [^{3}H]-quipazine binding by zacopride from 5-HT$_3$ sites in the rat cortex and neuroblastoma cells exhibited a similar degree of stereoselectivity. The (S)-isomer of zacopride was markedly more potent than the (R)-isomer. These observations were consistent with previous reports for other tissues in which [^{3}H]-(S)-zacopride was used as the radioligand (Pinkus *et al.*, 1990). The overall pharmacological specificity of 5-HT$_3$ binding sites observed in the rat cortex and neuroblastoma cells was consistent with that of 5-HT$_3$ receptors previously identified from functional data obtained in peripheral tissues (Richardson & Engel, 1986; Bradley *et al.*, 1986; Butler *et al.*, 1990).

[^{3}H]-GR65630 is a selective, high affinity ligand that has been previously employed to label 5-HT$_3$ binding sites in rodent tissue homogenates and sections including rat cerebral cortex (Kilpatrick *et al.*, 1987; 1989). In order to verify that [^{3}H]-quipazine interacted with 5-HT$_3$ receptors in the neuroblastoma cells, [^{3}H]-GR65630 binding was tested in NCB-20 cell homogenates. The excellent correlation between the pharmacological specificity of [^{3}H]-quipazine and [^{3}H]-GR65630 binding in these cells, as well as the similar stereoselectivity obtained with isomers of zacopride, confirmed that [^{3}H]-quipazine labelled 5-HT$_3$ binding sites in the neuroblastoma cells. The (S)-isomer of zacopride exhibited a much greater affinity for [^{3}H]-GR65630 binding in NCB-20 cell homogenates than the (R)-isomer. These data demonstrated an appropriate degree of stereoselectivity of the 5-HT$_3$ binding sites in these cells and confirmed the results obtained with [^{3}H]-quipazine.

In conclusion, [^{3}H]-quipazine labelled 5-HT$_3$ receptors under experimental conditions when its affinity for the 5-HT uptake sites was eliminated. NG108-15 and NCB-20 cell homogenates exhibited a high density of 5-HT$_3$ receptors as determined by [^{3}H]-quipazine and [^{3}H]-GR65630 binding. These cell lines constitute a source with a high density of 5-HT$_3$ receptors suitable for future biochemical and molecular biological investigations.

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