from these two sites with markedly different affinities. Further studies are currently in progress to assess the significance of these findings and their possible relationship with the DA receptor.

The authors thank the Wellcome Trust for financial support.

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Pitfalls in the assessment of the specific binding of (−)[^{3}H]-dihydroalprenolol to β-adrenoceptors

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The use of radiolabelled ligands to identify receptors directly has been particularly useful in the study of the β-adrenoceptor (β-AR). However, the binding characteristics (K_D, B_{max}, Hill coefficients) vary considerably between laboratories even when workers are utilizing identical tissues and labelled ligands. We have made a careful assessment of the binding of (−) [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) to bovine lung membranes and have estimated the apparent specific binding to the β-AR using various competing cold

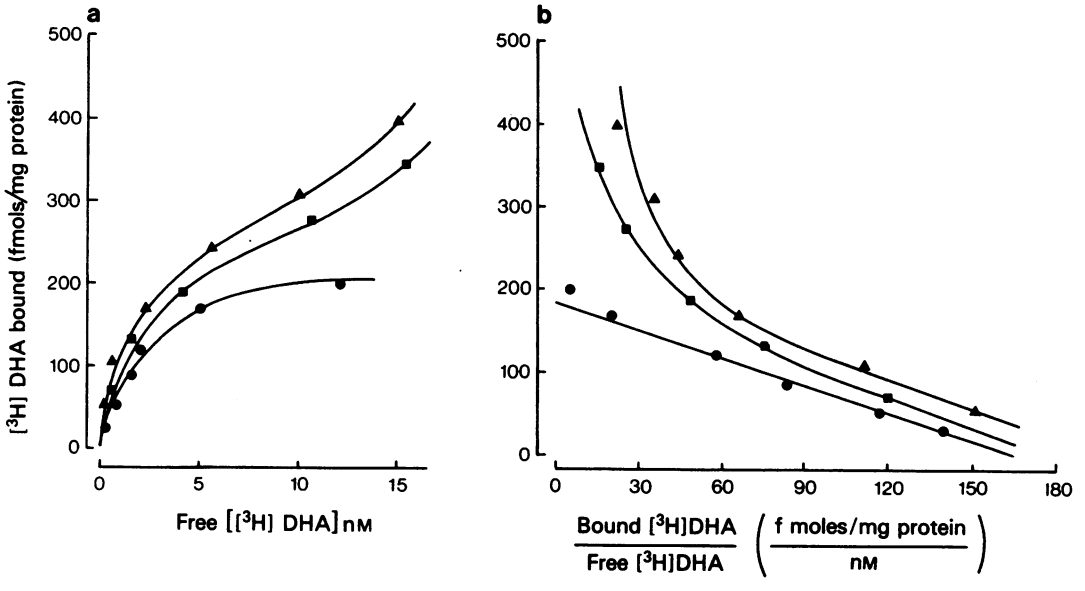

Figure 1 (a) Bovine lung membranes were incubated with increasing concentrations of [^{3}H]-DHA in the presence and absence of (−)-isoprenaline (2×10^{-4} M), (−)-alprenolol (1×10^{-5} M), and (−)-propranolol (1×10^{-5} M), and binding determined as previously described (Barnett, Rugg & Nahorski, 1978). Abscissa: Free [[^{3}H]-DHA] nM, Ordinate: [^{3}H]-DHA bound calculated as the difference between the total binding and that remaining in the presence of (−)-isoprenaline (●), (−)-alprenolol (■), or (−)-propranolol (▲). Each point represents the mean of four experiments. Variation around the points was not greater than ±5%. (b) The same data analysed by the method of Scatchard.

β-AR agents. We report that some of the discrepancies above may relate to an incorrect estimate of specific binding to the receptor.

Membranes were prepared from bovine lung parenchyma and binding assays performed as previously described for rat lung (Barnett, Rugg & Nahorski, 1978). Saturation curves for [^{3}H]-DHA were constructed using (−)-isoprenaline (2×10^{-4} M), (−)-alprenolol (1×10^{-5} M), and (−)-propranolol (1×10^{-5} M) to determine the specific binding. These drugs and concentrations were chosen as they represent the most common methods reported in the literature to assess specific β-adrenoceptor binding. The results (Figure 1a) show a large difference in the 'specific' binding assessed by these three methods. Only the 'specific' binding calculated using (−)-isoprenaline showed characteristics expected of a single homogeneous population of binding sites when analysed by the method of Scatchard (Figure 1b).

Detailed competition experiments for (−)-isoprenaline, (−)- and (+)-alprenolol, and (−)- and (+)-propranolol at a fixed [^{3}H]-DHA concentration (≃2 nM), showed that over a large concentration range (10^{-10} M → 10^{-3} M) only (−)-isoprenaline displaced [^{3}H]-DHA from a single site, whereas the β-AR antagonists displaced from an additional second site. The characteristics of the second site were not consistent with it being the β-AR, since no stereoselectivity was displayed and the β-antagonists still displaced [^{3}H]-DHA in the presence of 2×10^{-4} M (−)-isoprenaline.

Models of binding curves, based on law of mass action kinetics, were tested to determine the influence of incorrect specific binding on the parameters of this component. Selection of incorrect values for the specific binding resulted in the introduction of considerable error in the binding parameters for both saturation and competition models. The model accurately predicted the experimental results obtained.

These results indicate that great care must be exercised when assessing binding of radiolabelled ligands to receptor sites. Although in this communication the binding of [^{3}H]-DHA to β-adrenoceptors has been assayed, it is probable that similar pitfalls are inherent with several other ligand-receptor interactions.

A.R. is an ICI supported SRC (CASE) Award student.

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Ionic perturbation of agonist binding to brain muscarinic receptors

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The binding of potent agonists such as carbamoylcholine (CCh) to muscarinic receptors (MR) in subcellular preparations from both brain and peripheral tissues is characterised by Hill coefficients (H) having values much less than 1.0, indicating the presence of both high and low affinity agonist binding sites. In contrast, antagonists fail to distinguish between these sub-populations of receptors, and bind with H values of 1.0 (Birdsall & Hulme, 1976; Birdsall, Burgen & Hulme, 1978). We have studied the effects of high concentrations of NaCl and other inorganic salts on the interaction of agonists and antagonists with brain MR.

Measurements of agonist and antagonist binding to MR in homogenates of rat brain were made as described previously (Hulme, Birdsall, Burgen & Mehta, 1978). Two regions of brain were studied, medulla-pons, which contains predominantly high affinity agonist binding sites with only 25% low affinity sites, and cerebral cortex which contains 60% low affinity sites (Birdsall, *et al.*, 1978; Hulme & Birdsall, unpublished observations). Assays were performed at pH 7.0 in 20 mM Na-Hepes buffer, supplemented with inorganic salts. Similar results were obtained at both 4 and 30°C.

In medulla-pons, the concentration of CCh giving 50% receptor occupancy (ED_{50}) increased from 7×10^{-7} M in 0.1 M NaCl to 2×10^{-4} M in 1.0 M NaCl, and the Hill coefficient of the binding curve increased from 0.53 to 0.93. Similarly in the cortex the ED_{50} for carbachol increased from 1.5×10^{-5} M in NaCl (0.2 M) to 8×10^{-4} M in NaCl (2.0 M) with an accompanying increase in H from 0.32 to 0.90. Comparable results were obtained using other potent agonists. There was no loss of total binding sites, and the shifts were completely reversible on lowering the salt concentration. High NaCl concentrations exerted comparatively little effect on antagonist binding: affinity constants were reduced approximately 5-fold under the above conditions and the value of H remained 1.0.