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Relative Efficacies of Piperazines at the Phosphoinositide Hydrolysis-Linked Serotonergic (5-HT-2 and 5-HT-1c) Receptors[1]

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ABSTRACT

Serotonin (5-HT)-stimulated phosphoinositide hydrolysis is mediated by the 5-HT-2 receptor in rat cerebral cortex and by the 5-HT-1c receptor in rat choroid plexus. These systems were used to determine relative efficacies of piperazine derivatives at the 5-HT-2 and 5-HT-1c receptors. Both quipazine and 6-chloro-2-[1-piperazinyl]-pyrazine (MK-212) stimulated phosphoinositide hydrolysis in cerebral cortex, and these effects were blocked by ketanserin. The maximum responses to these agonists were 80% of the maximum response to 5-HT. *m*-Trifluoromethylphenylpiperazine (TFMPP), *m*-chlorophenylpiperazine (MCPP) and 1-(1-naphthyl)-piperazine (1-NP) did not stimulate phosphoinositide hydrolysis in cerebral cortex at concentrations that blocked the effect of 5-HT. In the choroid plexus, TFMPP and MCPP, as well as MK-212 and quipazine, increased phosphoinositide hydrolysis and mianserin blocked these effects. MK-212 had an efficacy which was equal to that of 5-HT, whereas quipazine, MCPP and TFMPP were partial agonists in the choroid plexus. 1-NP did not stimulate phosphoinositide hydrolysis in choroid plexus but completely blocked the effect 5-HT. On the basis of these data, we conclude that quipazine and MK-212 are partial agonists at 5-HT-2 receptors in cerebral cortex, whereas 1-NP, TFMPP and MCPP are pure antagonists of the cortical 5-HT-2 receptor. However, TFMPP and MCPP as well as quipazine and MK-212 are agonists at the 5-HT-1c receptor, while 1-NP is a pure antagonist of the 5-HT-1c receptor in choroid plexus.

Central 5-HT receptors have been classified into two major subtypes (5-HT-1 and 5-HT-2) based upon radioligand binding studies (Peroutka and Snyder, 1979). The 5-HT-1 site has been further subdivided into 5-HT-1a, 5-HT-1b and 5-HT-1c sites (Pedigo *et al.*, 1981; Pazos *et al.*, 1984). The classification of the 5-HT binding sites is based on the capacity of drugs to compete selectively for one or another subsite. However, unless affinity differences span several orders of magnitude, establishment of selectivity based on binding affinities is of limited value in functional studies because of the difficulty in relating doses in a whole tissue preparation or in the whole animal to the concentrations in membrane binding assays. Several drugs are selective by several orders of magnitude for the 5-HT-2 site *vs.* the 5-HT-1a and 5-HT-1b sites, including ketanserin (Leysen *et al.*, 1982), mianserin (Leysen *et al.*, 1981; Sills *et al.*, 1984), LY 53847 (Cohen *et al.*, 1983) and ritanserin (Leysen *et al.*, 1985). This selectivity is, however, lost with the recent discovery of yet another 5-HT-1 subtype, the so-called 5-HT-1c site (Pazos *et al.*, 1984; Yagaloff and Hartig, 1985, 1986). This new site actually has an antagonist profile which more closely resembles the 5-HT-2 site than the 5-HT-1 sites, but the name 5-HT-1c was chosen because, in common with other 5-HT-1 sites, the 5-HT-1c site has a high affinity for 5-HT.

When highly selective antagonists are not available, another way to determine the receptor subtype which mediates a response in whole animals or in tissue preparations is to use drugs with different efficacies at the different receptors. For instance, if an agent is found to be an agonist at either the 5-HT-2 or 5-HT-1c receptor subtype but an antagonist at the other, this would provide a valuable tool for determining which subtype mediates a given response to 5-HT. Two major classes of compounds are thought to act as agonists at 5-HT receptors: indole derivatives, such as 5-HT and structurally related compounds, and piperazine derivatives, including among others, quipazine, MK-212, TFMPP and MCPP. Although the piperazines are commonly classified as agonists at central 5-HT receptors, efficacy at the various 5-HT receptor subtypes has not been tested rigorously. This has been due largely to the lack of appropriate models which reflect activation of the different receptor subtypes in brain.

Recent studies suggest that 5-HT-stimulated phosphoinositide hydrolysis is coupled to two different 5-HT receptor sub-

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ABBREVIATIONS: 5-HT, serotonin; MK-212, 6-chloro-2-[1-piperazinyl]-pyrazine; TFMPP, *m*-trifluoromethylphenylpiperazine; MCPP, *m*-chlorophenylpiperazine; 1-NP, 1-[1-naphthyl]piperazine; DR, dose-ratio; IP, inositol phosphate.

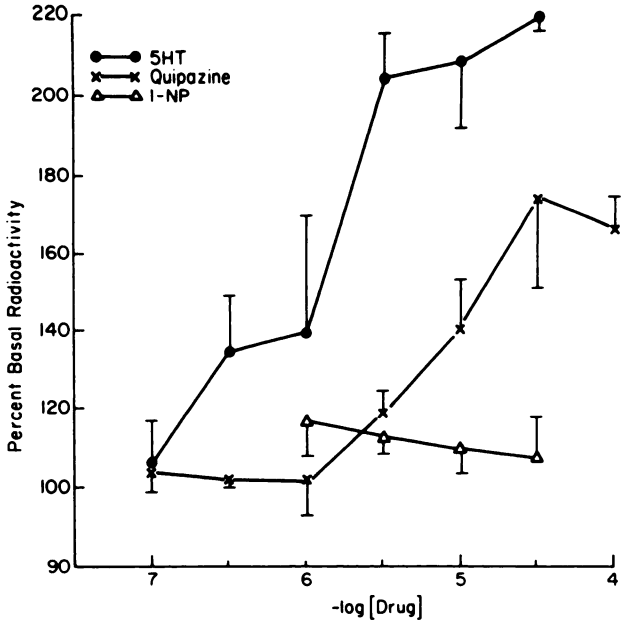


Fig. 1. Effect of 5-HT, quipazine and 1-NP on the accumulation of [^{3}H]IP in cerebral cortical slices. [^{3}H]Inositol-labeled slices were incubated with 10 mM LiCl, 10 μM pargyline and increasing concentrations of agonists. The percentage of basal [^{3}H]IP radioactivity present at the end of a 45-min incubation is plotted on the *Y* axis. The mean basal [^{3}H]IP release was 559 cpm. The data are representative of two separate experiments each done in triplicate. The vertical bars represent S.E.M.

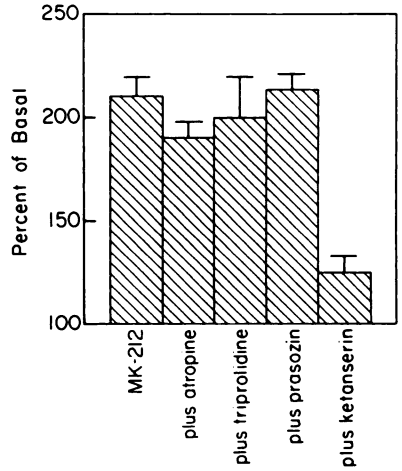


Fig. 2. Antagonist profile of MK-212-stimulated phosphoinositide hydrolysis in cortical slices. Conditions were the same as in figure 1. MK-212 was added at a concentration of 32 μM with or without the indicated antagonists. Antagonists were added 15 min before the addition of MK-212 at a concentration of 10 μM, except ketanserin, which was 1 μM. The mean basal release of [^{3}H]IP was 812 ± 29 cpm. The vertical bars represent the S.E.M., n = 6–8.

types, 5-HT-2 and 5-HT-1c receptors. The 5-HT-2 mediated phosphoinositide hydrolysis response has been characterized in cerebral cortex (Conn and Sanders-Bush, 1984, 1985, 1986a), platelets (de Chaffoy de Courcelles *et al.*, 1985; Schachter *et al.*, 1985), aorta (Roth *et al.*, 1984, 1986) and cultured cells (Cory *et al.*, 1986; Doyle *et al.*, 1986; Ananth *et al.*, 1987). On the other hand, the 5-HT-1c receptor coupled to phosphoinositide hydrolysis is highly localized in the choroid plexus (Conn *et al.*, 1986; Conn and Sanders-Bush, 1986b). In the present study, the 5-HT-stimulated phosphoinositide hydrolysis responses in choroid plexus and cerebral cortex were used to determine the

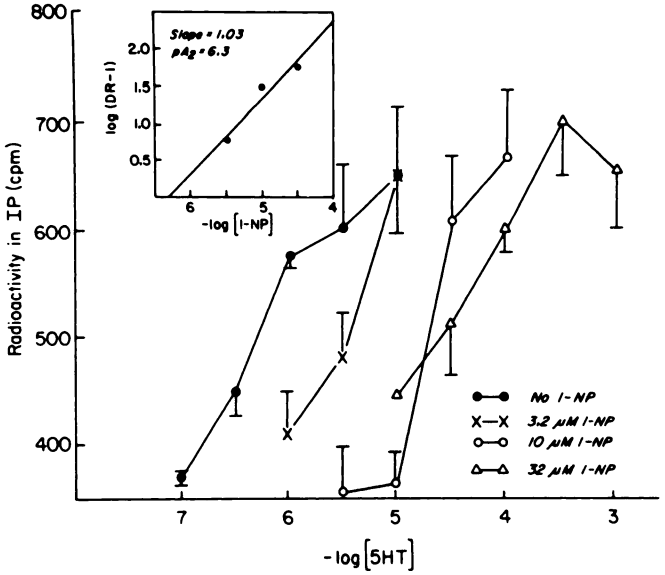


Fig. 3. Effect of 1-NP on 5-HT-stimulated phosphoinositide hydrolysis in cortical slices. Conditions were the same as in figure 1. The inset depicts a Schild analysis of the same data. The DR was determined by dividing the concentration of 5-HT needed to elicit an EC_{50} response in the presence of 1-NP by the concentration of 5-HT needed to elicit the same response in the absence of 1-NP. Log (DR-1) is plotted as a function of −log molar concentration of 1-NP. The data presented are representative of three separate experiments, each done in triplicate. The vertical bars represent S.E.M.

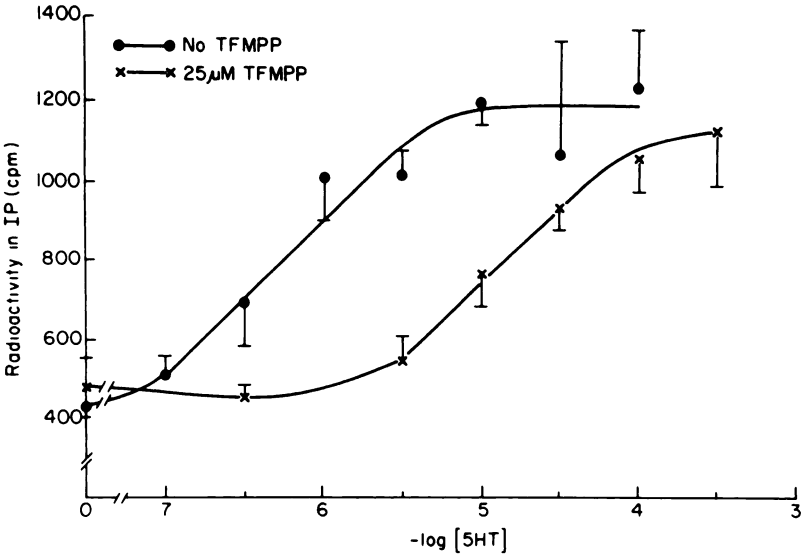
relative efficacies of piperazine derivatives at the 5-HT-1c and 5-HT-2 receptors, respectively. These studies show that MK-212 and quipazine are agonists at the 5-HT-2 receptor in cerebral cortex, whereas TFMPP, 1-NP and MCPP are pure antagonists of the cortical 5-HT-2 receptor. However, only 1-NP is a pure antagonist of the choroid plexus 5-HT-1c receptor.

Methods

Drugs. 5-HT creatinine sulfate was purchased from Sigma Chemical Co. (St. Louis, MO); MCPP HCl from Aldrich Chemical Co, Inc. (Milwaukee, WI); quipazine maleate from Miles Laboratories, Inc (Elkhart, IN) and mianserin HCl, TFMPP HCl and 1-NP HCl from Research Biochemicals, Inc. (Wayland, MA). The following drugs were kindly donated by the indicated manufacturers: MK-212 from Merck, Sharp and Dohme (West Point, PA); ketanserin tartrate from Janssen Pharmaceutica (Beerse, Belgium); and pargyline HCl from Abbott Laboratories (North Chicago, IL). [^{3}H]Ketanserin HCl (78.6 Ci/mmol) was purchased from New England Nuclear (Boston, MA) and [^{3}H]-myo-inositol (14 Ci/mmol) from American Radiolabeled Chemicals (St. Louis, MO). [^{3}H]Inositol was routinely stored with a small amount of Dowex-1 anion exchange resin in the formate form in order to maintain purity.

Radioligand binding. The binding of [^{3}H]ketanserin was measured in buffer containing physiological salts as described previously (Conn and Sanders-Bush, 1985). IC_{50} values were determined from log-logit plots of competition binding data. K_i values were calculated by the method of Cheng and Prusoff (1973).

Phosphoinositide hydrolysis. Measurement of agonist-induced phosphoinositide hydrolysis in cerebral cortex was as described previously (Conn and Sanders-Bush, 1985) except that 25 μl of gravity packed tissue was labeled for 3 hr in tubes containing 1 μCi of [^{3}H] inositol in 200 μl of Krebs-bicarbonate buffer with 10 mM glucose. Drugs were added directly to these tubes and subsequent incubations were in the continuous presence of [^{3}H]inositol. Pargyline was added routinely to the incubation medium. Pargyline shifts the 5-HT concentration-response curve leftward and eliminates the nonketanserin sen-



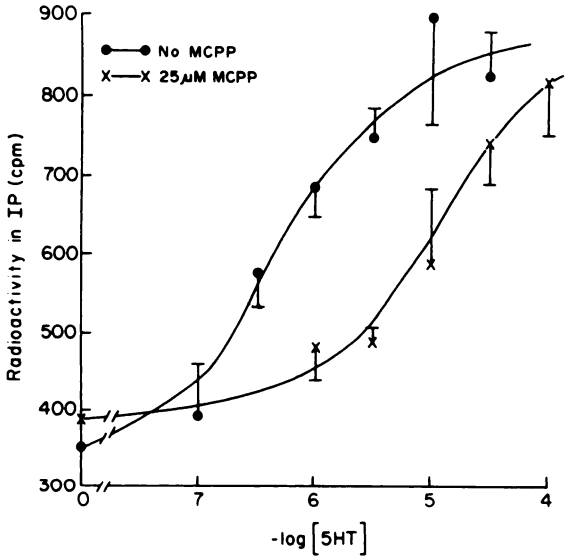


Fig. 5. Effect of 5-HT on phosphoinositide hydrolysis in the presence and absence of 25 μM MCPP. Conditions were the same as in figure 1. The data are representative of three separate experiments each done in triplicate. The vertical bars represent S.E.M.

sitive effects of high concentrations of 5-HT (Conn and Sanders-Bush, 1985). Measurement of phosphoinositide hydrolysis in excised choroid plexus was the same as in cerebral cortex except that each tube contained one choroid plexus and the incubation durations for prelabeling and agonist stimulation were for 90 and 30 min, respectively.

Estimates of antagonists affinities. The K_d value of 1-NP was estimated using the method of Arunlakshana and Schild (1959). Briefly, the concentration-response curve of 5-HT was determined in the absence and presence of various concentrations of 1-NP. The 5-HT concentration-response curves were shifted progressively to the right with increasing concentrations of 1-NP. EC_{50} values of 5-HT were determined at each antagonist concentration and DR values were calculated by dividing the EC_{50} of 5-HT in the absence of 1-NP by the EC_{50} of 5-HT in the presence of 1-NP. The regression line of log (DR-1) *vs.* −log antagonist concentration was extrapolated to the *X* axis to give a value theoretically equal to the negative log of the K_d of 1-NP for the phosphoinositide-linked receptor (pA_2).

Some antagonists had nonspecific effects at high concentrations in cerebral cortex, which prevented the use of increasing concentrations for complete Schild analyses. For these drugs, K_d values were estimated by using single antagonist-induced shifts of the 5-HT concentration-response curve and the Schild equation:

Fig. 4. Effect of 5-HT on phosphoinositide hydrolysis in the presence and absence of 25 μM TFMPP. Conditions were the same as in figure 1. The data are representative of five separate experiments each done in triplicate. The vertical bars represent S.E.M.

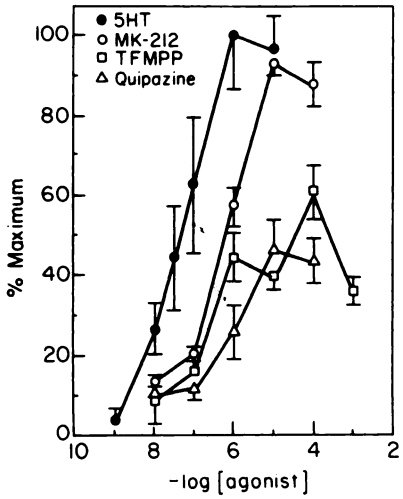


Fig. 6. Effect of MK-212, quipazine, TFMPP and 5-HT on phosphoinositide hydrolysis in choroid plexus. Labeled choroid plexi were incubated for 30 min with 10 mM LiCl, 10 μM pargyline and the indicated agonists. The values are expressed as percentage of the maximal 5-HT effect on [^{3}H]IP formation, determined in dose-response analyses run concurrently. Representative basal [^{3}H]IP formation was 1249 ± 53 cpm (mean ± S.E.M., n = 7) and the maximal 5-HT response was 9437 ± 257 (n = 4) cpm. The values plotted are the means of three to six separate determinations. The vertical bars represent S.E.M.

$$K_i = \frac{[A]}{(EC_{50a}/EC_{50b}) - 1}$$

where $[A]$ = concentration of antagonist, $EC_{50a} = EC_{50}$ in the absence of antagonist and $EC_{50b} = EC_{50}$ in the presence of antagonist.

Results

Effect of piperazine derivatives in cerebral cortex. Quipazine increased phosphoinositide hydrolysis in a concentration-dependent manner in cerebral cortical slices (fig. 1) with an EC_{50} value of 10 μM. Neither quipazine (fig. 1) nor MK-212 (Conn and Sanders-Bush, 1985) was as efficacious as was 5-HT. The effect of MK-212 (fig. 2) and of quipazine (data not shown) in cerebral cortex was blocked by the addition of ketanserin, but not by *alpha*-1 adrenergic, muscarinic or histamine-H_2 antagonists.

The addition of increasing concentrations (3–32 μM) of 1-

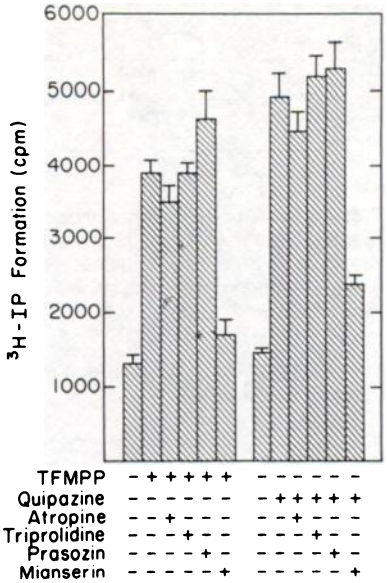


Fig. 7. Effect of antagonists on quipazine- and TFMPP-stimulated phosphoinositide hydrolysis in choroid plexus. Experimental conditions were the same as those described in figure 6. Quipazine and TFMPP were added in a concentration of 31 and 1 μM, respectively. Antagonists were added 15 min before the agonists at a concentration of 10 μM, except mianserin which was 0.1 μM. Each value represents the mean of four to eight separate determinations. The vertical bars represent S.E.M.

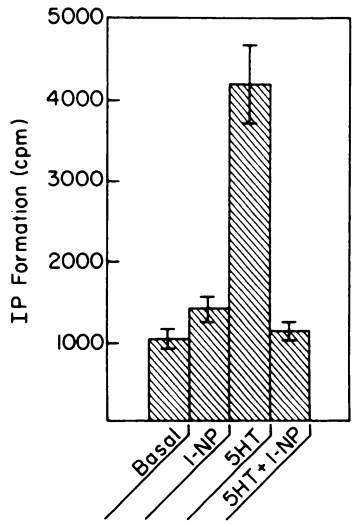


Fig. 8. Effect of 1-NP on 5-HT-stimulated phosphoinositide hydrolysis in choroid plexus. Slices were incubated with 5-HT in the absence or presence of 100 μM 1-NP. Experimental conditions were the same as those described in figure 6 except the concentration of 5-HT was 50 nM. Each bar represents the mean of four to five separate determinations. The vertical bars represent S.E.M.

NP induced parallel rightward shifts of the 5-HT concentration-response curve (fig. 3). These concentrations of 1-NP had no effect on basal activity (fig. 1). The mean slope (±S.E.M.) of three separate Schild plots (inset, fig. 2) was 1.25 ± 0.2, suggesting that 1-NP is a simple competitive antagonist of the 5-HT-2 receptor. 1-NP was less potent as an antagonist of phosphoinositide hydrolysis (K_i = 745 ± 278 nM) than it was in competition for [^{3}H]ketanserin labeled 5-HT-2 sites (K_d = 110 ± 20 nM). Similar potency differences were found for other 5-HT-2 antagonists (Conn and Sanders-Bush, 1986c). These differences may be related to the different tissue preparations which are required for the two assays. TFMPP (fig. 4) and MCPP (fig. 5) also induced parallel rightward shifts of the 5-HT concentration-response curve, demonstrating that these drugs are competitive antagonists of the 5-HT-2 receptor. Neither TFMPP (fig. 4) nor MCPP (fig. 5) had an effect on basal IP release at a concentration of 25 μM or less. However, consistent with previous reports (Conn and Sanders-Bush, 1985), higher concentrations (>100 μM) of TFMPP and MCPP increased [^{3}H]IP formation (data not shown). This effect was not inhibited by ketanserin and did not reach asymptote at concentrations as high as 1 mM. A number of other compounds have similar effects in cerebral cortex at high concentrations. These include mianserin, cinanserin, amitriptyline, propranolol, phenolamine and quipazine (data not shown). The mechanism(s) of these effects are unknown but may involve direct activation of phospholipase *C*, as has been shown for detergents (Manning and Sun, 1983), or possible inhibition of phosphatidate phosphatase, as apparently is the case with propranolol and other cationic amphiphilic drugs (Abdel-Latif *et al.*, 1983).

Effect of piperazine derivatives in choroid plexus. In agreement with previous studies (Conn *et al.*, 1986), 5-HT elicited a 8- to 9-fold increase in [^{3}H]IP formation in choroid plexus. Similarly, MK-212, quipazine and TFMPP induced dose-related increases in phosphoinositide hydrolysis in this tissue (fig. 6). MCPP (100 μM) induced a 7-fold increase in [^{3}H]IP formation (data not shown). The efficacies of MK-212 and MCPP were close to the efficacy of 5-HT, whereas quipazine and TFMPP had lower efficacies. The piperazine derivatives were less potent than was 5-HT, with EC_{50} values of 830, 550 and 800 nM for MK-212, TFMPP and quipazine, respectively, compared with an EC_{50} value of 50 nM for 5-HT (fig. 6). The response elicited by TFMPP and quipazine (fig. 7) was inhibited by the addition of mianserin but not by atropine, triprolidine or prazosin. The addition of 100 μM 1-NP did not stimulate phosphoinositide hydrolysis in choroid plexus, but completely blocked the effect of an EC_{50} concentration of 5-HT (fig. 8). Unlike cerebral cortex, high concentrations of the piperazines did not elicit nonketanserin sensitive effects in choroid plexus (data not shown).

Discussion

The 5-HT-stimulated phosphoinositide response in cerebral cortex (Conn and Sanders-Bush, 1985, 1986a) and in choroid plexus (Conn *et al.*, 1986; Conn and Sanders-Bush, 1986b) was used to determine the relative efficacies and potencies of several piperazine derivatives at the 5-HT-2 and 5-HT-1c receptors, respectively (table 1). Quipazine and MK-212 stimulated phosphoinositide hydrolysis in cerebral cortex and these effects were blocked by the 5-HT-2 antagonist, ketanserin, but not by antagonists of other phosphoinositide hydrolysis-linked receptors. These drugs gave lower maximal responses than did 5-HT, suggesting that they are partial 5-HT-2 agonists. Conversely, 1-NP, MCPP and TFMPP failed to stimulate phosphoinositide hydrolysis in cerebral cortex at concentrations that inhibited completely the response to 5-HT, suggesting that these compounds are pure 5-HT-2 antagonists. Although 1-NP, MCPP and TFMPP are pure antagonists of the cortical 5-HT-2 receptor, they have many properties that are consistent with central 5-HT agonist effects, including anorexia (Samanin *et al.*, 1979; Fuller *et al.*, 1981a), reduction of 5-HT turnover (Fuller *et al.*, 1981b, 1986) and neuroendocrine effects (Aloi *et al.*, 1984; Fuller *et al.*, 1981b, 1986). These effects *in vivo* must

TABLE 1

Piperazine effects at phosphoinositide-linked receptors in cerebral cortex and choroid plexus

Drug	Cerebral Cortex			Choroid Plexus		
	Agonist (EC_{50})[a]	Antagonist (K_i)[b]	Relative Efficacy[c]	Agonist (EC_{50})[a]	Antagonist (K_i)[b]	Relative Efficacy[c]
	nM	*nM*		*nM*	*nM*	
5-HT	1400		1.0	50		1.0
Quipazine	10,000		0.8	800		0.5
MK-212	18,000		0.8	820		0.9
TFMPP		760	0.0	550		0.5
MCPP		860	0.0	ND[d]		0.9
1-NP		745	0.0		ND[d]	0.0

[a] EC_{50}, that concentration of agonist that elicits a half-maximum response.
[b] Apparent K_i values were determined from the data in figures 3 to 5 using the Schild equation for MCPP and TFMPP and a Schild analysis for 1-NP.
[c] Relative efficacy is the maximum release of [^{3}H]IP relative to the maximum effect of 5-HT, which is assigned a value of 1.
[d] ND, not determined.

therefore be mediated by 5-HT receptor subtypes other than the 5-HT-2 site in cerebral cortex. The current findings are consistent with previous reports in which responses which are clearly mediated by the 5-HT-2 site were used. Quipazine is a partial agonist at the 5-HT-2 receptor on vascular smooth muscle (Cohen *et al.*, 1981), but MCPP, TFMPP (Cohen and Fuller, 1983) and 1-NP (Cohen *et al.*, 1983) are pure antagonists in this preparation. Furthermore, MK-212 and quipazine induce head twitches in mice (Malick *et al.*, 1977; Clineschmidt *et al.*, 1977), but TFMPP, 1-NP and MCPP have not been demonstrated to be agonists in this model of 5-HT-2 receptor activation. Indeed, a recent preliminary report (Simansky and Schechter, 1987) states that all three of these piperazines block head twitches elicited by L-5-hydroxytryptophan in mice, a response presumably mediated by activation of 5-HT-2 receptors in brainstem. These behavioral results reflecting interactions in the brainstem combined with the present biochemical studies in cerebral cortex suggest that the central 5-HT-2 blocking effects of 1-NP, MCPP and TFMPP are widespread and functionally significant. Interestingly, the profile of the piperazines at the terminal 5-HT-1-like autoreceptor which modulates 5-HT release is the opposite of the profile at the 5-HT-2 receptor; quipazine is a pure antagonist, whereas MCPP and TFMPP are agonists at this site (Bauman and Waldmeier, 1981; Martin and Sanders-Bush, 1982). Furthermore, *in vitro* addition of MCPP and TFMPP induces the release of 5-HT from hypothalamic slices (Pettibone and Williams, 1984). Similar effects occurring *in vivo* could play a role in mediating the physiological and behavioral effects of these agents.

The profile of efficacies of the piperazine derivatives at the 5-HT-1c receptor in choroid plexus was clearly distinct from the profile at the 5-HT-2 receptor in cerebral cortex. In choroid plexus, TFMPP, MCPP, quipazine and MK-212 were all agonists, but only MK-212 had an efficacy equal to that of 5-HT. 1-NP, on the other hand, was a pure antagonist at the 5-HT-1c receptor. Thus, the effects of the piperazine derivatives at the 5-HT-1c receptor are more consistent with the commonly held beliefs concerning piperazine actions at central 5-HT receptors.

In conclusion, the current studies of functional responses linked to 5-HT-2 and 5-HT-1c receptor sites demonstrate that two piperazine derivatives, TFMPP and MCPP, have opposite effects at these receptors. They are partial agonists at the 5-HT-1c site in choroid plexus, but pure antagonists at the 5-HT-2 receptor in cerebral cortex. These findings are of practical value and suggest that the piperazine derivatives may prove to be valuable tools for discriminating between 5-HT-2- and 5-HT-1c-mediated behavioral and physiological responses.

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