Refractoriness in blood platelets: effect of prior exposure to aggregating agents on subsequent aggregation responses

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Low concentrations of adenosine diphosphate (ADP), 5-hydroxytryptamine (5-HT) or collagen added to stirred platelet-rich plasma (PRP) induce the formation of reversible aggregates which disperse over 2-5 min to yield single platelets again. Following reversible ADP-induced aggregation in human PRP, a second addition of ADP is less effective in causing aggregation (O'Brien, Etherington & Jamieson, 1971).

We have investigated this phenomenon ('refractoriness') in rabbit platelets. All experiments were performed at 37°C and similar results were obtained when either heparin or acid-citrate-dextrose were used as anti-coagulants. Reversible aggregation was induced by ADP (0.5-7 μM) and the responses were measured photometrically (Born, 1962). Ten min later, aggregation responses to 5 μM ADP were reduced, and the degree of refractoriness was proportional to the logarithm of the initial concentration of ADP added. The duration of refractoriness was also concentration-dependent. After prior exposure to 5 μM ADP, platelets were refractory for 50-75 min, while after exposure to 2 μM ADP the refractory period lasted 30-40 minutes. In each case, the responses increased rapidly after the refractory period, returning to the control values after a further 15-20 minutes. ADP did not cause aggregation in unstirred samples, but induction of refractoriness by ADP was the same in stirred and unstirred samples—that is, refractoriness was produced by ADP directly, and was not a consequence of prior aggregation.

Following initial exposure to ADP (5 μM), aggregation responses to collagen (3.5 μg/ml) were also reduced, but aggregation induced by Zymosan (0.2 mg/ml), a preparation of bacterial cell wall polysaccharide, was unaffected, and aggregation responses to 5-HT (10 μM) were increased. Initial exposure to 5-HT (20 μM) depressed subsequent aggregation responses to 5-HT, but aggregation induced by ADP was not significantly altered.

The observation that platelets refractory to ADP are hypersensitive to 5-HT indicates that 5-HT-induced aggregation is not mediated by ADP (Haslam, 1967), and also suggests that 5-HT receptors may be exposed or altered in ADP-refractory platelets. Similarly, the lack of interdependence between aggregation responses to Zymosan and collagen suggests that these agents, although both particulate, have separate sites or modes of action.

The mechanisms responsible for refractoriness in platelets are still unclear, but the differential changes in sensitivity to agonists, after induction of refractoriness by one agent, indicate that refractoriness does not represent a non-specific depression of platelet reactivity. Further study of this phenomenon may provide information about the basic mechanisms involved in platelet aggregation.

References

BORN, G.V.R. (1962). Quantitative investigations into the aggregation of blood platelets. *J. Physiol. (Lond.)*, **162**, 67P.

HASLAM, R.J. (1967). Mechanisms of blood platelet aggregation. In: *Physiology of Hemostasis and Thrombosis.* Ed. Johnson, S.A. & Seegers, W.H. Springfield, Illinois: Charles C. Thomas.

O'BRIEN, J.R., ETHERINGTON, M. & JAMIESON, S. (1971). Refractory state of platelet aggregation with major operations. *Lancet*, ii, 741-743.

Histamine receptors in the cardiovascular system of the cat

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Two receptor populations, defined as H_1- and H_2-receptors (Ash & Schild, 1966; Black, Duncan, Durant, Ganellin & Parsons, 1972) have been described for histamine.

Both H_1- and H_2-receptors have been demonstrated in the cardiovascular system of the rabbit (Black, Owen & Parsons, 1973; Parsons & Owen, 1974), the dog (Parsons & Owen, 1974) and the cat (Folkow, Haeger & Kahlson, 1948; Black *et al.*, 1972). In the rabbit, interaction of histamine with H_1- and H_2-receptors results in pressor and depressor responses respectively, whereas in the