Binding of arylpiperazines to 5-HT_3 serotonin receptors: results of a structure-affinity study

Richard A. Glennon [1], Abd El-Kader M. Ismaiel [1], Bruce G. McCarthy [2], and Stephen J. Peroutka [2]

[1] Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0581, U.S.A. and [2] Department of Neurology, Stanford University Medical Center, Stanford, CA 94305-5235, U.S.A.

Received 26 January 1989, revised MS received 16 June 1989, accepted 27 June 1989

The binding affinities of a series of arylpiperazine derivatives at [^{3}H]quipazine-labeled central 5-HT_3 sites were investigated. Features determined to be important for binding include the N4 piperazine nitrogen atom (but not the N1 piperazine nitrogen), and a quinolinyl group. The quinoline nitrogen atom of quipazine also contributes to affinity and its replacement by carbon reduces affinity by 20-fold. The entire quinoline nucleus is not necessary for binding, and certain monocyclic arylpiperazines, particularly those with a chloro group meta to the position of the piperazine ring (e.g. mCPP, MK-212), also bind at 5-HT_3, sites; however, the affinities of these agents are at least an order of magnitude less than that of quipazine itself. Taking advantage of the fact that tertiary amines are not well tolerated at 5-HT_{1B} sites, but that N-methyl substituents have little effect on 5-HT_3 binding, we designed and synthesized a tertiary amine analog of quipazine, i.e., N-methylquipazine (NMQ). NMQ binds at 5-HT_3 sites with an affinity similar to that of quipazine; however, unlike quipazine, NMQ shows very little affinity (IC_{50} > 10 000 nM) for central 5-HT_{1B} sites.

5-Hydroxytryptamine (5-HT, serotonin); 5-HT_3 (sites); Quipazine; Arylpiperazines; (Radioligand binding)

1. Introduction

Three major classes of serotonin (5-hydroxytryptamine; 5-HT) receptors have been identified: 5-HT_1-like, 5-HT_2 and 5-HT_3 (Bradley et al., 1986; Glennon, 1989; Osborne and Hamon, 1988; Peroutka, 1988). Initially, 5-HT_3 receptors were described only in peripheral tissue; however, evidence suggested the possible existence of central 5-HT_3 receptors (for reviews, see Costall et al., 1988; Richardson and Buchheit, 1988). More recently, central 5-HT_3 sites have been identified using [^{3}H]GR6530 (Kilpatrick et al., 1987), quaternized [^{3}H]ICS 205-930 (Watling et al., 1988), [^{3}H]zacopride (Barnes et al., 1988), and the arylpiperazine [^{3}H]quipazine (Peroutka and Hamik, 1988) as radioligands. [^{3}H]ICS 205-930 has also been used to label 5-HT_3 sites in a neuroblastoma cell line (Hoyer and Neijt, 1988).

To date, very little is known about the structure-activity relationships of 5-HT_3 agents, and nothing is known about the structure-affinity relationships for the binding of arylpiperazines at central 5-HT_3 sites. Indeed, arylpiperazines (depending upon the presence and location of pendant substituent groups) are known to bind at 5-HT_1 and 5-HT_2 sites (Glennon, 1989). Quipazine itself, for example, displays nanomolar affinities for these sites and, in particular, for a sub-

Correspondence to: R.A. Glennon, Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0581, U.S.A.

population of 5-HT_1 sites (i.e., 5-HT_{1B} sites: K_i = 100-300 nM). Thus, it was of interest to conduct a structure-affinity investigation in order to determine which portions of the quipazine molecule are important for binding. A long range goal of these studies is the development of arylpiperazines with greater selectivity for central 5-HT_3 binding sites; for this reason, several quipazine-related analogs were also examined.

2. Materials and methods

2.1. Radioligand binding studies

Radioligand binding studies were performed as described previously (Peroutka and Hamik, 1988; Milburn and Peroutka, in press). Briefly, rat brains (Pel-Freeze Biologicals, Rogers, AR) were stored at −70°C until needed. On the day of study, the samples were thawed in Tris-HCl buffer (50 mM, pH 7.7 at 25°C). The tissue was homogenized in 20 volumes Tris-HCl buffer using a Brinkmann Polytron and then centrifuged in a Sorvall centrifuge (at 17000 × *g*) for 10 min. The supernatant was discarded and the pellet was resuspended in the same volume of Tris-HCl buffer and incubated for 10 min prior to a second centrifugation (at 17000 × *g*) for 10 min. The final pellet was resuspended in 80 volumes of Tris-HCl buffer containing 10 μM pargyline, 4 mM calcium chloride and 0.1% ascorbic acid. The suspensions were immediately used in the binding assay. Rat cortical membranes were used in all experiments. Protein concentrations averaged 5 (3-7) μg/ml of homogenate by Lowry (Lowry et al., 1951) assay. Radioligand binding studies consisted of 0.1 ml of tritiated radioligand, 0.1 ml of buffer or displacing drug, and 0.8 ml of tissue suspension. The following radioligands were used: 0.2 nM [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin ([^{3}H]8-OH-DPAT) for 5-HT_{1A} sites, 1.4 nM [^{3}H]5-HT in the presence of 10^{-7} M 8-OH-DPAT and 10^{-6} M mianserin for 5-HT_{1B} sites, 0.8 nM [^{3}H]mesulergine for 5-HT_{1C} sites, 0.2 nM [^{3}H]ketanserin for 5-HT_2 sites, 0.8 nM [^{3}H]quipazine for 5-HT_3 sites, 0.9 nM [^{3}H]WB-4101 in the presence of 10^{-7} M 8-OH-DPAT for α_1-adrenergic sites, 0.9 nM [^{3}H]rauwolscine for α_2-adrenergic sites, 0.2 nM [^{3}H]dihydroalprenolol hydrochloride (DHA) for β-adrenergic sites, 0.1 nM [^{3}H]quinuclidinyl benzilate (QNB) for muscarinic cholinergic sites, 0.1 nM [^{3}H]flunitrazepam for benzodiazepine sites. Following incubation at 25°C for 30 min, the assays were rapidly filtered under vacuum through No. 32 glass fiber filters (Schleicher and Schuell; Keene, NH) with two 5-ml washes using 50 mM Tris-HCl buffer. Radioactivity was measured by liquid scintillation spectrometry in 2.5 ml of 3a70 Counting Cocktail (Research Products International; Mt. Prospect, IL) at 61% efficiency for tritium. Specific binding was defined as the excess taken over blanks in the presence of 10^{-5} M 5-HT for 5-HT_{1A} and 5-HT_{1B} sites, 10^{-6} M cinanserin for 5-HT_2 sites, 10^{-6} M ICS 205-390 for 5-HT_3 sites, 10^{-6} M prazosin for α_1 adrenergic sites, 10^{-4} M yohimbine for α_2 adrenergic sites, 10^{-6} M propranolol for β adrenergic sites, 10^{-6} M scopolamine for muscarinic cholinergic sites and 10^{-6} M diazepam for benzodiazepine sites. Specific 5-HT_3 binding was generally 35-45% of total [^{3}H]quipazine binding. All tested compounds competed for 100% of the [^{3}H]quipazine binding as defined by ICS 205-390.

2.2. Drugs

All drugs were dissolved and diluted in assay buffer. Radioligand sources were as follows: [^{3}H]5-HT (20 Ci/mmol), [^{3}H]WB-4101 (17.6 Ci/mmol), [^{3}H]rauwolscine (78.5 Ci/mmol), [^{3}H]DHA (95.0 Ci/mmol), [^{3}H]QNB (39 Ci/mmol), [^{3}H]flunitrazepam (78 Ci/mmol), [^{3}H]8-OH-DPAT (142.9 Ci/mmol), [^{3}H]ketanserin (61.8 Ci/mmol), [^{3}H]quipazine (52.3 Ci/mmol), from Dupont-New England Nuclear; Boston, MA. Drug sources were as follows: 5-HT, diazepam, scopolamine and yohimbine (Sigma Chemical Company, St. Louis, MO), cinanserin (Squibb & Sons, Princeton, NJ), ICS 205-930 [3-(1aH,3a,5aH-tropan-3-yl)indole carboxylate] (Sandoz, East Hanover, NJ), prazosin (Pfizer, Brooklyn, NY), propranolol (Ayerst, New York, NY), mianserin (Organon, Oss, The Netherlands), 8-OH-DPAT (Research Biochemicals, Wayland, MA). With the exception of NMQ, NPQ and

AMI-098, all of the other agents examined in the competition studies were previously prepared in our laboratories (Lyon et al., 1986; Glennon et al., 1986); either an original sample was used, or, in several cases, the agent was re-synthesized according to our published procedure.

N^1-Methyl-N^4-(2-quinolinyl)piperazine dimaleate (NMQ) was prepared by the alkylation of N-methylpiperazine with 2-chloroquinoline; the crude free base (m.p. 111-112° C) was treated with maleic acid and recrystallized from absolute ethanol to afford the product as shiney white crystals (m.p. 171-172° C). Although a crude salt had been previously reported in the patent literature (Rodriguez, 1972), there was no indication as to its purity or elemental analysis. As a consequence, NMQ dimaleate was submitted to Atlantic Microlab for elemental analysis (calculated/found): carbon (57.51/57.43%), hydrogen (5.48/5.51%), nitrogen (9.15/9.15%). Spectral data were also consistent with the assigned structure.

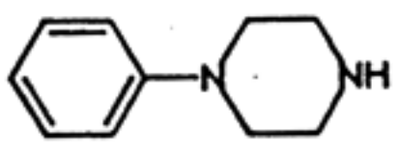
N^1-Methyl-N^4-(2-quinazol-4-onyl)piperazine (AMI-098) (m.p. 225-226° C) was prepared in a similar manner from 2-chloroquinazoline-4-one and crystallized, after recrystallization from 95% ethanol/anhydrous ether, with 0.25 moles of water; elemental analysis (calculated/found): carbon (62.74/62.64%), hydrogen (6.69/6.69%), nitrogen (22.53/22.45%).

N^1-n-Propyl-N^4-(2-quinolinyl)piperazine dimaleate (NPQ) was prepared by alkylation of quipazine (free base) with 1-propanol-p-toluene sulfonate; the product (mp 175-176° C after recrystallization from absolute ethanol/anhydrous ether) analyzed correctly for carbon (59.13/59.19%), hydrogen (6.00/6.16%), and nitrogen (8.62/8.66%). All new products were homogeneous in multiple thin-layer chromatographic systems.

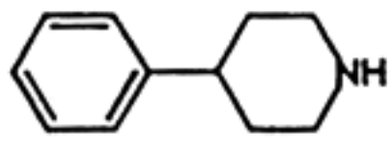
3. Results

Structures of the agents examined are shown in fig. 1; the results of the radioligand binding studies are shown in table 1. The simplest arylpiperazine examined is phenylpiperazine (**1**); the affinity of this agent (IC_{50} = 430 nM) is several hundred times less than that of quipazine, but is, nonetheless, similar to the affinity of 5-HT itself (Peroutka and Hamik, 1988). Replacement of the N1 piperazine nitrogen atom with a methylene group has no effect on affinity (e.g. compare IC_{50} values of **1** and **2**). Because replacement of the phenyl group by either a benzyl, (1-BzP; **3**), benzoyl (i.e., **4**), or 2-pyrimidinyl group (1-PP; **5**) significantly decreases affinity (IC_{50} values > 1000 nM), further changes were restricted to phenyl-related analogs. Incorporation of a lipophilic electron-withdrawing chloro group at the 3-position, such as in 1-(3-chlorophenyl)piperazine (mCPP; **6**), increases affinity by 20-fold (IC_{50} value = 20 nM), whereas incorporation of this group at the 4-position (i.e., pCPP; **7**) increases affinity by only about 4-fold (IC_{50} value = 120 nM). The chloro derivative MK-212 (**8**) also displays a significant affinity (IC_{50} value = 29 nM) for 5-HT_3 sites. On the other hand, incorporation of an electron-donating methoxy group, such as in 2-MPP (**9**) and 4-MPP (**10**), have essentially no effect on affinity (IC_{50} values = 324 and 340 nM, respectively) relative to that of the unsubstituted parent 1-phenylpiperazine **1** (IC_{50} value = 430 nM). Benz-fusion at the b-face of phenylpiperazine results in a 4-fold increase in affinity (i.e., **11**; IC_{50} value = 110 nM) whereas fusion at the c-face (i.e., **12**; IC_{50} value = 30 nM) results in about a 15-fold increase. Agent **12** may be viewed as the 1-deaza analog of quipazine.

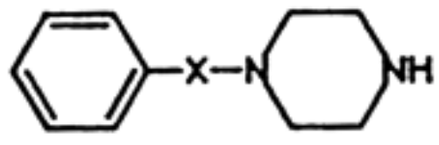
Unfortunately, none of the monocyclic analogs, the b-fused analog, nor the 1-deaza analog bind as well as the quinoline derivative quipazine itself (IC_{50} value = 1.5 nM). Additional quinoline derivatives were examined in order to determine the importance of various features of quipazine. The low affinity (i.e., circa 1/1000 that of quipazine) of the methylene derivative **13**, the dialkylamine derivative **14** and the primary amine **15**, attest to the importance of the N4 piperazine nitrogen. Incorporation of the methoxy group, as in **16** and **17**, reduces affinity by more than 200-fold. However, the presence of the N-methyl group, comparing the IC_{50} values for **16** and **17**, seems to have little effect on affinity. The quinazoline derivative AMI-098 (**18**) possesses only a moderate affinity for 5-HT_3 sites. The N-methyl ana-



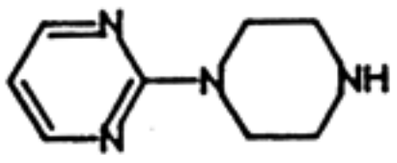
1



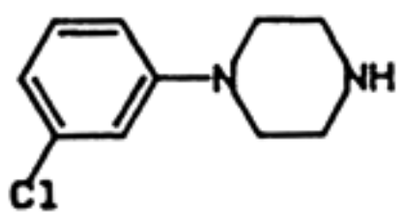
2



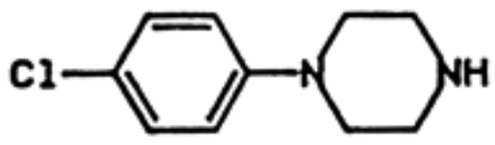
3 X= CH_2
4 X= C=O



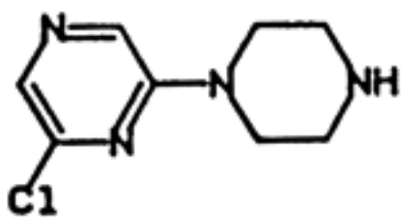
5



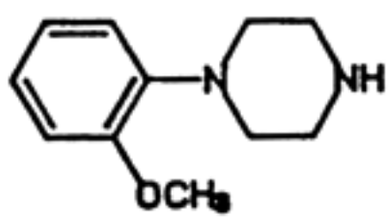
6



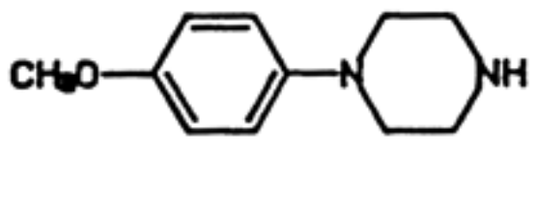
7



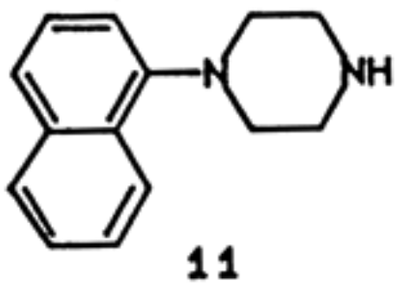
8



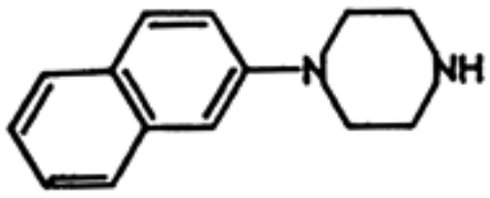
9



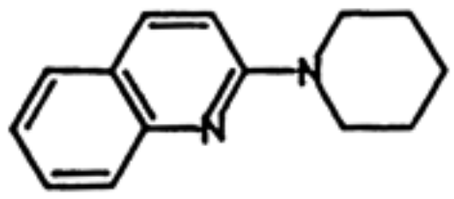
10



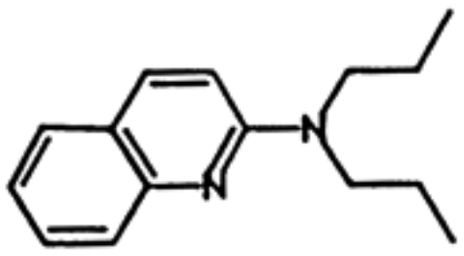
11



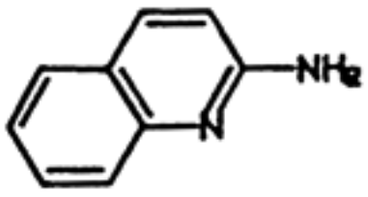
12



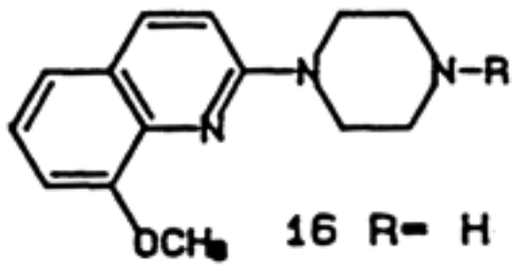
13



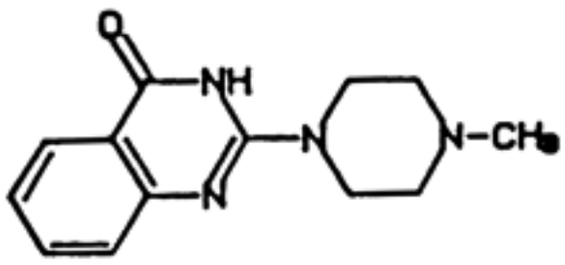
14



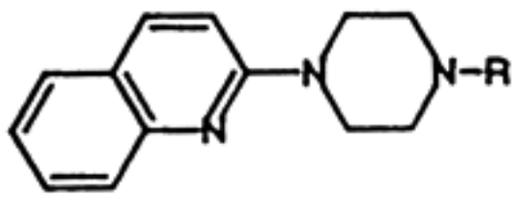
15



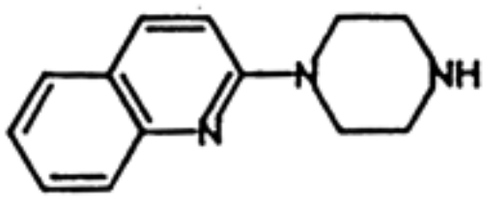
16 R= H
17 R= CH_3



18



19 R= CH_3
20 R= C_3H_7



21 QUIPAZINE

Fig. 1. Structures of the arylpiperazines examined in the present study. 1-Phenylpiperazine (**1**), 4-phenylpiperidine (**2**), 1-benzylpiperazine (1-BzP; **3**), 1-benzoylpiperazine (**4**), 1-(2-pyrimidinyl)piperazine (1-PP; **5**), 1-(3-chlorophenyl)piperazine (mCPP; **6**), 1-(4-chlorophenyl)piperazine (pCPP; **7**), MK-212 (**8**), 1-(2-methoxyphenyl)piperazine (2-MPP; **9**), 1-(4-methoxyphenyl)piperazine (4-MPP; **10**), 1-(1-naphthyl)piperazine (1-NP; **11**), 1-(2-naphthyl)piperazine (2-NP; **12**), N4-deazaquipazine (**13**), 2-(N,N-dipropylamino)quinoline (**14**), 2-aminoquinoline (**15**), 8-methoxyquipazine (**16**), 8-methoxy-N-methylquipazine (**17**), AMI-098 (**18**), N-methylquipazine (NMQ; **19**), N-propylquipazine (NPQ; **20**) and quipazine (**21**).

log of quipazine (i.e., NMQ; **19**, $IC_{50} = 4.7$ nM) binds with an affinity similar to that of quipazine (**21**) itself, whereas its N-propyl homolog (i.e., NPQ; **20**, $IC_{50} = 15$ nM) binds with an affinity of 1/10 that of quipazine.

4. Discussion

With the exception of certain tryptamine derivatives, agents that bind at 5-HT_3 sites normally display a low affinity for 5-HT_1 and 5-HT_2

TABLE 1

Interaction of arylpiperazines with [^{3}H]quipazine-labeled 5-HT_3 serotonin sites.

Agent	IC_{50} value (nM)[a,b]
1 Phenylpiperazine	430 (±50)
2 4-Phenylpiperidine	440 (±60)
3 1-BzP	>1000
4 1-Benzoylpiperazine	>1000
5 1-PP	4000 (±400)
6 mCPP	20 [c]
7 pCPP	120 (±30)
8 MK-212	29 (±10)
9 2-MPP	324 (±100)
10 4-MPP	340 (±30)
11 1-NP	110 (±60)
12 2-NP (1-deazaquipazine)	30 (±20)
13 N4-Deazaquipazine	>1000
14 2-(N-dipropylamino)quinoline	>1000
15 2-Aminoquinoline	>1000
16 8-Methoxyquipazine	360 (±90)
17 8-Methoxy-N-methylquipazine	440 (±300)
18 AMI-098	270 (±70)
19 N-Methylquipazine (NMQ)	4.7 (±1)
20 N-Propylquipazine (NPQ)	15 (±2)
21 Quipazine	1.5

[a] Radioligand blinding assays were performed using rat cortex membranes as described in Materials and methods. IC_{50} values are ±S.E.M. of three experiments, each performed in triplicate; S.E.M. was not normally determined if K_i values were >1000 nM. [b] For purposes of comparison, K_i values for the 5-HT_3 antagonists ICS 205-930 and MDL 72222, determined using the present conditions, are 0.38 and 6.2 nM, respectively (Milburn and Peroutka, in press). [c] From Peroutka and Hamik (1988).

sites, and visa versa. Arylpiperazines, depending on what substituents are present in the molecule, can bind with varying degrees of affinity to all of the 5-HT binding sites. From this standpoint, arylpiperazines appear to be useful templates for the design and synthesis of novel serotonergic agents. With regard to 5-HT_3 sites, it is necessary to better understand the structure-affinity relationships of these agents before any rational drug design can be attempted. For this reason, we conducted the present investigation.

Phenylpiperazine itself binds at 5-HT_3 sites with an affinity comparable to that of 5-HT. Incorporation of the electron-donating methoxy group has little effect on affinity, whereas incorporation of the lipophilic electron withdrawing chloro group at the 3-position enhances affinity by 20-fold. A structurally related chloro derivative, MK-212, also binds with high affinity. The N1 piperazine nitrogen atom of the arylpiperazines does not seem to make a major contribution to affinity whereas the presence of the N4 piperazine nitrogen atom appears critical. In general, the quinoline nucleus of quipazine imparts a higher affinity than that noted for any of the monocyclic derivatives, and the quinoline nitrogen atom, though not essential, is important for binding.

One of the serious shortcomings of quipazine as a 5-HT_3 ligand is its moderately high affinity for 5-HT_{1B} sites. This could be a potential problem in future autoradiographic or in vivo studies. On the basis of the structure-affinity data generated from the first 18 agents shown in table 1, we attempted to prepare a quipazine analog with reduced affinity for 5-HT_{1B} sites. Comparing the affinity of **16** with that of its N-methyl analog **17**, it is clear that conversion of **16** to a tertiary amine (i.e., introduction the N-methyl group) has essentially no effect on affinity. It has been previously demonstrated that tertiary amines do not bind as well as their corresponding primary and secondary amines at 5-HT_{1B} sites (Glennon et al., 1988). Thus, it was thought possible to reduce the affinity of quipazine for 5-HT_{1B} sites by N-methylation. To test this hypothesis, we synthesized and evaluated N-methylquipazine (NMQ; **19**). Indeed, the affinity of NMQ is comparable to that of quipazine at 5-HT_3 sites (table 1), and yet NMQ displays essentially no affinity for 5-HT_{1B} sites (IC_{50} value > 10000) (table 2). Binding profiles for NMQ and quipazine are shown in table 2. The corresponding propyl homolog of NMQ (i.e., NPQ; **20**) displayed a lower affinity than NMQ for 5-HT_3 sites.

In summary then, the present study identifies specific structural features that are important for the binding of arylpiperazines at [^{3}H]quipazine-labeled 5-HT_3 sites and should aid in future drug design. Furthermore, agents such as mCPP (**6**), MK-212 (**8**), 1-NP (**9**), and 2-NP (**10**), which are generally considered to be 5-HT_1 or 5-HT_2 agents, are shown to bind at 5-HT_3 sites with significant affinity; subsequent investigations will need to take the present results into account. The arylpiperazine 1-PP (**5**), a common metabolite of several

TABLE 2

A comparison of NMQ and quipazine binding at various sites.

Receptor	Radioligand	IC_{50} values (nM) [a]	
		NMQ	Quipazine [b]
Serotonin sites			
5-HT_3	[^{3}H]Quipazine	4.7 (±1)	1.5
5-HT_2	[^{3}H]Ketanserin	550 (±50)	180
5-HT_{1A}	[^{3}H]8-OH DPAT	1700 (±60)	2000
5-HT_{1B}	[^{3}H]5-HT	>10000	200 [c]
Adrenoceptors			
α_1	[^{3}H]WB-4101	15000 (±4000)	13000
α_2	[^{3}H]Rauwolscine	3800 (±420)	5000
β	[^{3}H]DHA	48000 (±2000)	2900
Other sites			
Muscarinic	[^{3}H]QNB	95000 (±9000)	33000
Benzodiazepine	[^{3}]Flunitrazepam	>100000	

[a] Radioligand binding assays were performed using rat cortex membranes as described in Materials and methods. IC_{50} values, followed by S.E.M. (in parentheses) of three experiments, were performed in triplicate. [b] Data previously reported (Milburn and Peroutka, in press); IC_{50} values were recalculated from reported K_i values using the Cheng-Prusoff equation and are included for comparative purposes. See also footnote c. [c] A K_i value for quipazine was not reported in the paper by Peroutka and Hamik (1988); however, numerous investigators have reported K_i values of between 100 and 300 nM (e.g. see Glennon et al., 1989, and references therein).

second-generation anxiolytic (SGA) agents such as buspirone, gepirone, and ipsapirone, displays a low affinity for 5-HT_3 sites. Several arylpiperazines possess significant affinity for 5-HT_3 sites, but studies are necessary in order to determine if these agents are 5-HT_3 agonists or antagonists. Finally, using these structure-affinity results, we designed and synthesized NMQ, a quipazine analog that was expected to display greater selectivity than quipazine for 5-HT_3 versus 5-HT_{1B} sites. NMQ, a tertiary amine analog of quipazine, binds at 5-HT_3 sites with nearly the same affinity as quipazine, but displays little affinity (IC_{50} > 10000 nM) for 5-HT_{1B} sites.

Acknowledgment

This work was supported, in part, by PHS Grant NS 23520.

References

Barnes, N.M., B. Costall and R.J. Naylor, 1988, [^{3}H]Zacopride: Ligand for the identification of 5-HT_3 recognition sites, J. Pharm. Pharmacol. 40, 548.

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, 1986, Proposals for the classification of functional receptors for 5-hydroxytryptamine, Neuropharmacology 25, 563.

Costall, B., R.J. Naylor and M.B. Tyers, 1988, Recent advances in the neuropharmacology of 5-HT_3 agonists and antagonists, Rev. Neurosci. 2, 41.

Glennon, R.A., 1989, Central serotonin receptors, in: Receptor Pharmacology and Function, eds. M. Williams, R.A. Glennon and P.B.M.W.M. Timmermans (Marcel Dekker, New York) p. 257.

Glennon, R.A., M.R. Slusher, R.A. Lyon, M. Titeler and J.D. McKenney, 1986, 5-HT_1 and 5-HT_2 binding characteristics of some quipazine analogs, J. Med. Chem. 29, 2375.

Hoyer, D. and H.C. Neijt, 1988, Identification of serotonin 5-HT_3 recognition sites in membranes of N1E-115 neuroblastoma cells by radioligand binding, Mol. Pharmacol. 33, 303 (and references therein).

Kilpatrick, G.J., B.P. Jones and M.B. Tyers, 1987, Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding, Nature 330, 746.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with Folin phenol reagent, J. Biol. Chem. 193, 265.

Lyon, R.A., M. Titeler, J.D. McKenney, P.S. Magee and R.A. Glennon, 1986, Synthesis and evaluation of phenyl- and benzoylpiperazines as potential serotonergic agents, J. Med. Chem. 29, 630.

Milburn, C.M. and S.J. Peroutka, Characterization of [^{3}H]quipazine binding to 5-hydroxytryptamine$_3$ receptors in rat brain membranes, J. Neurochem. (in press).

Osborne, N.N. and M. Hamon, 1988, eds, Neuronal Serotonin (John Wiley and Sons, Chichester).

Peroutka, S.J., 1988, 5-Hydroxytryptamine receptor subtypes: molecular, biochemical, and physiological characterization, Trends Neurosci. 11, 496.

Peroutka, S.J. and A. Hamik, 1988, [^{3}H]Quipazine labels 5-HT_3 recognition sites in rat cortical membranes, European J. Pharmacol. 148, 297.

Richardson, B.P. and K. Buchheit, 1988, The pharmacology, distribution and function of 5-HT_3 receptors, in: Neuronal Serotonin, eds. N.N. Osborne and M. Hamon (John Wiley and Sons, Chichester) p. 465.

Rodriguez, R., 1972, U.S. Patent 3, 737, 540, February 14, 1972; Chem. Abstr. 79, 20227.

Watling, K.J., S. Aspley, C.J. Swain and J. Saunders, 1988, [^{3}H]Quaternised ICS 205-930 labels 5-HT_3 receptor binding sites in rat brain, European J. Pharmacol. 149, 397.