Analysis of the activity and distribution of β-adrenoceptor antagonists in the rat using an *ex vivo* receptor binding assay

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The recent development of receptor-ligand binding assays has led to the development of novel radioreceptor assays for neuroleptics (Creese & Snyder, 1977), and β-adrenoceptor antagonists (Nahorski, Batta & Barnett, 1978) in plasma. In the present communication we describe a further application of receptor binding assays that allows the activity and tissue distribution of β-adrenoceptor antagonists to be easily determined. *Ex vivo* experiments were performed by first treating rats with different doses of β-adrenoceptor antagonists and then assessing the extent of receptor occupation under *in vitro* conditions.

Male Wistar rats (100–150 g) were used in all experiments. β-adrenoceptor antagonists were injected subcutaneously, the rats killed by decapitation, and several tissues (lung, heart, spleen, cerebral cortex and cerebellum) were rapidly removed, weighed and homogenised in 8 vols. of Tris-HCl (50 mM, pH 7.8). Homogenates were passed through cheesecloth and used directly in the binding assay. [^{3}H]-(−)-Dihydroalprenolol, a specific β-adrenoceptor ligand, was incubated with the crude tissue homogenates and specific binding assessed as the binding that could be displaced by (−)-isoprenaline (200 μM). This represented 65–95% of the total binding depending upon the tissue examined. In all experiments rats treated with saline were used as controls, and the binding of [^{3}H]-(−)-dihydroalprenolol to tissue membranes prepared from drug-treated animals was expressed as a percentage of these controls.

Rats treated with (−)-propranolol (0.1 μmole/kg) displayed peak plasma levels of biologically active drug (71.11 ± 1.85 pmole/ml) measured by a radioreceptor assay (Nahorski, *et al.*, 1978) 15 min after administration. On the other hand, the highest concentration of bioactive drug assessed by *ex vivo* assay, was observed at 30 min in all tissues examined. Three to six groups of rats were treated with different doses of (−)-propranolol (0.001–0.3 μmol/kg) or (+)-propranolol (0.03–30 μmole/kg) and the tissue distribution of active drug assessed at 30 minutes. ID_{50} values (dose of drug (μmole/kg) which reduces the specific binding of 1.5 nM [^{3}H]-dihydroalprenolol by 50%, with 95% confidence limits) for (−)- and (+)-propranolol respectively, were: lung, 0.0069 (0.0058–0.0077) and 0.66 (0.59–0.73); heart, 0.026 (0.021–0.033) and 1.98 (1.62–2.47); spleen, 0.0069 (0.0058–0.0082) and 0.63 (0.52–0.76); cerebral cortex, 0.018 (0.015–0.021) and 2.04 (1.58–2.65); cerebellum, 0.026 (0.021–0.033) and 1.91 (1.64–2.26). These data emphasise the stereoselective nature of the β-adrenoceptor and suggest that both isomers of propranolol exert a small (3 fold) degree of selectivity towards lung and spleen.

The inherent advantage of these *ex vivo* experiments is to allow an assessment to be made of the relative distribution and receptor selectivity of the drug and active metabolites between different tissues. We are presently examining the activity of β_1 and β_2 selective agents using these approaches.

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References

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