aggregation and lysis occurring in the Arthus reaction in the rabbit has been commenced.

Rabbits were immunized using alum precipitated ovalbumin (1.0 mg, s.c.) 6 weeks prior to challenge with aqueous ovalbumin (10 μg or 1 mg) injected s.c. at each of six sites on the back. Thrombocytopenia, measured as a fall in the circulating level of homologous ^{51}Cr-labelled platelets injected (i.v.) 24 h earlier occurred acutely following antigenic challenge. Its magnitude varied with the antigenic dose, so that after 6 mg or 60 μg challenges, the radioactivity in whole blood fell to 24.5 ± 1.9% and 74.3 ± 6.7% of control value, respectively. Similar results were obtained by absolute cell counts but the variation between animals was larger. The thrombocytopenia persisted for 4 h after both doses of antigen. An obligatory role for complement was confirmed since depletion of complement to 10% of control level through the use of anti-complementary factor from Cobra venom was found to result not only in the abolition of the Arthus reaction but in the thrombocytopenia as well.

Sulphinpyrazone (50, and 30 mg/kg), administered as the sodium salt intravenously 1 h before challenge with 60 μg of antigen completely inhibited the thrombocytopenia. Upon challenging with 6 mg of antigen, sulphinpyrazone (50 mg/kg) restored the platelet count significantly ($P < 0.01$) to 44.5 ± 4.9% of control, but a dose of 30 mg/kg was not active. The inhibitory effects persisted for at least 4 h in all cases.

These results confirm that sulphinpyrazone can act relatively quickly to protect platelets against complement-mediated immune lysis in the Arthus reaction, an effect which is possibly analogous to its action in endotoxin shock (Evans & Mustard, 1968) and the Forsmann reaction (Tsai, Taichman, Pulver & Schönbaum, 1973). However, it remains to be established whether such an effect is due to a direct action on platelets or on the endothelium of the vasculature.

References

EVANS, G. & MUSTARD, J.F. (1968). Inhibition of the platelet-surface reaction in endotoxin shock and the generalized Schwartzmann reaction. *J. Clin. Invest.* 47, 31a.

MUSTARD, J.F. & PACKHAM, M.A. (1975). Platelets, thrombosis and drugs. *Drugs*, 9, 19-76.

STEELE, P.P., WEILY, H.S. & GENTON, E. (1973). Platelet survival and adhesiveness in recurrent venous thrombosis. *New Eng. J. Med.*, 288, 1148-1152.

STEELE, P.P., WEILY, H.S., DAVIES, H. & GENTON, E. (1974). Platelet survival in patients with rheumatic heart disease. *New Eng. J. Med.*, 290, 537-539.

TSAI, C.C., TAICHMAN, N.S., PULVER, W.H. & SCHÖNBAUM, E. (1973). A role for platelets in the development of lethal vascular injury during Forsmann shock in guinea pigs. *Am. J. Path.*, 72, 179-200.

Inhibition of 5-hydroxy-[^{3}H]-tryptamine binding to rat blood platelets by 5-HT antagonists and uptake inhibitors

A.H. DRUMMOND* & J.L. GORDON

Department of Pathology, University of Cambridge

Rat blood platelets undergo a shape-change in response to sub-micromolar concentrations of 5-hydroxytryptamine (5-HT), and can also accumulate 5-HT against a high concentration gradient by active transport. We have recently shown that three [^{3}H]-5-HT binding sites exist on intact rat blood platelets (Drummond & Gordon, 1975), and in this present study we investigated the relationships between binding of the two highest affinity sites, production of the 5-HT-induced shape change and the active uptake of 5-HT. The experiments were performed with rat citrated platelet-rich plasma, and all the techniques used have been previously described in detail (Gordon & Drummond, 1974; Drummond & Gordon, 1975).

Table 1 Effects of 5-HT antagonists and uptake inhibitors on the high-affinity binding of [^{3}H]-5-HT to rat blood platelets and upon the platelet shape-change induced by 5-HT

Compound	IC_{50} value (nM) against 5-HT-induced shape-change	[^{3}H]-5-HT binding at 4° C
Pizotifen	1.2	1.3
D-LSD	5.5	1.2
Cyproheptadine	3.0	1.0
Cinanserin	2.8	1.4
Methysergide	5.5	2.0
Xylamidine	16.0	11.0
Chlorpromazine	32.0	24.0
Imipramine	250.0	100.0
Chlorimipramine	400.0	500.0
Lilly 110140 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine	4000.0	700.0
Lilly 103947 3-(p-trifluoromethylphenoxy)-3-phenylpropylamine		

Specific binding of [^{3}H]-5-HT was essentially instantaneous and was directly proportional to the number of platelets. Binding to the highest affinity site (Ka^{-1} = 10 nM) was prevented by 5-HT antagonists such as methergoline (IC_{50} = 0.7 nM). There was good correlation between inhibition of this [^{3}H]-5-HT binding and inhibition of the 5-HT-induced shape change (Table 1). Inhibitors of 5-HT uptake also affected shape change and binding to the highest affinity site, but only at micromolar concentrations. This provides direct evidence in support of the observation that inhibitors of 5-HT uptake can also act as 5-HT antagonists (Domenjoz & Theobald, 1959).

Binding of [^{3}H]-5-HT to the middle affinity site (Ka^{-1} = 0.1 μM) was insensitive to 5-HT antagonists but was blocked by 5-HT uptake inhibitors. There was good correlation between inhibition of 5-HT uptake and inhibition of binding to the middle affinity site for chlorimipramine (IC_{50} value against uptake, 0.2 μM), Lilly 103947 (0.25 μM), Lilly 110140 (0.5 μM), imipramine (0.7 μM), amitriptyline (1.2 μM) and desmethylimipramine (7.5 μM). Our results suggest that the binding of [^{3}H]-5-HT to the highest affinity site is involved in the production of the platelet shape change and that the middle affinity site may be related to the carrier for the active transport of 5-HT.

A.H.D. is an M.R.C. scholar.

References

DOMENJOZ, R. & THEOBALD, W. (1959). Zur Pharmakologie des Tofranil (N-(3-dimethylamino-propyl)-imino dibenzylhydrochlorid). *Arch. int. Pharmacodyn. Ther.*, **120**, 450-489.

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

GORDON, J.L. & DRUMMOND, A.H. (1974). A simple fluorimetric microassay for adenine compounds in platelets and plasma, and its application to studies on the platelet release reaction. *Biochem. J.*, **138**, 165-169.

A study of the binding of drugs of blood constituents

C.J. LIVSEY & G.M. SMITH*

School of Pharmacy, Robert Gordon's Institute of Technology, Aberdeen

This communication reports some preliminary results obtained in the study of the nuclear magnetic resonance spectroscopy (n.m.r.) of compounds that inhibit platelet aggregation.

Platelet suspensions were prepared from citrated rabbit and human blood by a modified procedure of Ardlie & Han (1974). To aid the resuspension of platelets after centrifugation, 1 vol of a 10% sucrose solution was added to 5 vol of platelet rich plasma (PRP). Submaximal aggregation was produced by adenosine diphosphate (ADP) 2 μM and the aggregation was recorded by the turbidimetric method of Born & Cross (1963). The n.m.r. spectra were obtained using a 60 or 100 MHz spectrometer with D_2O as a solvent.

ADP-induced platelet aggregation occurred to the same degree in platelet suspensions made up in water or D_2O. This enabled the NMR spectra of compounds to be studied in washed platelet suspensions without any impairment of the ability of platelets to aggregate.

Dipyridamole, its analogues 2,6-bis(diethanol-amino)-4-piperidinopyrimido-[5,4-d] pyrimidine (RA 233), 4-morpholino-2-piperazine-thiopheno-[3,2-b] pyrimidine (VK 774) and 2-[(2-amino-ethyl)amino] -4-morpholinothiopheno- [3, 2-b] pyrimidine (VK 744) were studied together with AG19417 (CIS 1, 2, 3, 4, 4a 10b-hexahydro-8, 9-dimethoxy-2-methyl-6-phenylbenzo [c] [1,6]-naphthyridine bis hydrogen maleate) (Ott & Smith, 1971) and its 4-acetoaminophenyl analogue (AH21132).

Preliminary binding studies were performed using four or five concentrations of bovine serum albumin (BSA). Dipyridamole and its analogues gave spectra that were unsuitable for quantitative study but the addition of increasing concentrations of albumin did cause the spectral peaks of dipyridamole and its analogues to broaden.

The spectra of AG19417 exhibited single isolated peaks which allowed calculations of relaxation rates to be made. An increase in BSA concentration resulted in a linear increase in the relaxation rates of the three main peaks. The increase for the phenyl group was significantly greater ($P > 0.95$) than the increase for either the two methoxy groups or the *N*-methyl group suggesting that the phenyl group is specifically involved in the binding process.

In washed platelet suspensions (4×10^6 platelets/mm^3) the peaks of both AG19417 and