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.

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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.

Analysis of binding curves at intermediate NaCl concentrations showed that the increase in H occurred over only a 5-fold range of NaCl concentration, and thus appeared cooperative, and was detectable at lower NaCl concentrations in the medullapons than in the cortex.

Preliminary experiments have shown that Li^+ is more effective, and Mg^{2+} less effective than Na^+ in perturbing agonist binding curves.

In summary, high concentrations of certain inorganic ions largely abolish the heterogeneity of agonist binding; the binding curves become close approximations to the simple Langmuir isotherm, with H values close to 1.0, and the ability of agonists to discriminate between high and low affinity binding sites is lost.

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Parameters of [^{3}H]-ouabain binding to human heart ($Na^+ + K^+$)-ATPase

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Human heart samples have been obtained from children operated for right ventricular outflow tract hypertrophy. Preparation of ($Na^+ + K^+$)-ATPase and determination of enzyme activities have been performed as described previously for guinea-pig heart (Godfraind, De Pover & Verbeke, 1977). The activity of human preparations was equal to 10–15 μmol Pi mg protein^{-1} h^{-1}. The preparations contained 5% of residual Mg^{2+}-ATPase. [^{3}H]-Ouabain binding was determined by a filtration technique (Godfraind, Sturbois & Verbeke, 1976). About 5 mg protein were usually incubated in 30 ml medium containing (mM): NaCl 100, $MgCl_2$ 3, Tris-ATP 2.5, EGTA 1, Tris-maleate 20 (pH 7.4, 37°C) and [^{3}H]-ouabain (0.1–12 Ci per mmole) 1 ml portions were filtered at various times on 0.45 μ Sartorius filters. The radioactivity retained was measured by liquid scintillation counting. Non specific binding was determined from samples incubated either in the presence of unlabelled ouabain (0.1 mM) or in the absence of ATP.

Table 1. Parameters of ouabain-human heart ($Na^+ + K^+$)-ATPase interaction

	High affinity sites	*Low affinity sites*
k_a (min^{-1} M^{-1})	3.7 × 10^6	3.4 × 10^6
k_d (min^{-1})	0.0092	0.058
K_D (μM) from k_d/k_a ratio	0.0025	0.017
K_D (μM) from Scatchard plots	0.0048	—
K_i (μM) from Hunter-Downs plots		0.013

Kinetic parameters of [^{3}H]-ouabain binding were calculated from Scatchard plots and from time-dependent binding data. k_a was calculated from [^{3}H]-ouabain binding at [^{3}H]-ouabain (0.2 μM) and k_d from its release following the addition of unlabelled ouabain (0.1 mM). Scatchard plots and dissociation kinetics indicated the presence of two classes of independent binding sites. The second order rate constants k_a were equal to 3.7 × 10^6 min^{-1} M^{-1} for high affinity binding sites and to 3.4 × 10^6 min^{-1} M^{-1} for low affinity binding sites. The first order rate constants k_d were equal, respectively, to 0.0092 min^{-1} and to 0.058 min^{-1} K_D calculated from k_d/k_a ratio and from Scatchard plots are reported in Table 1. For high affinity binding sites, these values lie close together. The inhibition constant (K_i) of ouabain, calculated from Hunter-Downs plots according to Akera, Larsen & Brody (1969), is several times higher than K_D of high affinity binding sites and is close to K_D of low affinity binding sites. This suggests that low affinity sites only are involved in the inhibition of ($Na^+ + K^+$)-ATPase.

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