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Agonist and Antagonist Activities of Arylpiperazines at Human Platelet Serotonin$_2$ Receptors[1]

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ABSTRACT

A series of arylpiperazines was examined for structure-function relationships at the human platelet serotonin (5-HT) receptor. Amplification of ADP-induced aggregation was used to measure 5-HT receptor activation. The platelet serotonergic agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), 5-HT and 5-methoxytryptamine (5-MeOT) and the antagonist ketanserin were used for comparison of potency and amplitude of response. All arylpiperazines, including the parent compound phenylpiperazine (PP) showed antagonist activity. The monosubstituted phenylpiperazines acted only as antagonists, and electron-withdrawing substituents markedly enhanced activity. Modification of PP by addition of another phenyl ring or benz-fusion also enhanced antagonist activity. Benz-fusion at the b face of PP (1-NP) yielded greater antagonist potency than benz-fusion at the c face (2-NP). The latter modification, however, also conferred a variable agonist activity with a very weak response. In contrast, the heteroaromatic piperazines consistently demonstrated concentration dependent mixed antagonist-agonist activity. These compounds were weak agonists compared with 5-HT, 5-MeOT and DOI, although the amplitude of the quipazine response was similar to DOI. This study demonstrates that the arylpiperazines, which are variably selective for the multiple brain 5-HT receptors, are all antagonists on the platelet 5-HT receptor. The antagonist activity is markedly increased by ring monosubstitution or aryl modification. Compared with the monosubstituted analogues, antagonist activity is decreased by heteroaromatic modification or by the addition of an *N*-aminophenethyl group to the 4-position nitrogen. Weak agonist activity can be conferred by heteroaromatic modification.

The blood platelets contain >90% of the circulating serotonin (5-HT) (Crawford, 1963). This endogenous 5-HT is released with ADP from cytoplasmic dense granules during platelet activation and serves to amplify the aggregation response. Under normal circumstances 5-HT alone cannot initiate aggregation, but in a significant percentage of patients with cardiovascular disease, 5-HT directly evokes biphasic irreversible platelet activation (De Cree *et al.*, 1985). In addition, 5-HT may mediate coronary artery reocclusion after thrombolytic therapy (Golino *et al.*, 1988). These studies emphasize the importance of 5-HT in platelet activation and the need for further pharmacologic characterizations of 5-HT platelet receptor-drug interactions.

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In contrast to the single 5-HT receptor type in platelets, the central nervous system (CNS) contains heterogenous 5-HT sites with distinct pharmacologic and physiologic properties (Bradley *et al.*, 1986, Titeler *et al.*, 1987). Although previous studies have demonstrated the pharmacologic similarities between the platelet 5-HT receptor and one of the brain 5-HT binding sites (5-HT$_2$), little is known about the structure-function relationships at the platelet receptor. The arylpiperazines are a class of ligands that have been examined for 5-HT structure-activity relationships in the CNS (Fuller *et al.*, 1980; Fuller and Mason, 1981; Huff *et al.*, 1985; Glennon, 1987 for review). Although generally classified as agonists with significant selectivity at brain 5-HT receptors, each drug appears to have distinct functional properties at the site subtypes (Cohen and Fuller, 1983; Clineschmidt, 1979; Glennon *et al.*, 1986b; Conn and Sanders-Bush, 1987). Although selectivity and efficacy of the arylpiperazines has been demonstrated at 5-HT sites outside the CNS (Glennon, 1987 for review; Cohen and Fuller, 1983), the arylpiperazines have not been examined for platelet 5-HT$_2$ activity.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-MeOT, 5-methoxytryptamine; CNS, central nervous system; citalopram, 1-(3-(di-methylamino)propyl)-1-(p-fluorophenyl)-5-phthalanecarbonitrile; DOI, (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; DPAT, (±)-8-hydroxy-2-(di-n-propylamino)tetralin; LSD, d-lysergic acid diethylamide; m-CPP, 1-(3-chlorophenyl)piperazine; PP, phenylpiperazine; MK-212, 6-chloro-2-[1-piperazinyl]-pyrazine; 1-NP, 1-(1-naphthyl)piperazine; 2-NP, 1-(2-naphthyl)piperazine; PAPP, 1-(4-aminophenylethyl)-4-(3-trifluoromethylphenyl)piperazine; PPP, platelet poor plasma; PRP, platelet rich plasma; quipazine, 2-(1-piperazino)quinoline; TFMPP, 1-(3-trifluoromethylphenyl)piperazine.

The homogeneous 5-HT receptor population on platelets presents a well-defined analytical system for the functional characterization of 5-HT_2 drugs. The aim of this investigation was to examine the structural properties that affect functional interactions of the arylpiperazines with the platelet 5-HT_2 receptor.

Methods

Drugs. Compounds purchased from commercial sources include PP-HCl and mCPP-HCl from Aldrich (Milwaukee, WI), 5-HT-creatinine-SO_4-H_2O from Regis (Morton Grove, IL), 5-MeOT from Sigma (St. Louis, MO) and 1-NP-HCl, (±)DOI-HCl, (±)DPAT-HBr, TFMPP-HCl and quipazine maleate from Research Biochemicals (Natick, MA). Compounds obtained as gifts include citalopram-HBr from Pfizer (New York, NY), ketanserin tartrate from Janssen Pharmaceutica (Beerse, Belgium), mianserin HCl from Organon Teknika-Cappel (Malvern, PA), MK-212 from Merck (Rahway, NJ) and 2-NP from Dr. Richard Glennon (Medical College of Virginia, Richmond, VA). PAPP-HCl was prepared in our laboratory by the method of Ransom *et al.* (1985). Other reagents were obtained from commercial sources.

Human platelet preparation. The platelet donations were performed in accordance with the Declaration of Helsinki. Venous whole blood (95 ml) was drawn from healthy volunteer donors with no history of recent aspirin usage, chronic medication or caffeine consumption. The first 5 ml were discarded, and the remainder was mixed with 10 ml sodium citrate (3.8% w/v). Platelet rich plasma (PRP) was prepared by centrifugation at 200 × *g* for 5 min at 20°C. The remaining packed cells were centrifuged at high speed to yield platelet poor plasma (PPP). PRP was adjusted to a cell count of 250,000 platelets/μl using a model S Plus IV cell counter (Coulter Electronics, Hialeah, FL) and rotated slowly in completely filled and sealed containers at 25°C for at least 30 min before use. In some studies the PRP was supplemented with 1.0 mg/ml glucose. All aggregation studies were completed within 6 hr after venipuncture. Platelet responses to 10 μM ADP (maximum responsiveness), to subthreshold amounts of ADP (0.25–1.0 μM) (base line) and to the combination 13 μM 5-HT/0.25-1 μM ADP (100% response) were monitored throughout the entire period to correct for any change in platelet activity.

Aggregation studies. Aggregation studies were performed in silanized glass cuvettes at 37°C using a Payton Scientific (Buffalo, NY) Dual Channel Aggregometer. The instrument was calibrated to measure turbidity as percent transmission (%T) (PPP = 100%T, PRP = 0% T). ADP at a concentration of 10 μM caused irreversible aggregation. For each donor on a given day, the subthreshold concentration of ADP was titrated (0.25 μM–1.0 μM) to evoke only a minimal change in turbidity (<5% of the 10 μM ADP response). 5-HT, in the absence of ADP, had no significant effect on aggregation. In the presence of subthreshold concentrations of ADP, 5-HT or a serotonergic agonist amplified the ADP effect into a full aggregation response. The maximum slope of the change in turbidity (aggregation response) was used to quantify the amplification. For 5-HT receptor agonist studies, varying concentrations of the drug and the subthreshold concentration of ADP in normal saline were added to PRP. Stock solutions of 5-HT also contained 1 mM ascorbic acid to prevent oxidation. Subsequent dilution of 5-HT-ascorbate-ADP into PRP did not affect the pH of the solution, and the presence of the ascorbic acid did not affect aggregation. The maximum amplification of ADP-induced aggregation was demonstrated at 1–15 μM 5-HT and 13 μM was used in antagonist studies as described below.

5-HT receptor antagonist studies were performed by preincubating 450 μl PRP with 25 μl antagonist dissolved in H_2O for 4 min at 37°C with stirring, followed by the addition of 25 μl of 5-HT/ADP in isotonic saline to give 13 μM 5-HT/0.25-1 μM ADP (final concentration). For each antagonist drug study, complete inhibition was defined by the response to subthreshold amounts of ADP (0.25–1.0 μM) alone, and maximal amplification was the response to the 13 μM 5-HT/0.25–1 μM ADP without antagonist.

The maximum slope of aggregation was measured as a function of added drug concentration. Aggregation studies were performed with at least eight drug concentrations in PRP from seven healthy adult volunteers. These studies were repeated on different days for a total of at least three complete concentration curves for each drug studied.

Data analysis. LogEC_{50} and logIC_{50} values for individual experiments were determined by fitting a curve to the maximum slope of the aggregation *versus* drug concentration by iterative nonlinear regression. The curve was fit to the equation

$$Y = A + \frac{B - A}{1 + \frac{(10^X)_D}{(10^C)^D}}$$

where X is log of the ligand concentration, A is bottom plateau log concentration, B is top plateau log concentration, C is log EC_{50} or IC_{50} concentration and D is slope factor (Hill coefficient). The percent error of the fitted dose-response curve for individual experiments averaged 2.9%.

Results

Antagonist studies. All arylpiperazines (fig. 1) antagonized 5-HT amplification of platelet aggregation. The data are summarized in table 1. Most significantly, all, except for the heteroaromatic piperazines and 2-NP, were strictly antagonists. For each compound, antagonist activity was reversible when the concentration of 5-HT was increased. The −logIC_{50} of these compounds ranged from 6.40 for mCPP to 4.18 for PP, compared with 7.68 for ketanserin, a potent platelet 5-HT_2 receptor antagonist (Leysen *et al.*, 1983; Victorzon *et al.*, 1986). The highly 5-HT_{1a} selective aminotetralin (DPAT) was only an antagonist in this system with low activity (−logIC_{50} = 4.65).

Electron-withdrawing substituents on the aromatic ring of PP (fig. 1A) significantly affected activity as demonstrated by the two monosubstituted phenylpiperazine analogues (mCPP, TFMPP). In contrast to monosubstitution on the phenyl ring, addition of an aminophenylethyl group at the 4-position nitrogen of TFMPP reduced activity (PAPP). Aromatic modification by addition of a phenyl ring (mianserin) or benz-fusion (1-NP and 2-NP) significantly increased antagonist activity. For the naphthylpiperazines, fusion at the b face (1-NP) resulted in slightly greater antagonist activity compared with fusion at the c face (2-NP). Of the modified phenylpiperazines (fig. 1B), mianserin was the most effective and PAPP the least effective antagonist.

The heteroaromatic piperazines (fig. 1C) MK-212 and quipazine which are structural analogues of m-CPP and 2-NP, respectively, demonstrated moderate antagonist activity. MK-212 was considerably less active than mCPP, whereas quipazine and 2-NP had similar potency. Thus, the quinoline nitrogen of quipazine did not significantly affect antagonist activity, although it substantially influenced agonist activity, as described below.

Agonist studies. Of the arylpiperazines evaluated in this investigation (to 580 μM), only quipazine and MK-212 had significant agonist activity. Quipazine had a maximal amplitude of response similar to DOI, whereas MK-212 induced a much weaker response (table 2). Compared with the nonselective indoleamine agonists (5-HT and 5-MeOT) and the 5-HT_2 selective agonist (DOI), quipazine and MK-212 were significantly less active. Maximal responses for quipazine and MK-212 occurred between 90 and 100 μM with slopes greater than one (≈1.5). Maximal responses occurred at 1–15 μM for DOI, 5-HT and 5-MeOT with slopes of 0.58, 1.10 and 1.07, respectively (fig. 2). Very high concentrations of these compounds were less effective in amplifying

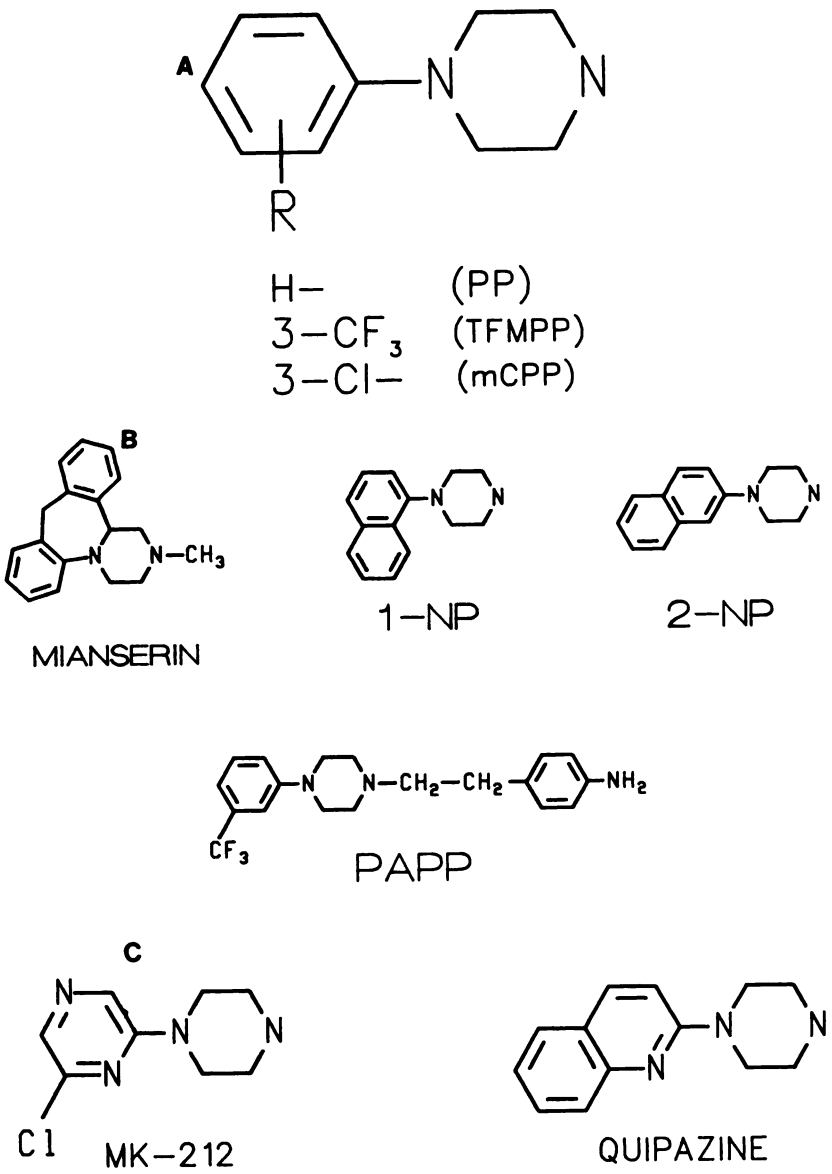

Fig. 1. Arylpiperazines active at the platelet 5-HT$_2$ receptor: *A*, monosubstituted phenyl piperazines; *B*, modified phenyl piperazines; *C*, heteroaromatic piperazines.

aggregation so that ~50% of the maximal response occurred at 500–580 μM. This result has been reported with other 5-HT agonists in platelets (Victorzon *et al.*, 1986). In contrast to quipazine, benz-fusion of PP at the c face (2-NP) conferred a highly variable ill-defined agonist activity with an extremely low response. Unlike the other agonist drugs, the agonist activity of 2-NP could only be erratically elicited (tested to concentrations ≥580 μM) at the highest subthreshold concentrations (1 μM) of ADP.

Specificity of the agonists quipazine, MK-212 and DOI for the platelet 5-HT receptor was studied in a series of competition experiments using known antagonists for 5-HT$_2$, α-adrenergic, β-adrenergic and 5-HT uptake sites (fig. 3). Quipazine and MK-212 appeared to amplify aggregation by interacting with the 5-HT$_2$ receptor. Amplification induced by quipazine and MK-212 was significantly attenuated (−84 and −56%, respectively) with 1 μM ketanserin and the reductions correlated with those for 5-HT (−81%) and DOI (−68%). The α-adrenergic antagonist phentolamine (1 μM) (Sogabe *et al.*, 1985) produced a paradoxical increase in the aggregation amplification of DOI (56%) and quipazine (32%). This statistically significant enhancement of the agonist responses of quipazine and DOI cannot be easily explained. If DOI had any α-adrenergic activity as an agonist, amplification of aggregation would most likely be diminished by phentolamine. No direct effects of phentolamine on the platelet aggregation response, unrelated to its α-adrenergic antagonist activities, were found. The β-adrenergic antagonist propranolol (0.8 μM) (Kerry and Scrutton, 1983) had no significant effect on the activity of these agonists. The 5-HT uptake inhibitor citalopram [1-(3-(dimethylamino)propyl)-1-(*p*-fluorophenyl)-5-phthalanecarbonitrile] (Hytell, 1982) and no significant effect on the amplification response of 5-HT, DOI and MK-212. The response to quipazine was reduced <20%.

Discussion

Serotonin interacts with platelets to cause many species-dependent changes (De Clerck *et al.*, 1982a). These include shape change (Laubscher and Pletscher, 1979; Pletscher and Affolter, 1983), aggregation (De Clerck *et al.*, 1982a) and amplification of the aggregation response evoked by other agents (De Clerck *et al.*, 1982b). These functional responses (De Clerck *et al.*, 1984a,b; Geaney *et al.*, 1984; Pletscher and Affolter, 1983; Pletscher, 1987) and the associated biochemical events (Affolter *et al.*, 1984; de Chaffoy de Courcelles *et al.*, 1984; Drummond and MacIntyre, 1987) appear to be evoked by a 5-HT$_2$ type receptor. In humans, 5-HT-induced platelet aggregation is normally weak and highly variable (Pletscher and Affolter, 1983).

TABLE 1

Arylpiperazine antagonist activities for 5-HT$_2$ amplification of ADP-induced aggregation in human platelets

Values are means ± S.E.; $n \geq 3$. Response was calculated from initial slope of aggregation in %T/min.

Drug	Structural Modification[a]	-logIC$_{50}$
		M
mCPP	monosubstitution	6.40 ± 0.10
Mianserin	aromatic modification	6.06 ± 0.11
1-NP	aromatic modification	5.86 ± 0.13
TFMPP	monosubstitution	5.76 ± 0.18
MK-212	heteroaromatic modification	5.69 ± 0.09
2-NP	aromatic modification	5.69 ± 0.09
Quipazine	heteroaromatic modification	5.68 ± 0.12
PAPP	*N*-aminophenethyl addition[b]	5.16 ± 0.15
PP	none	4.18 ± 0.27

[a] Structural modifications compared with phenylpiperazine as the starting compound.
[b] Modification of TFMPP.

TABLE 2

5-HT receptor agonist activities of heteroaromatic piperazines and DOI for amplification of ADP-induced aggregation in human platelets

Values are means ± S.E.; $n = 3$. Response was calculated from initial slope of aggregation in %T/min.

Drug	Drug Group	-logEC$_{50}$	E$_{max}$[a]
		M	
MK-212	heteroaromatic piperazine	4.43 ± 0.06	0.26 ± 0.01
Quipazine	heteroaromatic piperazine	4.54 ± 0.19	0.79 ± 0.02
DOI	phenylalkylamine	6.39 ± 0.05	0.71 ± 0.04
5-HT	indoleamine	6.52 ± 0.10	1.00

[a] E$_{max}$, maximum response of the agonist/maximum 5-HT response.

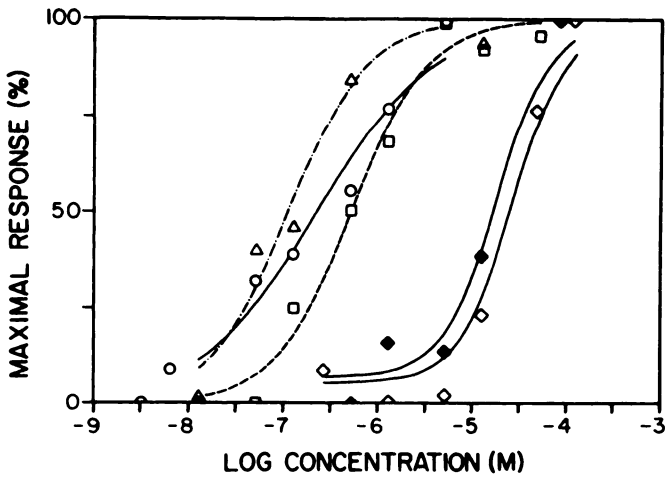

Fig. 2. Agonist dose-response curves for amplification of ADP-induced aggregation by arylpiperazines compared with phenylalkylamine and indoleamine drugs: Quipazine (♦—♦), MK-212 (◇—◇), DOI (○—○), 5-HT (□—□), 5-MeOT (△—△).

By contrast, 5-HT causes pronounced amplification of ADP-induced aggregation in human platelets. Therefore, amplification of aggregation can be used as a measurable physiologic response resulting from 5-HT receptor activation in human platelets. Although this technique has the advantage of monitoring receptor activity in a minimally manipulated system, platelet aggregation is a complex biologic process. It is affected by many other endogenous and exogenous agents and must be carefully and conservatively interpreted using well-controlled experimental conditions. The studies presented in this report use amplification of platelet aggregation for examining structure-function relationships of the arylpiperazines at the human 5-HT$_2$ receptor.

Of the arylpiperazines evaluated, all functioned as 5-HT antagonists at the platelet receptor. For the phenylpiperazine analogues, many features significantly affected antagonist activity. Monosubstitution with electron-withdrawing groups in the meta position enhanced activity. For the two monosubstituted analogues evaluated, factors other than the inductive and field effects of the substituents appeared to influence antagonist activity. Although the CF_3-group (TFMPP) conferred greater electron-withdrawing characteristics than Cl^- (mCPP) (σ^- constants = 0.41 and 0.37, respectively) (Hansch and Leo, 1979), the activity of TFMPP was lower. Differences in molecular volume and hydrophobicity may explain this discrepancy. The hydrophobicity (Π^- constant) (Fujita *et al.*, 1964) and molecular volume of TFMPP are approximately 1.4 and 1.2 times greater than mCPP. Increasing the hydrophobicity and molecular volume of TFMPP by addition of an aminophenethyl group (PAPP) to the 4-position nitrogen further lowered its potency as an antagonist.

Besides addition at the 4-position nitrogen, the presence of heteroaromatic nitrogen(s) (MK-212 *versus* mCPP) decreased the antagonist activity of the monosubstituted phenylpiperazines. For the fused ring systems, the presence of a heteroaromatic nitrogen had an insignificant effect (quipazine *versus* 2-NP). For the benz-fusion modifications of PP, the rank order of antagonist potencies between PP, 2-NP and 1-NP in human platelets correlates with the rank order of their reported K_i at 5-HT$_2$ cerebral cortical sites (Glennon *et al.*, 1986b).

The heterocyclic piperazines (quipazine and MK-212) and one arylpiperazine with a fused ring system (2-NP) also demonstrated agonist activity. 2-NP showed only erratic agonist activity that could not be quantitated. In contrast, the heterocyclic piperazines consistently amplified aggregation. Their potencies as weak agonists were comparable, whereas their response amplitudes differed significantly. The lower efficacy of MK-212 may reflect preferential interaction with the low-affinity uncoupled state of the 5-HT$_2$ receptor proposed by Lyon *et al.* (1987) and Glennon *et al.* (1988). The mixed agonist-antagonist actions of quipazine at vascular smooth muscle 5-HT$_2$ receptors have been reported (Cohen *et al.*, 1981). Only the agonist properties of quipazine and MK-212 have been reported in the CNS 5-HT$_2$ receptor system (Glennon *et al.*, 1986b; Conn and Sanders-Bush, 1987).

The aggregation studies with mCPP and MK-212 implicate the heteroaromatic nitrogens in conferring agonist properties at the platelet 5-HT$_2$ site. The nonheterocyclic analogue (mCPP) is exclusively a potent antagonist, whereas MK-212 has agonist activity. Likewise, quipazine with the quinoline nitrogen had consistent agonist activity with higher maximal responses than the analogue without the aromatic nitrogen (2-NP). This may be attributed to the increased facility with which the heteroaromatic nitrogen(s) of either MK-212 or quipazine coordinate a proton transfer by formation of hydrogen bonds. It has been postulated that proton transfer is important in simulated 5-HT congener-receptor interactions (Weinstein *et al.*, 1987). Once a heteroaromatic nitrogen is present, benz-fusion at the c face of PP enhances the amplitude of the agonist response. Quipazine, with the aromatic ring

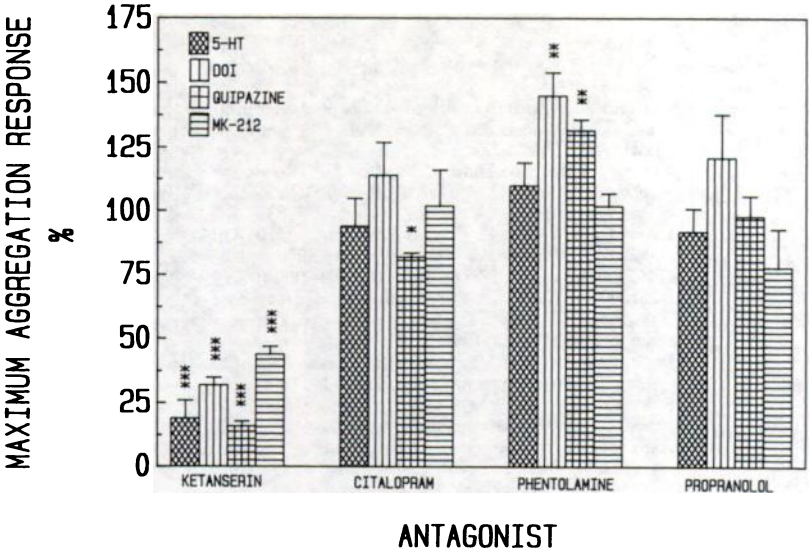

Fig. 3. Specificity of quipazine, MK-212 and DOI for platelet 5-HT$_2$ receptors. Values are percentage ± S.E. of maximum aggregation (100% determined in the absence of antagonist); $n = 3$. Values that differ significantly from 100% are indicated (*$P < .05$, **$P < .01$, ***$P < .001$), as determined by an unpaired two-tailed t test. Antagonists were preincubated for 4 min before addition of agonist/ADP (0.25 μM) mixture. Concentrations of agonists used were 13 μM 5-HT, 13 μM DOI, 90 μM quipazine, 90 μM MK-212. Concentrations of antagonists used were 1 μM ketanserin, 1 μM citalopram, 1 μM phentolamine, and 800 nM propranolol.

fusion at this face, evoked a significantly higher response than MK-212.

The potent platelet 5-HT agonists chosen for comparison with the heteroaromatic piperazines included drugs from two structural groups of 5-HT ligands, the indoleamines and the phenylalkylamines. Despite an apparent binding selectivity for 5-HT$_1$ sites in the CNS, the indoleamines have been characterized as platelet agonists (Laubscher and Pletscher, 1979). Conversely, DOI has been characterized as a selective high-affinity 5-HT$_2$ drug in the CNS (Shannon *et al.*, 1984; Glennon *et al.*, 1986a) with activity in feline platelets (Seggel *et al.*, 1987). The Hill coefficient for DOI in this study (0.58) and the reported values for the racemic mixture or stereoisomers of this compound in ^{3}H-ketanserin competition studies with CNS membranes (0.66–0.73) (Glennon *et al.*, 1986a; Shannon *et al.*, 1984) are consistently less than unity. In contrast, the weak arylpiperazine agonists in this study had Hill coefficients of ~1.5. The variations in slope among the different agonist compounds may reflect multiple states of 5-HT receptor agonist sites or receptor coupling in human platelets. These possibilities cannot be addressed, however, by the present data.

Of the drugs shown to be high-affinity agonists with variable selectivity at CNS 5-HT$_1$ receptors (DPAT, TFMPP, mCPP and PAPP) (Glennon *et al.*, 1986b; Glennon, 1987; Asarch *et al.*, 1985; Fuller *et al.*, 1986; Fuller *et al.*, 1981), none showed any agonist interactions with human platelets in this study. Significantly, they demonstrated different degrees of antagonism. This reversal of ligand action between 5-HT$_1$ and 5-HT$_2$ sites is also seen in vascular smooth muscle preparations (Cohen and Fuller, 1983; Cohen *et al.*, 1986) and in the CNS using phosphoinositide metabolism (Conn and Sanders-Bush, 1987). The extremely low potency of DPAT in the platelet system agrees with an earlier report (Victorzon *et al.*, 1986) and emphasizes its 5-HT$_{1a}$ site selectivity (Arvidsson *et al.*, 1981; Middlemiss and Fozard, 1983). In the case of PAPP, the data presented here do not support the earlier suggestion that PAPP is a 5-HT$_{1a}$ site selective drug (Asarch *et al.*, 1985). The present data on the functional activity of the phenylpiperazines, mCPP, TFMPP, PAPP, strengthen the idea that this group of compounds may generally interact with both 5-HT$_1$ and 5-HT$_2$ receptors, having agonist activity at 5-HT$_1$ sites and antagonist activity at 5-HT$_2$ sites.

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