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

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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

existing single methods in that it can monitor active metabolites of β-blockers that may contribute to the overall efficacy of the parent drug. Moreover, apart from being very sensitive and simple to perform, its use is not limited to a single β-blocking drug and application of the technique for routine plasma measurements can be anticipated.

M.I.B. is a British Council research fellow. We thank Dr. G.P. Mould for the kind gift of propranolol antisera.

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Effect of a phosphodiesterase inhibitor on cyclic GMP changes induced by muscarinic agonists in mouse neuroblastoma cells

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The potent muscarinic agonist, carbamoylcholine has been shown to elevate guanosine 3′:5′ phosphate (cyclic GMP) levels in mouse neuroblastoma cells, clone NIE 115 (Matsuzawa & Nirenberg, 1975; Strange, Birdsall & Burgen, 1977; Richelson, 1977). The response was inhibited by muscarinic antagonists but not by nicotinic antagonists. A phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine (MIX) was included in the assay system in order to obtain large (4–10 fold) and reproducible increases in cyclic GMP levels. These experiments have now been extended to include other muscarinic agonists (Table 1). Whereas agonist efficacy measurements indicated that a gradation of responses might have been expected, the agonists tested fell into two classes: potent agonists

Table 1 Effect of muscarinic agonists on cyclic GMP levels

Agonist	*Efficacy**	*Elevation of cyclic GMP over basal level produced by muscarinic agonists (10^{-3}M) expressed as a percentage of the elevation produced by carbamoylcholine (10^{-3}M)*	
		with MIX	*without MIX*
oxotremorine-M	N.D.	72	108**
carbamoylcholine	270	100**	100**
(+)-acetyl-β-methylcholine	107	88	N.D.
acetylcholine	71	107	N.D.
arecoline	32	2†	95**
oxotremorine	9	5	101**
pilocarpine	2.5	3	8†

Cyclic GMP was determined by a prelabelling method using [^{3}H]-guanine. Cyclic GMP elevations were measured by comparing duplicate or quadruplicate samples from cells stimulated with an agonist (applied for 60 s (with MIX) or 30 s (without MIX)) with duplicate or quadruplicate control samples (for basal cyclic GMP levels). The significance of the increase in cyclic GMP over the basal level was assessed where possible using a Wilcoxon test: ** significant at 5% level, † not significant at 5% level. The increase in cyclic GMP produced by agonists was expressed as a percentage of the increase produced by carbamoylcholine. N.D.—not determined. * N.J.M. Birdsall—unpublished results.