This marked potentiation effect by isoprenaline was also observed in the RPA reaction suggesting that the effect of vasodilatation on plasma exudation outweighed any possible β-adrenoceptor inhibition of endogenous mediator release. The potentiation phenomenon was abolished by locally-injected propranolol, but, using a range of β-adrenoceptor agonists and antagonists, we were not able to distinguish β_1 and β_2 effects.

Unlike in the rabbit, the exudation in RPA reactions of the guinea-pig was inhibited by locally-injected isoprenaline (in spite of an induced increase in blood flow in some experiments). This was not due merely to an inhibition of mediator release, since exudation induced by histamine was also reduced in some experiments, e.g. histamine (1.0 µg/0.1 ml) = 38.4 ± 2.0 µl, isoprenaline (0.5 µg/0.1 ml) = 1.0 ± 0.5 µl, histamine + isoprenaline = 13.1 ± 3.3 µl, $n = 6$ sites. This suggests a β-adrenoceptor inhibitory effect on vessel wall permeability in this species. A similar phenomenon has been observed previously in the hamster using the β-adrenoceptor agonist, terbutaline (Svensjö, Persson & Arfors, 1976), and more recently using the same compound in the guinea-pig (O'Donnell & Persson, 1978).

Inflammatory exudation can be modulated at three levels by adrenoceptor agonists: (a) by affecting mediator release, (b) by affecting vessel wall permeability responses and (c) by increasing or decreasing blood supply. In this study (a) was not clearly distinguished but examples of both (b) and (c) were demonstrated.

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References

O'DONNELL, S.R. & PERSSON, C.G.A. (1978). β-Adrenoceptor mediated inhibition by terbutaline of histamine effects on vascular permeability. *Br. J. Pharmac.*, **62**, 321–324.

SVENSJÖ, E., PERSSON, G.C.A. & ARFORS, K.-E. (1976). Effects of bradykinin and terbutaline on macromolecular leakage and its relation to other microvascular effects. *Microvasc. Res.*, **11**, 425.

WILLIAMS, T.J. (1976). Simultaneous measurement of local exudation and blood flow changes induced by intradermal injection of vasoactive substances using [^{131}I]-albumen and ^{113}Xe. *J. Physiol., Lond.*, **254**, 4–5P.

The effects of different particulate stimuli on the extracellular release of prostaglandins and lysosomal enzymes from mouse peritoneal macrophages *in vitro*

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The presence of macrophages is a characteristic feature of chronic inflammatory lesions. The ability of isolated macrophages to secrete lysosomal enzymes selectively on challenge with particulate inflammatory stimuli may contribute to their role in chronic inflammation and connective tissue damage (Page, Davies & Allison, 1974; Davies & Allison, 1976). In addition, macrophages from inflammatory exudates (Bray & Gordon, 1976) and normal or thioglycollate-stimulated macrophages exposed to zymosan (Davies, Bonney, Dahlgren, Pelus, Kuehl & Humes, 1977) will secrete prostaglandins *in vitro*.

Asbestos, zymosan and immune complexes have all been shown to stimulate the selective release of lysosomal enzymes from macrophages (Davies, Allison, Ackerman, Butterfield & Williams, 1974; Ringrose, Parr & McLaren, 1975; Cardella, Davies & Allison, 1974). However, the concomitant release of lysosomal enzymes and prostaglandins by these agents is not so well documented.

A comparison has been made of the effects of zymosan, asbestos and immune complexes (BSA-rabbit anti-BSA) on lysosomal enzyme (β-glucuronidase) release and prostaglandin production by normal mouse peritoneal macrophages exposed to these agents *in vitro*. Major differences have been observed in the patterns of release induced by the three materials, indicating that lysosomal enzyme release and prostaglandin secretion by phagocytosing macrophages are not directly related.

The effects of some antirheumatic drugs on the release of lysosomal enzymes by zymosan and asbestos have been studied. Gold (sodium aurothiomalate), flurbiprofen and indomethacin increased the intracellular levels of lysosomal enzymes without affecting significantly the absolute amount of enzyme released into the culture medium, irrespective of the stimulant used. In contrast, prednisolone inhibited zymosan-induced but not asbestos-induced lysosomal enzyme release, suggesting a difference in the modes of action of these agents.

These results will be discussed in relation to the effects of these drugs on prostaglandin release from macrophages.

References

BRAY, M.A. & GORDON, D. (1976). Effects of antiinflammatory drugs on macrophage prostaglandin biosynthesis. *Br. J. Pharmac.*, **57**, 466P.

CARDELLA, C.J., DAVIES, P. & ALLISON, A.C. (1974). Immune complexes induce selective release of lysosomal hydrolases from macrophages. *Nature, Lond.*, **247**, 46–48.

DAVIES, P. & ALLISON, A.C. (1976). The macrophage as a secretory cell in chronic inflammation. *Agents and Actions*, **6**, 60–72.

DAVIES, P., ALLISON, A.C., ACKERMAN, J., BUTTERFIELD, A. & WILLIAMS, S. (1974). Asbestos induces selective release of lysosomal enzymes from mononuclear phagocytes. *Nature, Lond.*, **251**, 423–425.

DAVIES, P., BONNEY, R.J., DAHLGREN, M.E., PELUS, L., KUEHL, F.A. & HUMES, J.L. (1977). Recent studies on the secretory activity of mononuclear phagocytes with special reference to prostaglandins. In "*Perspectives in Inflammation. Future trends and developments*", ed. Willoughby, D.A., Giroud, J.P. & Velo, G.P. p. 179–187.

PAGE, R.C., DAVIES, P. & ALLISON, A.C. (1974). Participation of mononuclear phagocytes in chronic inflammatory diseases. *J. Reticuloendothel. Soc.*, **15**, 413–438.

RINGROSE, P.S., PARR, M.A. & McLAREN, M. (1975). Effects of antiinflammatory and other compounds on the release of lysosomal enzymes. from macrophages. *Biochem. Pharmac.*, **24**, 607–614.

Development of a radioreceptor assay for β-adrenoceptor antagonists in plasma

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The effectiveness of β-adrenoceptor antagonists (β-blockers) in the treatment of a number of cardiovascular disorders is well established. However, variations in response to oral β-blockers have been frequently reported and in several cases related to individual variation in absorption and subsequent metabolism (Johnsson & Regardh, 1976). At the present time the assay methods for plasma levels of β-blockers are in some cases relatively insensitive and require tedious prior extraction of plasma samples (Shand, Nuckolls & Oates, 1970; Walle, 1974). In this communication we describe a new radio-receptor assay (RRA), applicable for all β-blockers, that is simple, very sensitive and can be performed on plasma without prior extraction. The principle of the assay depends upon the ability of the drug to compete with a radiolabelled β-adrenoceptor antagonist (−)[^{3}H]-dihydroalprenolol ([^{3}H]-DHA) for β-receptor binding sites on bovine living membranes.

Membranes were prepared from bovine lung parenchyma and binding assays were performed as previously described for the rat lung (Barnett, Rugg & Nahorski, 1978). Specific [^{3}H]-DHA binding to bovine lung membranes (binding displaced by 200 μM (−)isoprenaline) represented 80–90% of the total radioactivity bound and possessed characteristics that suggested that the labelled sites are in all probability the cell surface recognition site of the β-adrenoceptor. Thus the binding was of high affinity, saturable (K_D 0.95 ± 0.06 nM, B_{max} 277 ± 15 fmoles/mg protein) and a large number of β-adrenoceptor agonists and antagonists displaced the binding with affinities that matched their pharmacological potency. Using 200–300 μg of membrane protein and 1–2 nM [^{3}H]-DHA in a total volume of 250 μl (conditions that resulted in 10–20% binding of the ligand) a standard curve for (−)-propranolol demonstrated that the assay was sensitive 100 fmol. Addition of up to 20 μl of drug-free plasma had no effect on the standard curve and the recovery of propranolol (2 pmoles) following addition and incubation with plasma was 93.5%.

To further assess the validity of the RRA, plasma samples were taken from volunteers who had received (±)-propranolol (40 mg) orally and a comparison was made of the RRA and a recently developed radioimmunoassay (RIA) (Marks, Mould, Stout & Williams, 1978). Peak plasma levels with both assays were observed at 1½–2 h though the RIA gave levels twice those of the RRA (subject 1 RRA 110 ± 10, RIA 260 ± 10; subject 2 RRA 140 ± 12, RIA 313 ± 13.9 pmoles/ml plasma ± s.e. mean of three repeated determinations). This discrepancy related to the inability of the RIA to distinguish between the (−) and (+) isomers of propranolol whereas the RRA only measures the biologically active (−)-isomer. A volunteer receiving (+)-propranolol (40 mg) had peak plasma levels of 145 ± 3 whereas the RRA gave levels of only 3 ± 0.01 pmoles/ml plasma. Since the RRA measures the biological activity of β-blockers at the receptor, it has the substantial advantage over all