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CHARACTERIZATION OF RECEPTORS MEDIATING 5-HYDROXYTRYPTAMINE- AND CATECHOLAMINE-INDUCED PLATELET AGGREGATION, ASSESSED BY THE ACTIONS OF α- AND β-BLOCKERS, BUTYROPHENONES, 5-HT ANTAGONISTS AND CHLORPROMAZINE

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1 Blood platelets from normal human volunteers were isolated and aggregated *in vitro* with 5-hydroxytryptamine (5-HT), noradrenaline (NA), dopamine and N-dimethyldopamine (DMDA).

2 The effects of 5-HT antagonists, α- and β-adrenoceptor blocking agents, butyrophenones and chlorpromazine upon aggregation induced by the four amines were investigated.

3 Only the 5-HT antagonists, chlorpromazine and spiroperidol were potent inhibitors of 5-HT-induced aggregation, and only phentolamine was a potent inhibitor of the catecholamine-induced aggregation.

4 Evidence was obtained for two populations of receptors, one for 5-HT and one for the three catecholamines.

Introduction

It is well established that the monoamines 5-hydroxytryptamine (5-HT), noradrenaline (NA) and adrenaline cause platelet aggregation (Mitchell & Sharp, 1964; O'Brien, 1964).

Gaddum & Picarelli (1957) showed that the 5-HT receptors of the smooth muscle of the guinea-pig ileum are of two kinds: those blocked by dibenzyline, dihydroergotamine and lysergic acid diethylamide (LSD), the D type receptor and those blocked by atropine, morphine and cocaine, the M type receptor. Michal (1969) found that in sheep platelet-rich plasma, 5-HT-induced aggregation is inhibited by LSD and dibenzyline in dose-dependent fashion but not by morphine or cocaine, suggesting that the platelet 5-HT receptors are of the D type. Born, Juengjaroen & Michal (1972) obtained similar results with human platelets.

Adrenaline and noradrenaline are known to produce platelet aggregation mediated by α-adrenoceptors (O'Brien, 1964) since phentolamine blocks the response whilst propranolol (β-adrenoceptor blocker) has no effect (Mills & Roberts, 1967a).

Earlier studies in this laboratory have also shown that dopamine and N-dimethyldopamine (DMDA) cause human platelets to aggregate *in vitro* (Boullin, Green & Grimes, 1975b). Whether these amines are acting on separate receptors or on either 5-HT or α-adrenoceptors has not been established.

In the present study we have compared the potencies of nine potential antagonists on platelet aggregation induced by 5-HT, NA, DMDA and dopamine. Evidence is presented for two populations of separate receptors, one for 5-HT and one for the catecholamines NA, dopamine and DMDA.

Methods

Blood, obtained from 15 normal volunteers was collected into 3.8% trisodium citrate. Platelet-rich plasma (PRP) was prepared and platelet aggregation was carried out as previously described (Boullin, Grahame-Smith, Grimes & Woods, 1975a). Samples of PRP (1 ml) were incubated at 37°C for 2 min, trans-

ferred to a platelet aggregometer (Corning-EEL, Evans Electroselenium Ltd, Halstead, Essex) stirred at 800 rev/min and the aggregation responses were recorded on a Tekman TE200 recorder (Tekman Electronics Ltd, 120 Churchill Road, Bicester). Concentrations of antagonists tested were varied from 1 nmol/litre to 200 μmol/litre and these were added 3 min before the agonist. The antagonists tested were methysergide, LSD and BW501C67, all potent 5-HT inhibitors, the dopamine-receptor blockers, haloperidol and spiroperidol, the adrenoceptor antagonists phentolamine (α-blocker) and propranolol (β-blocker), and chlorpromazine which is known to block 5-HT-induced aggregation *in vitro* (Mills & Roberts, 1967b) and enhances aggregation *in vivo* (Boullin, Woods, Grimes, Grahame-Smith, Wiles, Gelder & Kolakowska, 1975c). None of the antagonists induced aggregation when added alone.

Experiments were carried out with constant concentrations of agonists (5-HT 20 μmol/litre, NA 20 μmol/litre, DMDA 20 μmol/litre and dopamine 50 or 200 μmol/litre) which we find produce maximal aggregation responses. Each aggregation record was measured as the total change in optical density (ΔOD, arbitrary units) with a Kent compensating polar planimeter. From these results the percentage inhibition in relation to the control response was obtained for each concentration of antagonist. Log dose-response relationships were then plotted as percentage inhibition of aggregation (ordinate scale) against log antagonist concentration (abscissa scale).

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate, noradrenaline bitartrate and dopamine hydrochloride (Sigma Chemical Co.); haloperidol and spiroperidol (donated by Janssen Pharmaceutical Ltd); methysergide and lysergic acid diethylamide (donated by Sandoz Products Ltd); phentolamine hydrochloride (Ciba Laboratories); (+)- and (−)-propranolol (donated by ICI); chlorpromazine hydrochloride (May & Baker, Ltd.); BW501C67 (donated by Wellcome Research Laboratories) and N-dimethyldopamine (donated by Dr J. Kitzen, Department of Medical Chemistry and Pharmacology, University of Iowa, Iowa City, U.S.A.).

Results

In all the blood platelet samples tested, 5-HT produces a transient reversible aggregation (Boullin *et al.*, 1975b) whereas NA induces irreversible aggregation in two phases (Mills & Roberts, 1967a). DMDA produces irreversible responses similar to those of NA, at similar concentrations (see Boullin & Grimes, 1976). Responses to dopamine were much harder to produce and frequently high concentrations of 200 μmol/litre only produced small responses (Boullin & Grimes, 1976). Braunstein *et al.* (1977) have also found that dopamine induces platelet aggregation in similar concentrations (see Discussion). All the amines were made up fresh daily in ascorbic acid (1 mg/ml). The inhibitory effects of all the antagonists of amine-induced aggregation are expressed graphically in terms of inhibition of ΔOD (ordinate scale) plotted against logarithm of antagonist concentration (abscissa scale). Thus we can compare the relative potencies of the various inhibitors on the basis of IC_{50} values even though the graphs are not all parallel (see Discussion). Table 1 shows the absolute values in μmol/litre of IC_{50} values (concentration of antagonist producing 50% inhibition) for each amine-antagonist interaction.

Inhibition of 5-hydroxytryptamine-induced aggregation

Figure 1 shows that the inhibitors of 5-HT-induced aggregation fall into two groups. The first group consists of the most potent inhibitors which are effective at doses of 1 nmol/litre to 1 μmol/litre, and these are LSD, methysergide, BW501C67 and surprisingly, spiroperidol. The latter is the most potent, being marginally more potent than methysergide and BW501C67.

The second group consists of the least potent antagonists which are effective only at concentrations greater than 10 μmol/litre and this group contains

Table 1 IC_{50} values for inhibition of amine-induced platelet aggregation

	IC_{50} values (μmol/litre)			
Antagonist	*5-HT*	*NA*	*DMDA*	*DA*
Spiroperidol	0.018	20	44	14
Methysergide	0.074	26	25	7
BW501C67	0.034	8.8	11	5
LSD	0.15	—	6.4	—
Chlorpromazine	3.4	13.5	22	23
Haloperidol	15	16	26	23
(−)-Propranolol	56	28	45	14
(+)-Propranolol	33	31	46	28
Phentolamine	31	0.22	0.1	0.14

The values are based on measurements of platelet aggregation expressed in terms of changes in optical density of platelet-rich plasma (ΔOD, see Methods section). 5-HT: 5-hydroxytryptamine; NA: noradrenaline, DMDA: N-dimethyldopamine; DA: dopamine.

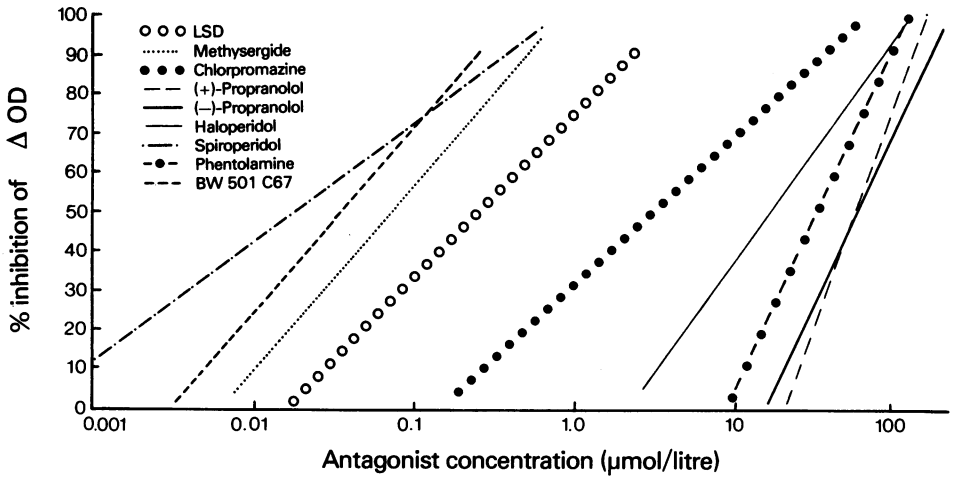

Figure 1 Dose-response relationships for inhibition of 5-hydroxytryptamine-induced platelet aggregation by various drugs. Inhibition of aggregation (% inhibition of ΔOD, ordinate scale) is plotted against log drug concentration (antagonist concentration, abscissa scale). The lines shown are based on experimental data obtained from 4 subjects. They are calculated by the method of least squares. For reasons of clarity, actual experimental values are not shown. The standard errors of the mean of the observation were in the range of 0–50%. Details for calculation of ΔOD and initial rate are described in the Methods section.

(+) and (−)-propranolol, phentolamine, and haloperidol. Chlorpromazine is intermediate in activity between these two groups being effective in concentrations from 0.1 μmol/litre to 10 μmol/litre.

Inhibition of noradrenaline, N-dimethyldopamine and dopamine-induced aggregation

Figures 2, 3 and 4 show log dose-response curves for inhibition of NA, DMDA and dopamine-induced aggregation respectively. The data for inhibition of aggregation of the three amines again can be divided into two groups on the basis of concentrations producing 50% inhibition. Phentolamine is clearly the most potent inhibitor producing complete inhibition at about 1 μmol/litre. All other drugs are effective only at much higher concentrations, 10 μmol/litre to 200 μmol/litre.

Discussion

In order to compare potencies the slopes of the graphs should be the same. The fact that the graphs are not parallel may indicate involvement of different receptor populations. However, a comparison of values for 50% inhibition can be made to show the difference in potency between the drugs. Based on the relative potencies of the various inhibitors of aggregation responses, the data presented here suggest that there are two populations of receptors mediating amine-induced platelet aggregation responses: D-type 5-HT receptors display preferential sensitivity to the 5-HT blockers LSD, methysergide, and BW501C67 as shown in Table 1. LSD and methysergide are well-known 5-HT antagonists (Aghajanian and Haigler, 1974; Bradley & Briggs, 1974) and BW501C67 is also known to be a 5-HT antagonist (Mawson & Whittington, 1970) which in this system is nearly equipotent to methysergide.

Earlier work by Born *et al.* (1972) has shown the effects of several 5-HT analogues on platelet shape change, aggregation and uptake. Some of these analogues were potent aggregators. They behaved like 5-HT itself; they were self-inhibitory and their responses were blocked by methysergide. Other analogues were specific inhibitors of 5-HT-induced shape-change and aggregation, although less potent than methysergide. This suggests there is only one type of receptor, of low structural specificity, mediating 5-HT-induced aggregation.

The second population of receptors are most sensitive to the α-blocker phentolamine. All three catecholamines, NA, DMDA and dopamine, thus stimulate α-receptors. There is no evidence for specific dopamine-sensitive, haloperidol-blocked receptors, such as occur in brain neurones (Kebabian, Petzold & Greengard, 1972). Clearly dopamine has purely an action on α-adrenoceptors, like NA, in this system. Similarly

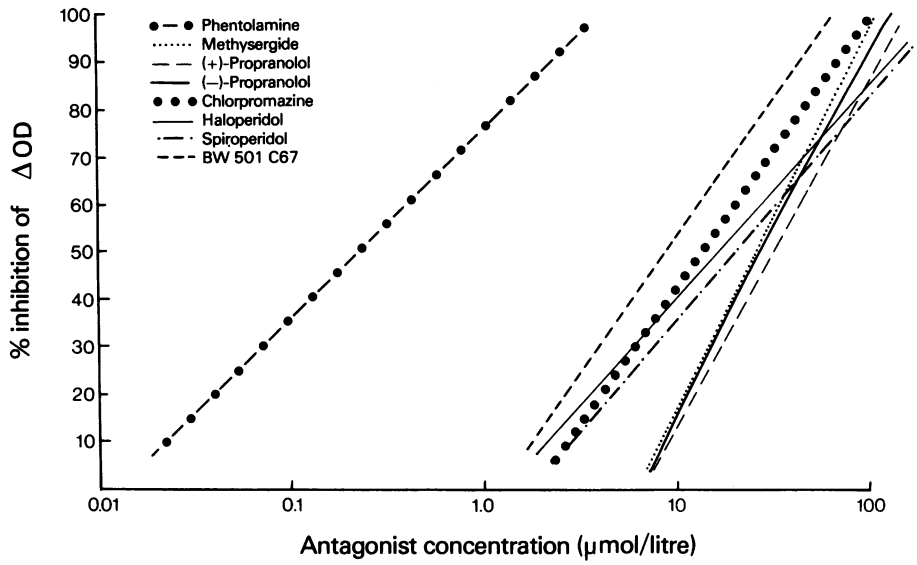

Figure 2 Dose-response relationships for inhibition of noradrenaline-induced platelet aggregation by various drugs. Inhibition of aggregation (% inhibition of ΔOD, ordinate scale) is plotted against log drug concentration (antagonist concentration, abscissa scale). The data are presented in the same format as in Figure 1.

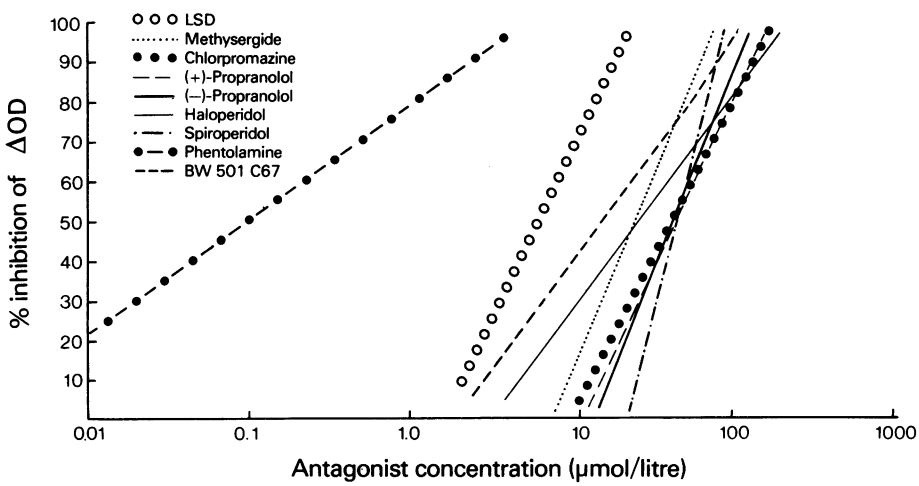

Figure 3 Dose-response relationships for inhibition of N-dimethyldopamine-induced platelet aggregation by various drugs. Inhibition of aggregation (% inhibition of ΔOD, ordinate scale) is plotted against log drug concentration (antagonist concentration, abscissa scale). The data are presented in the same format as in Figure 1.

dopamine also acts on α-receptors in the peripheral vascular bed (Goldberg, 1972) but on the other hand haloperidol-sensitive receptors are present in cerebral arteries (Boullin, Adams, Mohan, Green, Hunt, Du Boulay and Rogers, 1977).

Braunstein, Sarji, Kleinfelder, Schraibman, Colwell & Eurenius (1977) have seen similar results with phentolamine, haloperidol and propranolol against dopamine potentiation of adenosine diphosphate (ADP) aggregation with a slightly different experi-

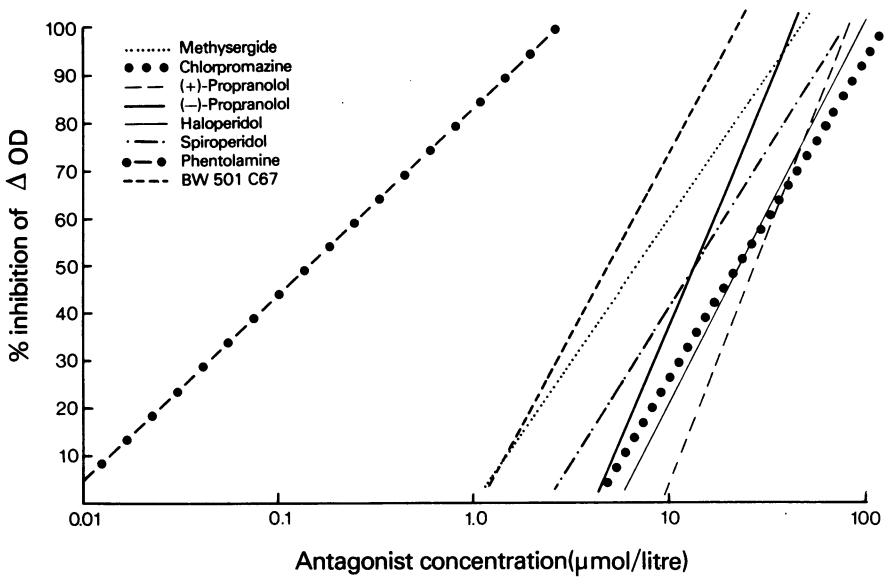

Figure 4 Dose-response relationships for inhibition of dopamine-induced platelet aggregation (% inhibition of ΔOD, ordinate scale) is plotted against log drug concentration (antagonist concentration, abscissa scale). The data are presented in the same format as in Figure 1.

mental protocol. In their experiments dopamine in low doses (which produced slight aggregation) enhances ADP aggregation. This effect is blocked by phentolamine in concentrations of 0.5 μg/ml (1.8 μmol/litre) or above, but is not affected by propranolol or haloperidol. The concentration of phentolamine used in the experiments by Braunstein *et al.* (1977) is comparable to that which inhibits dopamine-induced platelet aggregation in our experiments (1 μmol/litre). They suggest that there may be two receptor sites for dopamine, a high affinity α-receptor, inducing platelet aggregation and a low affinity site, possibly a β-receptor. We have found that dopamine-induced irreversible aggregation can be evoked in some volunteers, and we have confirmed and extended the findings of Braunstein *et al.* that the actions of dopamine and DMDA are mediated by α-receptors.

Our view is that inhibition by propranolol of platelet aggregation is not mediated via a specific β-receptor. The (+)- and (−)-isomers show similar potency, and the drug is only active in high concentrations known to have local anaesthetic effects (Barrett & Cullum, 1968). Also haloperidol and chlorpromazine, which have diverse chemical structures have comparable potency to propranolol against NA, dopamine and DMDA.

Chlorpromazine does have some anti-5-HT actions in this system. It is of intermediate potency against 5-HT-induced aggregation, being 23 to 188 times less potent than spiroperidol, BW501C67, methysergide and LSD, and is 10 to 16 times more potent than (+)- and (−)-propranolol, phentolamine and haloperidol. Anti-5-HT actions of chlorpromazine have also been described in some central and peripheral systems (Grahame-Smith, 1971; Boullin & Grimes, 1976).

Most surprisingly, spiroperidol was an extremely potent inhibitor of 5-HT-induced platelet aggregation, in contrast to the closely related compound, haloperidol, which is 840 times less potent.

Consequently it is concluded that drug specificity in blocking receptors mediating human platelet aggregation is only relative, i.e. methysergide which is a 'classical' 5-HT inhibitor, will block catecholamine responses at concentrations up to 350 times greater than against 5-HT. BW501C67 also has comparable anticatecholamine activity, but is 100 to 350 times more potent on 5-HT receptors than catecholamine receptors. Conversely phentolamine is 140 times less potent against 5-HT than against NA-induced aggregation.

Ball, Boullin & Glenton (1977) have demonstrated interactions between the two populations of receptors mediating amine-induced aggregation responses. Thus catecholamines can potentiate 5-HT aggregation responses, overcome the self-inhibitory effect of 5-HT and even cause 5-HT to produce irreversible aggregation. This interaction may occur at the drug receptor binding site or at a stage subsequent to this, by affecting platelet aggregation trigger mechanisms.

In spite of these interactions our data show two distinct populations of receptors mediating aggregation. This conclusion is based on the comparative inhibitory effects of methysergide, BW501C67 and phentolamine. Our view is that there is a 5-HT receptor with low structural specificity (Born *et al.*, 1972) and a catecholamine α-adrenoceptor.

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