presence of Procion Brilliant Red (1% w/v; -5×10^{-3} M) in the bath had no effect on membrane potential, miniature end-plate potentials, 'input resistance', or action potentials elicited either by indirect stimulation or direct excitation of individual muscle fibres, 1 h after its application.

In another series of experiments individual muscle fibres were injected with Procion Brilliant Red by passing hyperpolarizing direct currents of 40 nA through an intracellular micropipette containing a 4% (w/v) solution of the dye. In these experiments the dye-filled micropipette was used as the current passing electrode when measuring 'input resistance' or generating muscle fibre action potentials. Action potentials were measured immediately following penetration of the dye-filled micropipette and again 1-2 min later.

In many fibres dye injection was associated with a localized swelling of the muscle fibre and sometimes this was accompanied by a rapid fall in membrane potential. In most fibres, however, there was no significant change in any measured parameter. Satisfactory marking of the muscle fibres was attained within 15-20 s of penetration.

The results suggest that Procion Brilliant Red H3BN is a suitable agent for marking individual muscle fibres, and within the context of these experiments, is devoid of appreciable pharmacological activity.

Details of the techniques used to prepare the micropipettes, and of the techniques of injection and post-injection fixation are described elsewhere (Harris & Ribchester, 1975).

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The mechanism of 'adrenaline reversal' in the anaesthetized cat and rabbit

H.C. GRUNDY* & H.F. GRUNDY

Westminster Medical School, London and Department of Pharmacology, University of Cambridge

α-Adrenoceptor blockers share the ability to produce adrenaline reversal in anaesthetized laboratory animals (Dale, 1906; Nickerson, 1949). Adrenaline activates vasoconstrictor (α-) and positive inotropic and chronotropic (β_1-) adrenoceptors which mediate pressor responses, and vasodilator (β_2-)adrenoceptors which are depressor. Therefore, α-blockade will only produce adrenaline reversal if the β_2-effect transcends the β_1-action. This could result from three possibilities:— (1) the β_2-action intrinsically exceeds the β_1-effect; (2) the β_2-response is potentiated by α-blockade; (3) the α-blocker antagonizes the β_1-action.

We examined these hypotheses in 41 adult cats anaesthetized with i.p. chloralose (80 mg/kg) or sodium pentobarbitone (35 mg/kg). In 17 cats, by selective blockade with phentolamine, practolol or propranolol, we determined the contributions of α-, β_1-, and β_2-adrenoceptor responses to the BP changes elicited by i.v. noradrenaline, adrenaline or isoprenaline doses up to 10 μg/kg. The log dose-response curves showed for adrenaline:— firstly, the β_1-threshold is highest; secondly, the β_2-effect often predominates at low doses giving depressor actions but at higher doses the α- and β_1-effects cause a pressor response; thirdly, at all doses and in all cats the β_2-action exceeds the β_1-effect. The α- and β_1-components of noradrenaline are similar to those of adrenaline but β_2-activation is weak (see later). Isoprenaline shows β-effects greater than those of adrenaline: but the α-blocker left the isoprenaline response unchanged which indicates that phentolamine has little significant action upon β-components. In further experiments, using ten different α-blockers, we found that neither β_1-depression nor β_2-potentiation provides a satisfactory explanation for adrenaline reversal.

Adrenaline reversal is invariably associated with a marked degree of vasodilator (β_2-) stimulation.

This activation also explains why noradrenaline is more pressor than adrenaline in the cat—their α- and β_1-actions are similar but usually (see later) only adrenaline has an appreciable depressor (β_2-) influence. Corroborative signs of substantial β_2-adrenoceptor stimulation are the depressor effects of low doses of adrenaline and secondary hypotensive phases following the initial pressor responses to higher doses. With noradrenaline, the above signs only occurred, and even then infrequently, in three cats and one rabbit (see later). In these animals alone noradrenaline reversal sometimes happened (Karim, 1964).

In the rabbit, adrenaline reversal is uncommon (Harvey & Nickerson, 1953) yet β_2-effects (diastolic BP falls) undoubtedly occur with isoprenaline. In 5 out of 8 New Zealand White rabbits (i.p. urethane, 2 g/kg), the β_2-activation produced by adrenaline was negligible and adrenaline and noradrenaline were equipressor. But, in the other 3 rabbits, adrenaline had a stronger β_2-action so that low doses were depressor and pressor effects less than those of noradrenaline: these rabbits were the only ones to show adrenaline reversal.

Therefore, in both cats and rabbits, 'adrenaline reversal' is invariably linked with the extent of β_2-adrenoceptor stimulation, and is explicable solely as a phenomenon of α-adrenoceptor blockade unveiling a β_2-action which intrinsically exceeds the corresponding β_1-effect.

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45-Calcium uptake in rat peritoneal mast cells

J.C. FOREMAN, M.B. HALLETT* & J.L. MONGAR

Department of Pharmacology, University College, London

When sensitized rat peritoneal mast cells are challenged with antigen, the resulting secretion of histamine is dependent upon calcium in the external medium (Foreman & Mongar, 1972). Calcium entry into the cell appears to provide the trigger for histamine release (Foreman, Mongar & Gomperts, 1973). Indirect evidence suggests that calcium entry as well as histamine release is blocked by dibutyryl cyclic AMP (Foreman, Mongar, Gomperts & Garland, 1975).

Changes in the amount of calcium associated with the cell have been measured in a purified suspension of rat peritoneal mast cells from Lister Hooded rats, sensitized with egg albumin and *Bordetella pertussis* adjuvant 2-3 weeks previously. The cells were purified by centrifugation through a human serum albumin gradient (20% and 26% albumin). The pellet contained 70-90% mast cells. After washing, the cells were incubated for 5 min in 100 μl Tyrode solution, containing 45-calcium chloride, above 100 μl silicon oil in a micro-centrifuge tube. The tubes were spun for 30 s at 15,000 *g* to separate the cells from the radioactive medium. The pellet, after removal by freezing and cutting off the bottom of the micro-centrifuge tube, was dissolved in Triton-X–100 and the radioactivity measured by liquid scintillation counting.

On average antigen caused 94 nmol histamine release and 0.3 nmol calcium uptake per million cells. With unstimulated cells the corresponding values were 28 nmol histamine release and 0.55 nmol calcium uptake. When pre-incubated with metabolic inhibitors, antimycin A and cyanide, histamine release was abolished but calcium uptake was only slightly reduced (Figure 1). In the absence of inhibitors part of the calcium uptake is possibly due to the increase in surface area of the cells that accompanies histamine release. When this is inhibited a small inhibition of calcium uptake may occur. With dibutyryl cyclic AMP both histamine release and 45-calcium uptake were inhibited.

These results suggest that the increase in 45-calcium associated with cells after antigen challenge is due to an increase in membrane permeability to calcium. Opening of the calcium