## 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 $\mu$M) and the responses were measured photometrically (Born, 1962). Ten min later, aggregation responses to 5 $\mu$M 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 $\mu$M ADP, platelets were refractory for 50-75 min, while after exposure to 2 $\mu$M 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 $\mu$M), aggregation responses to collagen (3.5 $\mu$g/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 $\mu$M) were increased. Initial exposure to 5-HT (20 $\mu$M) 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.

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## 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

dog and cat both receptors mediate depressor responses.

This communication describes further experiments on the role of $H_1$- and $H_2$-receptors in the depressor response to histamine in the cat.

Experiments have been made in cats, body weight 1.5-3.1 kg, anaesthetized with pentobarbitone sodium 60 mg/kg i.p. Blood pressure was measured from a cannula inserted into the right carotid artery. Histamine was administered by i.v. injection. Mepyramine was administered i.v. 10 min before repeating the doses of histamine. Metiamide (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973) was administered by continuous i.v. infusion for 30 min before and during the injection of histamine.

Histamine, over the dose-range $10^{-10}$ to $10^{-7}$ mol/kg (30 ng/kg to 30 $\mu$g/kg), caused dose-dependent depressor responses. Administration of mepyramine, $2.5 \times 10^{-5}$ mol/kg (1 mg/kg), caused a shift, to the right, in the histamine dose-response curve, with a dose-ratio of less than 10. Administration of larger doses of mepyramine, up to $2.5 \times 10^{-5}$ mol/kg (20 mg/kg), caused no further shift in the dose-response curve. When the maximal blocking effect of mepyramine had been achieved, metiamide, $4 \times 10^{-7}$ and $2 \times 10^{-6}$ (mol/kg)/min (100 and 500 ($\mu$g/kg)/min), caused further parallel, dose-dependent shifts, to the right, of the histamine dose-response curve.

In contrast, metiamide alone, up to $1 \times 10^{-5}$ (mol/kg)/min (2.5 (mg/kg)/min), had no significant effect on the histamine dose-response curve. Administration of mepyramine, $2.5 \times 10^{-6}$ to $8.25 \times 10^{-5}$ mol/kg (1 to 33 mg/kg), during continued infusion of metiamide caused parallel, dose-dependent shifts, to the right, of the dose-response curve. These were bigger than the maximal shifts caused by mepyramine in untreated animals but comparable to the shifts caused by the same doses of mepyramine and metiamide when the order of antagonist administration was reversed.

The results reported in this communication indicate that histamine can interact with both $H_1$- and $H_2$-receptors within the cardiovascular system of the cat. In the cat, like the dog, stimulation of either receptor elicits hypotension. The different effects of the selective antagonists suggest that histamine has different $ED_{50}$ for the two receptors.

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## The inhibition of α-bungarotoxin binding to denervated rat muscle by tubocurarine and other drugs


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The experiments reported here were done in order (a) to test the specificity of α-bungarotoxin (BuTX) binding in skeletal muscle in the light of reports (Berg, Kelly, Sargent, Williamson & Hall, 1972; Porter, Chiu, Wieckowski & Barnard, 1973) that much of the BuTX binding to the endplates cannot be inhibited by (+)-tubocurarine (TC), and (b) to provide information about extrajunctional receptors in denervated muscle for comparison with endplate receptors in normal muscle. Because BuTX combines virtually irreversibly with acetylcholine (ACh) receptors, no inhibition of BuTX binding at all would be expected *at equilibrium*. In this situation it is appropriate to measure the effect of inhibitors on the rate of binding.

The simplest model for inhibition of binding of an irreversible ligand by a fast-acting competitive inhibitor predicts that the rate constant, and, *a fortiori*, the initial rate, will be reduced by a factor