FOREMAN, J.C., MONGAR, J.L. & GOMPERTS, B.D. (1973). Calcium ionophores and movement of calcium following the physiological stimulus to a secretory process. *Nature, Lond.*, **245**, 249–251.

GORDON, J.L. (1975). Blood platelet lysosomes and their contribution to the pathophysiological role of platelets. In *Lysosomes in Biology and Pathology*, vol. 4, ed. Dingle, J.T. & Dean, R.T. pp. 3–31, North Holland: Amsterdam.

GORDON, J.L., MacINTYRE, D.E. & McMILLAN, R.M. (1977). Stimulation of platelets and macrophages by carrageenin. *Br. J. Pharmac.*, (in press).

HENSON, P.M. (1969). The adherence of leucocytes and platelets induced by fixed IgG antibody or complement. *Immunology*, **16**, 107–121.

HENSON, P.M. (1974). Mechanisms of mediator release from inflammatory cells. In *Mediators of Inflammation*, ed. Weissmann, G., pp. 9–50. Plenum Press: New York and London.

LAY, W.H. & NUSSENZWEIG, V. (1968). Receptors for complement on leukocytes. *J. exp. Med.*, **128**, 991–1009.

UNANUE, E.R. (1976). Secretory function of mononuclear phagocytes. *Am. J. Path.*, **83**, 396–417.

WEISSMANN, G., DUKOR, P. & ZURIER, R.B. (1971). Effect of cyclic AMP on release of lysosomal enzymes from phagocytes. *Nature New Biol.*, **231**, 131–135.

WROBLEWSKI, F. & LA DUE, J.S. (1955). Lactic dehydrogenase activity in blood. *Proc. Soc. Exp. Biol. Med.*, **90**, 210–213.

Binding of 5-hydroxytryptamine to human blood platelets

D.J. BOULLIN, P.A.M. GLENTON, D. MOLYNEUX, J.R. PETERS & B. ROACH

MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE

Drummond & Gordon (1975) described the binding of tritiated 5-hydroxytryptamine ([^{3}H]-5-HT) to rat blood platelets at 4°C. Total and specific binding were measured, the latter being defined as the component of [^{3}H]-5-HT bound which was displaceable by non-radioactive 5-HT. We wished to use human platelets and to determine whether specific [^{3}H]-5-HT binding could be demonstrated. Our aim was then to characterize 5-HT binding sites and assess their relationship to platelet aggregation. The physiological response to specific [^{3}H]-5-HT binding is likely to be platelet aggregation.

Platelet rich plasma (PRP) was prepared in EDTA (Boullin, Green & Price, 1972) and cooled to 2°C. The [^{3}H]-5-HT binding technique was essentially that of Drummond & Gordon (1975); 1–200 nmol/l [^{3}H]-5-HT was incubated with PRP for 45 s to 30 minutes. Thereafter platelets were separated from plasma by centrifugation.

At 2°C [^{3}H]-5-HT was bound extremely rapidly, attaining equilibrium after 120 seconds. 30–50% of [^{3}H]-5-HT was specifically bound being displaced by non-radioactive 5-HT.

Scatchard analysis of values for specifically bound [^{3}H]-5-HT showed at least 2 binding sites: a high affinity (K_1) low capacity (C_1) site, and a low affinity (K_2) high capacity (C_2) site. Individual variations in the values of K and C were measured in several subjects. There was greater variation in K_1 and K_2 than in the capacities of the 2 binding sites (C_1 and C_2). Subject 1 (values are the mean ± s.e. of the mean of 4 determinations made on different days): K_1 $1.58 \pm 0.036.10^{-11}$ mols; C_1 1288 ± 160 molecules/platelet; K_2 $15.1 \pm 2.8.10^{-11}$ mols; C_2 7089 ± 94 molecules/platelet. The capacities of the two binding sites defined in our experiments are similar to two of the three sites described in the rat. The affinity constants of our two specific binding sites are, however greater by about four orders of magnitude.

Subsequent experiments have attempted to measure total and specific [^{3}H]-5-HT binding at 20° and 37°C. To diminish platelet 5-HT transport, PRP was preincubated with metabolic inhibitors, (1 mmol/l N-ethyl maleimide, ouabain, and NaCN), for 30–90 min prior to assessing [^{3}H] 5-HT binding. Under these conditions the values for the affinity constants and binding capacities for specifically bound [^{3}H]-5-HT were comparable to those seen at 2°C.

Preliminary results indicate that the non-displaceable component of total [^{3}H]-5-HT binding may correspond to 5-HT transport.

References

BOULLIN, D.J., GREEN, A.R. & PRICE, K.S. (1972). The mechanism of adenosine diphosphate induced platelet aggregation: binding to platelet receptors and inhibition of binding and aggregation by prostaglandin E_1. *J. Physiol. (Lond.)*, **221**, 415–426.

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