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Structural relationships in the inhibition of [^{3}H]serotonin binding to rat brain membranes *in vitro* by 1-phenyl-piperazines

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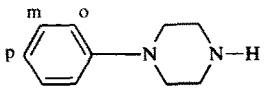
Binding of radioligands to membrane fractions is being widely used as a means of studying the interactions of endogenous neurotransmitters and hormones or of drugs with receptors. The high affinity binding of radioactive serotonin to brain membranes *in vitro* appears to represent attachment to post-synaptic receptors for serotonin, inasmuch as raphe lesions that destroy pre-synaptic elements of serotonin neurons in forebrain do not diminish serotonin binding [1]. Various indoles thought to interact with post-synaptic serotonin receptors inhibit the binding of radioactive serotonin to brain membranes *in vitro* [1–5]. In this paper, we describe the inhibition of [^{3}H]serotonin binding by a series of substituted 1-phenyl-piperazines. These compounds were studied because of earlier evidence that: (a) 1-(*m*-trifluoromethylphenyl)-piperazine stimulates serotonin receptors directly [6]; (b) 1-(*m*-chlorophenyl)-piperazine, a metabolite of trazodone, may interact with serotoninergic systems in rat brain [7]; and (c) quipazine [8] and MK-212 [9], both 1-aryl-piperazines, are serotonin receptor agonists that compete against serotonin binding [5].

The methods of Bennett and Snyder [1] were used to study the binding of [^{3}H]serotonin to synaptic membranes from rat brain *in vitro*, except that membranes were preincubated at 37° in the absence of pargyline to destroy endogenous serotonin. Each compound was tested in triplicate at molar concentrations differing by a factor of 10. The data were plotted as per cent antagonism of specific binding of [^{3}H]serotonin concentration of competitor; from this graph the IC_{50} value (concentration producing 50 per cent inhibition) was determined by interpolation. The concentration of [^{3}H]serotonin was 2 nM; specific binding represented 45–55 per cent total binding in the assay conditions. Specific binding is defined as the difference between total radioactivity bound and that bound in the presence of 10 μM unlabeled serotonin. Fenfluramine was a gift from the A. H. Robins Co. (Richmond, VA). The other compounds were synthesized in the Lilly Research Laboratories or were purchased from commercial sources.

Table 1. Inhibition of [^{3}H]serotonin binding to rat brain membranes *in vitro* by 1-phenyl-piperazines with substituents on the phenyl ring



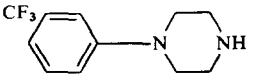
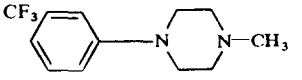
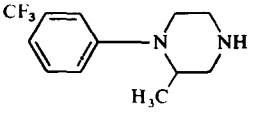
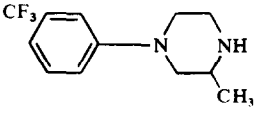
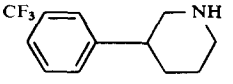
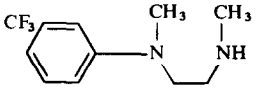
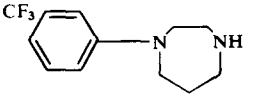
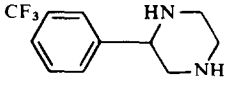
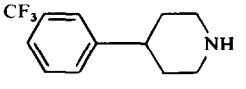
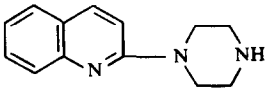
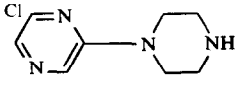
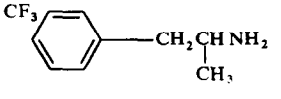
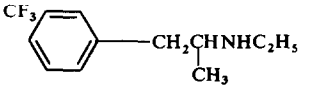
	IC_{50} (nM)
(1) *meta*-substituents	
m-CF_3	190
m-chloro	230
m-bromo	270
m-ethyl	320
m-methyl	540
m-methoxy	600
m-hydroxy	1050
m-fluoro	1140
(2) *para*-substituents	
p-chloro	580
p-methyl	1700
p-CF_3	1700
p-methoxy	50,000
(3) *ortho*-substituents	
o-chloro	630
o-methoxy	1010
o-methyl	1040
(4) No substituent	1050

Table 1 shows the structure–activity relationships among phenyl-piperazines with substituents on the aromatic ring as inhibitors of [^{3}H]serotonin binding. *Meta*-substituted compounds were more active than *para*- or *ortho*-substituted compounds. The trifluoromethyl, chloro, bromo and ethyl derivatives were the most active in the *meta*-substituted series. The methyl, methoxy, hydroxy and fluoro compounds were about one-third to one-fifth as active as the *meta*-trifluoromethyl compound. In the *para*- and *ortho*-substituted groups, fewer compounds were studied, but none were as active as the corresponding *meta*-substituted compounds. The IC_{50} value for unlabeled serotonin was 3–4 nM. This value and those for some of the substituted piperazines are lower than those we have obtained earlier with membranes not preincubated to remove endogenous serotonin [6].

Since 1-(*m*-trifluoromethylphenyl)-piperazine was the most potent inhibitor of [^{3}H]serotonin binding among the compounds listed in Table 1, several other structural variants of this compound were compared (Table 2). Addition of a methyl to the secondary amine nitrogen or to one of the carbons in the piperazine ring reduced the potency to one-eighth or less that of the parent compound. The piperidine compounds (with carbon in place of the nitrogen to which the *m*-trifluoromethylphenyl group is connected) were relatively inactive, though the 3-substituted piperidine was more active than the 4-substituted piperidine. Increasing the size of the piperazine ring, cleavage of that ring, or attachment of the *m*-trifluoromethylphenyl group to the 2- instead of the 1-position of the piperazine markedly reduced the potency from that of the parent compound. Two agents previously reported as serotonin agonists are included, quipazine and MK-212. Quipazine was less than one-third and MK-212 was only about one-eightieth as potent as 1-(*m*-trifluoromethylphenyl)-piperazine in inhibiting [^{3}H]serotonin binding. The bottom two compounds in Table 2, norfenfluramine and fenfluramine, also contain a *m*-trifluoromethylphenyl moiety and an amino group as does 1-(*m*-trifluoromethylphenyl)-piperazine. These compounds were compared because of numerous suggestions in the literature [10–14] that fenfluramine and/or its metabolite, norfenfluramine, may stimulate serotonin receptors directly in addition to their better known pre-synaptic effects on serotoninergic systems. Our data reveal that norfenfluramine has some ability to compete with [^{3}H]serotonin for binding to rat brain membranes, though fenfluramine is relatively inactive. Whether there is any direct receptor stimulation by norfenfluramine when it or fenfluramine is injected at doses that are effective *in vivo* in releasing serotonin and depleting serotonin content in rat brain is questionable.

Although 1-(*m*-trifluoromethylphenyl)-piperazine was

Table 2. Inhibition of [^{3}H]-serotonin binding to rat brain membranes *in vitro* by 1-(*m*-trifluoromethylphenyl)-piperazine and some structurally related compounds

Structure		IC_{50}, nM
		190
		1450
		1480
		4010
		1860
		2090
		3090
		7940
		7000
	(Quipazine)	680
	(MK-212)	3800
	(Norfenfluramine)	6000
	(Fenfluramine)	71.000

only about one-fiftieth as potent as serotonin in inhibiting [^{3}H]serotonin binding *in vitro*, this compound appears to be more potent as an inhibitor of such binding than any previously reported non-indole compound thought to act as a serotonin receptor agonist. In particular, the compound was more potent than either quipazine or MK-212, two compounds that have been reported to have numerous effects *in vivo* suggested to result from activation of serotonin receptors [8, 9, 15–17]. 1-(*m*-Trifluoromethylphenyl)-piperazine itself has actions consistent with serotonin agonist activity *in vivo* in rats. For instance, it decreases brain serotonin turnover [6] and elevates serum hormones (corticosterone and prolactin) whose secretion has previously been found to be increased by agents that enhance central serotoninergic function [18, 19]. The elevation of serum prolactin by this compound was at one time considered possibly to result from dopamine agonist activity [20], but *in vitro* binding studies (D. T. Wong and L. R. Reid, personal communication) indicated that 1-(*m*-trifluoromethylphenyl)-piperazine had very little ability to inhibit the binding of either [^{3}H]dopamine (IC_{50} 35,000 nM) or [^{3}H]spiperone (IC_{50} 4300 nM) to rat brain membrane receptors. These findings indicate some specificity of this agent for the serotonin receptor.

1-(*m*-Trifluoromethylphenyl)piperazine was a more potent inhibitor of [^{3}H]serotonin binding than of [^{3}H]-D-lysergic acid diethylamide) ([^{3}H]LSD) binding to brain membranes *in vitro*. Its IC_{50} value as an inhibitor of [^{3}H]LSD binding was 300 nM. The same was true for quipazine, another serotonin agonist, whose IC_{50} value as an inhibitor of [^{3}H]LSD binding was 1700 nM. In contrast, the IC_{50} values of serotonin receptor antagonists like metergoline and methysergide were found to be lower with [^{3}H]LSD binding than for [^{3}H]serotonin binding. These differences have been reported previously for serotonin receptor agonists and antagonists [1, 4] and support the idea that 1-(*m*-trifluoromethylphenyl)-piperazine acts on brain serotonin receptors as an agonist rather than an antagonist.

In summary, various 1-phenyl-piperazines and related compounds have been shown to be effective in inhibiting the binding of [^{3}H]serotonin to rat brain membranes *in vitro*. The most effective compound was 1-(*m*-trifluoromethylphenyl)-piperazine. Variation in the piperazine moiety of this compound greatly diminished the ability of this compound to inhibit [^{3}H]serotonin binding. Some variation in the nature of the *meta*-substituent retained activity but compounds with substituents in other positions of the phenyl ring were less active.

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Increased total and high density lipoprotein cholesterol with apoprotein changes resembling streptozotocin diabetes in tetrachlorodibenzodioxin (TCDD) treated rats

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Hyperlipidemia has been frequently mentioned among clinical findings in subjects exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In particular, a 56 per cent incidence of hypercholesterolemia was described [1] in the follow-up of Czechoslovakian factory workers contaminated with TCDD; more recently, a type IIA hypercholesterolemia was diagnosed in three young scientists exposed to TCDD [2]. In other studies, however, a significant prevalence of hyperlipidemia among intoxicated subjects was not detected [3, 4].

These controversial reports, related to a documentedly important prognostic factor for the development of coronary vascular disease [5], suggested that animal studies on this topic be carried out within the special program on long term effects of TCDD in Seveso, Italy. Particular interest was devoted to lipoprotein changes induced by