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THE BINDING OF HALOPERIDOL TO HUMAN BLOOD PLATELETS AND INTERACTIONS WITH 5-HYDROXYTRYPTAMINE AND DOPAMINE

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1 The binding of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT), [^{3}H]-haloperidol and [^{3}H]-dopamine to human blood platelets was investigated and the effects of unlabelled haloperidol on [^{3}H]-5-HT binding and (+)- and (−)-butaclamol on [^{3}H]-haloperidol were studied.

2 Scatchard analysis did not show any specific binding of [^{3}H]-haloperidol or [^{3}H]-dopamine to platelets, but two binding sites were identified for binding of [^{3}H]-5-HT.

3 Unlabelled haloperidol reduced the binding of 5-HT in concentrations comparable to those inhibiting 5-HT-induced platelet aggregation; (+)- and (−)-butaclamol did not affect the binding of [^{3}H]-haloperidol.

4 It is concluded that haloperidol binding represents saturation of the platelet membrane, and that the platelet is not a suitable model for investigation of dopamine-drug interactions.

Introduction

Although haloperidol is considered to be the classic anti-dopamine drug in regard to actions of dopamine on the central nervous system it does possess other properties. For example it inhibits 5-hydroxytryptamine (5-HT)-induced aggregation of normal human blood platelets (Boullin & Glenton, 1978).

Recently, binding of [^{3}H]-5-HT to platelets has been demonstrated in rat platelets (Drummond & Gordon, 1975a) and human platelets (Boullin, Glenton, Molyneux, Peters & Roach, 1977). This has led to a partial characterization of the 5-HT receptors on the platelet surface (Drummond & Gordon, 1975a), and in particular to the suggestion that there are at least two 5-HT binding sites with different affinities and binding capacities. Inhibitor studies on rat platelets (Drummond & Gordon, 1975a) have suggested that one site (site 'A') is responsible for platelet aggregation, whilst binding of 5-HT to the other site (site 'B') leads to uptake of 5-HT into the platelet.

The observation (Boullin & Glenton, 1978) that haloperidol will inhibit 5-HT-induced aggregation led us to investigate the interaction of haloperidol with human platelets. In particular, we have investigated whether binding of haloperidol to the platelet involved binding to specific receptors, either 5-HT or dopamine receptors, and whether this could account for the inhibition of 5-HT-induced aggregation.

Another reason for our investigation of the platelet-haloperidol interaction is that binding of haloperidol has been demonstrated to dopamine-sensitive cells of the calf and rat caudate nucleus and rat corpus striatum (Seeman, Chau-Wong, Tedesco & Wong, 1975; Burt, Enna, Creese & Snyder, 1975). As platelets are aggregated by dopamine and this response is blocked by haloperidol (Boullin, Green & Grimes, 1975) it was important to know whether platelets possess specific dopamine receptors sensitive to haloperidol.

The investigation involved binding of [^{3}H]-5-HT, [^{3}H]-dopamine, and [^{3}H]-haloperidol separately to human platelets and the effects of unlabelled haloperidol and (+)- and (−)-butaclamol on this binding. We have found that at low concentrations (1 to 200 nmol/l), haloperidol does not appear to bind specific receptors on the platelet surface. At higher concentrations (30–50 μmol/l), haloperidol interferes with the binding of 5-HT to the platelet.

Methods

Blood was collected into disodium edetate (EDTA) as described by Boullin & O'Brien (1969) and Boullin & Green (1972). After centrifugation at 200 *g* for 15 min, platelet rich plasma (PRP) was separated. The platelet count was obtained by use of a Coulter Counter Industrial D Model.

The procedure for measuring the binding of [^{3}H]-5-HT to human blood platelets was based on

the method used by Drummond & Gordon (1975a) to demonstrate binding of [^{3}H]-5-HT to rat platelets. In the present experiments [^{3}H]-5-HT, [^{3}H]-haloperidol and [^{3}H]-dopamine were diluted with EDTA (0.1 g/100 ml of 0.9% w/v NaCl solution, adjusted to pH 7.4) to give a range of concentrations of 0.1 to 10 pmol/μl; 10 μl of the appropriate dilution was added to 1 ml of PRP to give a final concentration of 1 to 200 pmol/ml. These concentrations are in the same range as those used by Drummond & Gordon in their studies on 5-HT binding to rat platelets. (+)- and (−)-Butaclamol were diluted in distilled water to give a final concentration in PRP of 1 nmol/ml.

Aliquots (1 ml) of PRP were cooled to 0°C. Labelled ligand of the required concentration was added followed by either 0.9% w/v NaCl solution (saline) or excess unlabelled ligand, and the tubes were incubated on ice for 45 s to 4 minutes. After centrifugation at 20000 *g* for 2 min, the supernatant was decanted and the pellet washed with EDTA and sonicated (20000 MHz, 5 s pulses for a total of 30 s) in 1 ml EDTA. Aliquots of supernatant and pellet were counted in Instagel in a Beckman LS-250 liquid scintillation counter. When inhibitors were used these were added to PRP at 0°C, 3 min before addition of the appropriate labelled ligand.

The experiments were carried out on blood from 1 male and 3 female normal volunteers aged 20 to 23 years. The data shown in Results are based on mean values from 3 or 4 experiments unless stated otherwise. The standard error of the mean of the observations did not exceed 12%.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate and dopamine hydrochloride (Sigma Chemical Co.), [^{3}H]-5-hydroxytryptamine creatinine sulphate (14 Ci/mmol) and [^{3}H]-dopamine hydrochloride (6.2 Ci/mmol) (Radiochemical Centre, Amersham, Bucks), unlabelled and [^{3}H]-haloperidol donated by Janssen Pharmaceutical Ltd.; (+)- and (−)-butaclamol donated by Dr Leslie Iversen, MRC Neuropharmacology Unit, University of Cambridge.

Results

The results have been expressed as amount of ^{3}H-ligand bound as a proportion of the total ^{3}H-ligand added. All results have been corrected for the platelet count, and are expressed per 10^8 platelets. These data were then plotted as a Scatchard analysis where bound/free (B/F) is plotted on the ordinate scale against Bound (B) (pmol/ml) on the abscissa scale. The intercept on the y axis is C/*K*a where C = capacity and *K*a = affinity constant and the x intercept = C. From the values of the two intercepts *K*a can be calculated.

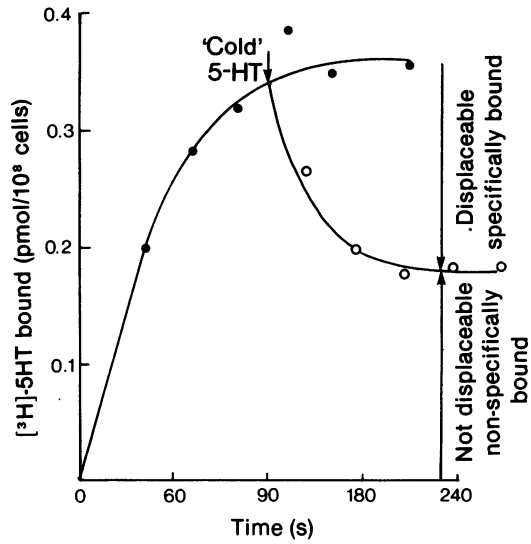

Figure 1 Time course of binding of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) and displacement by nonradioactive 5-HT. Platelets were incubated with [^{3}H]-5-HT 10 nmol/l for 45–210 s at 0°C (●). After 90 s incubation, non-radioactive 5-HT 100 μmol/l was added to some samples (○) and specific and non-specific binding of [^{3}H]-5-HT were determined according to the criterion of displacement of radioactive by non-radioactive 5-HT (see Results and Drummond & Gordon, 1975a). Each point is the mean of 9 replicates obtained in 3 experiments.

5-Hydroxytryptamine binding to platelets

Figure 1 shows the time course of 5-HT accumulation by platelets incubated at 0°C with [^{3}H]-5-HT 10 nmol/litre. The data are plotted as pmol of [^{3}H]-5-HT bound per 10^8 cells (ordinate scale) against incubation time (abscissa scale). The figure shows that binding is very rapid and reaches equilibrium after about 150 seconds. At the arrow, after 90 s incubation, non-radioactive (cold) 5-HT was added in a concentration of 10 mmol/litre. This concentration of non-radioactive material is 10^6 times in excess of the concentration of radioactive 5-HT. The procedure caused a very rapid displacement of a proportion of the labelled compound; 60 s after addition of non-radioactive ligand the amount of radioactive 5-HT remaining in the platelets remained constant, and there was no further displacement. Figure 1 shows that a maximum of approximately 50% of the radioactive 5-HT was displaced by the non-radioactive compound. The displaceable proportion of [^{3}H]-5-HT represents the binding of 5-HT to specific

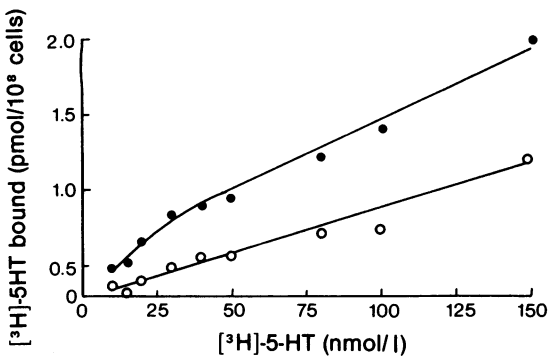

Figure 2 Binding of [³H]-5-hydroxytryptamine ([³H]-5-HT) to human platelets. Platelets were incubated with [³H]-5-HT (10 to 150 nmol/l) for 3 min at 0°C. At the end of incubation, total [³H]-5-HT binding was determined (●) and specific binding (○) by addition of 100 μmol/l unlabelled ligand as discussed in Methods. Each point is the mean of 9 replicates obtained in 3 experiments.

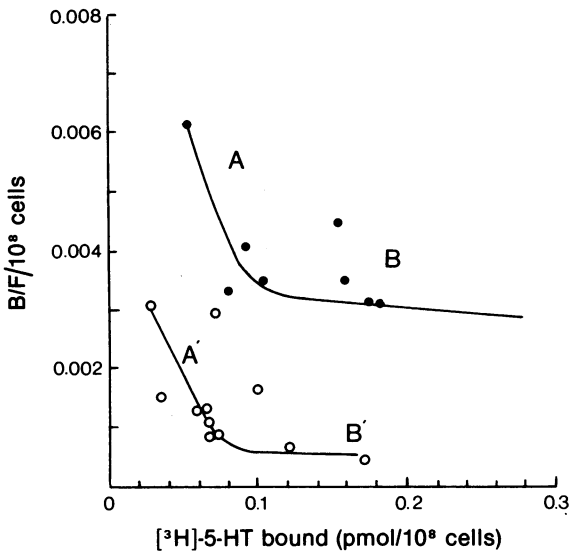

Figure 3 Specific 5-hydroxytryptamine (5-HT) binding by platelets, and inhibition by haloperidol. Platelets were incubated with haloperidol 25 μmol/l for 3 min at 0°C (○) before addition of [³H]-5-HT 1–200 nmol/l for 3 min incubation at 0°C. Thereafter specific 5-HT binding was determined as described in Methods. The data are plotted in Scatchard format (B/F per 10^8 cells, ordinate scale; 5-HT bound, pmol/10^8 cells, abscissa scale). Each point is the mean of 9 replicates obtained with platelets from 3 subjects.

binding sites, which at 37°C are involved in various parameters of platelet function including change in shape, aggregation and intracellular 5-HT transport (Drummond & Gordon, 1975a).

Figure 2 shows the relationship between [³H]-5-HT bound and the initial plasma concentration at the beginning of incubation. It is clear that except at extremely low substrate concentrations (below 25 nmol/l) the proportion of 5-HT specifically bound is approximately constant at 50 to 60%.

Figure 3 shows the specific binding of 5-HT to platelets expressed in kinetic terms according to a Scatchard analysis. The data can be resolved into at least 2 exponentials, A and B. Extrapolation of the exponentials to the X and Y axis enable calculations to be made of the affinity and capacity of the 5-HT binding sites.

Platelets appear to have two sites for specific binding of 5-HT. Table 1 shows the binding capacities and affinity constants for the 2 sites. The site represented by exponential A has a high affinity of the order of 10^{-9} mol/l and a capacity in the region of 600 molecules of 5-HT per platelet. The second binding site (B) has a lower affinity approximately 20 times less than that of Site A, but with a capacity 5 times greater. These values compare with those described by Drummond & Gordon (1975a) for 5-HT binding to rat platelets. These workers described three 5-HT binding sites in rat platelets (A, B and C) with respective affinities of 23, 150 and 2000 nmol/l, and respective binding capacities of 660, 1800 and 57,300 molecules per cell.

From values for human platelets given in Table 1 it can be seen that the affinity constants for 5-HT binding to human platelet binding sites are comparable to those described above for rat platelets in respect of sites A and B. With regard to site A, the capacities of human and rat binding sites for 5-HT are very similar. In the case of site B, the binding capacity for human platelets is double the capacity of the rat, while there is no evidence for a very low affinity high capacity site in human platelets, which could be equated with site C in rat platelets (Drummond & Gordon, 1975a).

Table 1 Affinity constant (1/*Ka*) and binding capacity (C) for specific [³H]-5-hydroxytryptamine ([³H]-5-HT) binding to 2 sites in human platelets

	Site A (high affinity, low capacity)	*Site B (low affinity, high capacity)*
Affinity constant (nmol/l)	mean 5.2 s.e. ± 1.1	101 5.4
Binding capacity (molecules/cell)	mean 627 s.e. ± 111	3919 1113

Values are the mean ± s.e. mean of 12 replicates obtained with 4 subjects.

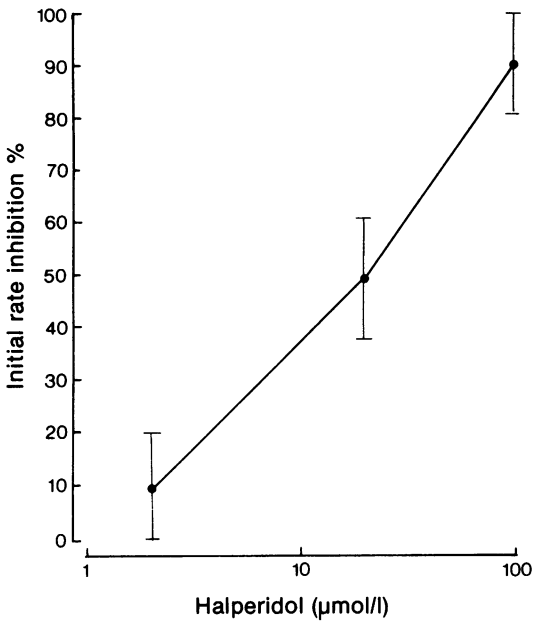

Figure 4 Dose-response relationship for inhibition of platelet aggregation by haloperidol. Aggregation induced by 5-hydroxytryptamine (5-HT) 20 μmol/l was measured as the initial rate. Haloperidol was added to platelet rich plasma 3 min before challenge with 5-HT. Inhibition of aggregation (initial rate, ordinate scale) is plotted against haloperidol concentration (μmol/l, abscissa scale). Values are the mean of 6 observations with 4 subjects, vertical lines show s.e.means. Platelet aggregation was studied as described previously (see Boullin, Green & Grimes, 1975).

Figure 4 shows the dose-response relationship for haloperidol inhibition of platelet aggregation induced by 5-HT 20 μmol/litre. The ID_{50} for haloperidol inhibition is 25 μmol/litre.

The effect of haloperidol 25 μmol/l upon specific 5-HT binding by platelets was studied and the results are presented in Figure 3. From the figure it can be seen that haloperidol reduces the binding capacity of both A and B sites without producing any dramatic change in the affinity constants. Thus the data can be described by two exponentials A′ and B′ which have similar slopes to A and B $(-1)/Ka$; the changes in the intercept on the abscissa scale demonstrate diminished binding capacity.

Having demonstrated an interaction between 5-HT and haloperidol involving specific 5-HT binding sites, [^{3}H]-haloperidol was investigated.

Binding of haloperidol to platelets

Figure 5 shows the relationship between [^{3}H]-haloperidol binding and plasma concentration; there is

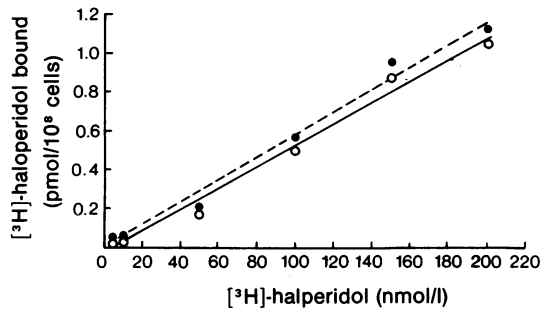

Figure 5 Platelets were incubated with [^{3}H]-haloperidol 5 to 200 nmol/l for 120 s and then the total binding of labelled ligand was determined as described in Methods. Each point is the mean value obtained in 3 replicates. Values (○——○) and (●---●) refer to data from 2 subjects.

no evidence for saturation up to concentrations of 200 μmol/litre. Due to the limited amounts of labelled haloperidol available it was not possible to make an extensive study of the time course of binding of haloperidol or the effects of different concentrations.

It will be noted from a comparison of Figure 5 and Figure 2 that the actual amounts of [^{3}H]-haloperidol bound to platelets are much greater than for 5-HT. Furthermore, [^{3}H]-haloperidol binding could not be displaced by addition of excess non-radioactive haloperidol; thus there was no indication for specific binding of [^{3}H]-haloperidol.

Total [^{3}H]-haloperidol binding was subjected to Scatchard analysis as shown in Figure 6. The data can again be resolved into two exponentials A and B. The binding capacity for Site A is extremely low (see Table 2 below) whereas the capacity for site B is virtually infinitely high since the slope of B is almost parallel with the abscissa scale. It was not possible to obtain sufficient experimental data to determine the statistical significance of the deviation of the line from parallelism.

On the other hand, Site A has an extremely high affinity approaching infinity (slope parallel to ordinate scale). The actual existence of Site A is doubtful since the significance of the deviation of binding from zero could not be assessed. Since Site B has a virtually infinite capacity it is possible that this binding represents solution of haloperidol in platelet membranes and other structures.

In spite of the lack of evidence suggesting that haloperidol was localized in any discrete site in platelets, some experiments were done involving the effects of (−)- and (±)-butaclamol. Seeman *et al.* (1975) used the two isomers of butaclamol to differentiate between specific and non-specific binding of haloperidol to dopamine receptors in the caudate nucleus. (+)-Butaclamol is the therapeutically active form of the neuroleptic drug, and the same isomer displays

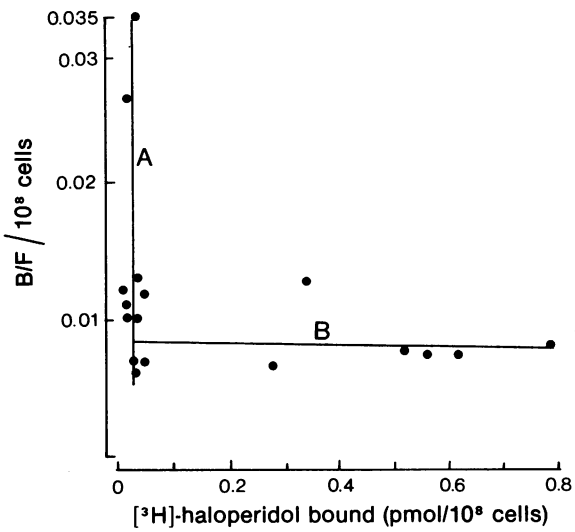

Figure 6 Haloperidol binding to platelets. Results plotted in Scatchard format (B/F per 10^8 cells, ordinate scale; B pmol/10^8 platelets, abscissa scale). Platelets were incubated with [^{3}H]-haloperidol 10 to 200 nmol/l for 3 min at 0°C as described in Methods. Each point is the mean of 6 replicates obtained in two experiments.

a similar pattern of pharmacological activity towards dopamine receptors. Thus (+)-butaclamol blocks [^{3}H]-dopamine binding to specific receptors in the rat caudate nucleus, whereas (−)-butaclamol has no such specific blocking action (Seeman *et al.*, 1975).

In our studies 1 µmol/l of (+)- or (−)-butaclamol added to platelets in plasma at 0°C 3 min before measurement of [^{3}H]-haloperidol binding failed to have any effect.

Dopamine binding to platelets

In view of the interactions between haloperidol and central dopamine receptors in the rat described above, [^{3}H]-dopamine binding to platelets was also investigated.

Table 2 Binding of 5-hydroxytryptamine (5-HT), dopamine and haloperidol to human platelets at a concentration of 100 nmol/litre

Substance	*'Specific' binding* (µmol/10^8 platelets)	*'Non-specific' binding* (µmol/10^8 platelets)	*Total binding* (µmol/10^8 platelets)
5-HT	182	72	254
Dopamine	2.8	6.3	9.1
Haloperidol	0	565	565

The values shown are based on results obtained in 6 experiments for 5-HT, 2 experiments for dopamine and 3 experiments for haloperidol.

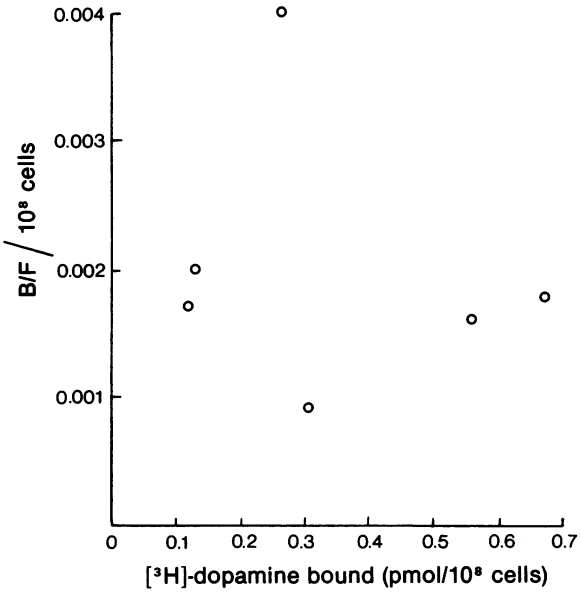

Figure 7 Binding of [^{3}H]-dopamine by platelets. Platelets were incubated with [^{3}H]-dopamine 1 to 200 µmol/l for 3 min at 0°C. At the end of incubation total and specific binding were determined as described in Methods. Each point is the mean of 6 replicates obtained in 2 experiments.

Figure 7 shows the binding of [^{3}H]-dopamine to platelets, the data being plotted according to Scatchard. There are clearly no kinetically identifiable parameters to this binding, at least in the range of concentrations where substantial specific 5-HT binding could be demonstrated (1 to 20 nmol/litre). In the presence of excess unlabelled dopamine small amounts of labelled amine were displaced.

The total and specifically bound 5-HT, dopamine and haloperidol following incubations with 100 µmol/l for 120 s are shown in Table 2.

Haloperidol shows the greatest degree of binding but there is no drug specifically bound; in the case of 5-HT, about 72% is specifically bound. In contrast very little dopamine is bound to platelets and of that amount only 31% can be considered specifically bound. Also previous addition of haloperidol (100 µmol/l) failed to influence [^{3}H]-dopamine binding. This leads to the conclusion that haloperidol and dopamine do not interact on platelet receptors.

Discussion

In contrast to the situation in rat brain, human blood platelets do not appear to have any specific

binding sites for haloperidol although they do have such sites for 5-HT and possibly to a very small degree for dopamine.

We have previously described the daily variation in binding of [^{3}H]-5-HT to platelets from 1 individual with values of approximately 6 and 100.10^{-11} mol ml^{-1} (or 10^{-8} mol l^{-1} in conformity with the units used in this paper) and binding capacities of 4000 and 10,000 molecules/cell for the 2 binding sites. The values given here for 4 subjects are somewhat similar to the earlier data, but it does appear that there is considerable variation between individuals, in addition to daily individual variations (see Boullin *et al.*, 1977).

Regarding dopamine, there is no evidence that it binds to specific receptors upon the platelets which can be identified as a separate receptor population. Although this amine aggregates platelets and the dopamine response is blocked by haloperidol (Boullin *et al.*, 1975) more recent evidence indicates that dopamine stimulates catecholamine α-receptors rather than specific dopamine receptors (Boullin & Glenton, 1978). Much further work is required on dopamine binding to platelets but at least it is possible to conclude that we have not found dopamine-haloperidol interactions comparable to those seen in brain tissue.

On the other hand, haloperidol does display interactions with 5-HT receptors. Haloperidol inhibits platelet aggregation (Boullin & Glenton, 1978) and also reduces the binding capacity of the two 5-HT sites demonstrated in platelets (Figure 3). In spite of this interaction, haloperidol does not appear to bind to platelets by kinetic parameters with finite terms. Although the results can be shown to comply with a Scatchard analysis, the biological significance of these kinetic data is doubtful. Firstly, the binding capacity of the high affinity Site A is so small that the significance of this binding from zero would need to be determined in a large number of experiments. As regards the second, low-affinity site with virtually unlimited capacity, this may represent the physicochemical combination of haloperidol with tissues, since the drug possesses high lipid solubility. It seems likely that the 5-HT-haloperidol interaction in platelets involves non-specific saturation by haloperidol of all platelet proteins.

In conclusion, these experiments show that haloperidol binding with platelets does not involve specific receptor structures comparable to those found in brain tissue. Platelets possess specific 5-HT receptors but haloperidol does not interact with these receptors in any kinetically definable way: the interaction between haloperidol and 5-HT does not occur at the molecular level of 5-HT receptors. Much further work would be required to define the nature of the interaction.

Finally, platelets bound dopamine to a very small degree but there is no interaction between haloperidol and dopamine in this respect. Consequently the platelet cannot be considered as a suitable model system for investigating dopamine-drug interactions at the molecular level, but it may be useful for studying 5-HT-drug interactions, as shown by Drummond & Gordon (1975b) with rat platelets.

References

BOULLIN, D.J. & GLENTON, P.A.M. (1978). Characterisation 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. *Br. J. Pharmac.*, **62,** 537–542.

BOULLIN, D.J., GLENTON, P.A.M., MOLYNEUX, D., PETERS, J.R. & ROACH, B. (1977). Binding of 5-hydroxytryptamine to human blood platelets. *Br. J. Pharmac.*, **61,** 453P.

BOULLIN, D.J. & GREEN, A.R. (1972). Mechanisms by which human blood platelets accumulate glycine, gaba and amino acid precursors of putative neurotransmitters. *Br. J. Pharmac.*, **45,** 83–94.

BOULLIN, D.J., GREEN, A.R. & GRIMES, R.P.J. (1975). Human blood platelet aggregation induced by dopamine, 5-hydroxytryptamine and analogues. *J. Physiol.*, **252,** 46–47P.

BOULLIN, D.J. & O'BRIEN, R.A. (1969). The accumulation of guanethidine by human blood platelets. *Br. J. Pharmac.*, **35,** 90–102.

BURT, D.R., ENNA, S.J., CREESE, I. & SNYDER, S.H. (1975). Dopamine receptor binding in the corpus striatum of mammalian brain. *Proc. natn Acad. Sci., U.S.A.*, **72,** 4655–4659.

DRUMMOND, A.M. & GORDON, J.L. (1975a). Specific binding sites for 5-hydroxytryptamine on rat blood platelets. *Biochem. J.*, **150,** 129–132.

DRUMMOND, A.H. & GORDON, J.L. (1975b). Inhibition of 5-hydroxy-{^{3}H}-tryptamine binding to rat blood platelets by 5-HT antagonists and uptake inhibitors. *Br. J. Pharmac.*, **55,** 257P.

SEEMAN, P., CHAU-WONG, M., TEDESCO, J. & WONG, K. (1975). Brain receptors for antipsychotic drugs and dopamine: direct binding assays. *Proc. natn. Acad. Sci., U.S.A.*, **72,** 4376–4380.

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