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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Antinociceptive actions of morphine and buprenorphine given intrathecally in conscious rats

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Morphine injected into the spinal subarachnoid space in animals produces focal analgesia (Yaksh & Rudy, 1977; Yaksh, 1978). In the present study the antinociceptive effects of morphine and buprenorphine injected intrathecally or subcutaneously were compared in the rat (male, 200–270 g, AH-PVG/C). Chronically implanted cannulae (PP10, Portex Ltd) allowed injections (total 15 µl) of drugs or placebo (artificial cerebrospinal fluid in g/l NaCl 7.46; KCl 0.19; $MgCl_2$ 0.19; $CaCl_2$ 0.14) to be made into the subarachnoid space at the level T_{13}–L_1. Antinociceptive activities were determined in dose groups of 6 rats against hind-paw 'lick' response latencies in the hot-plate (55°C) test and in the hind-paw pressure ('analgesimeter', Ugo Basile) test. Drugs were injected either intrathecally at 10, 30 or 60 min or subcutaneously at 30 min prior to determination of nociceptive thresholds. Different groups of rats were used for each pre-treatment time. Experiments were carried out blind using a randomised dosing schedule. Data were analysed for linearity and regression using methods of Finney (1964). Results obtained are given in Figure 1.

After intrathecal injection the peak (30 min) antinociceptive potencies of buprenorphine or morphine were similar in both tests but the buprenorphine

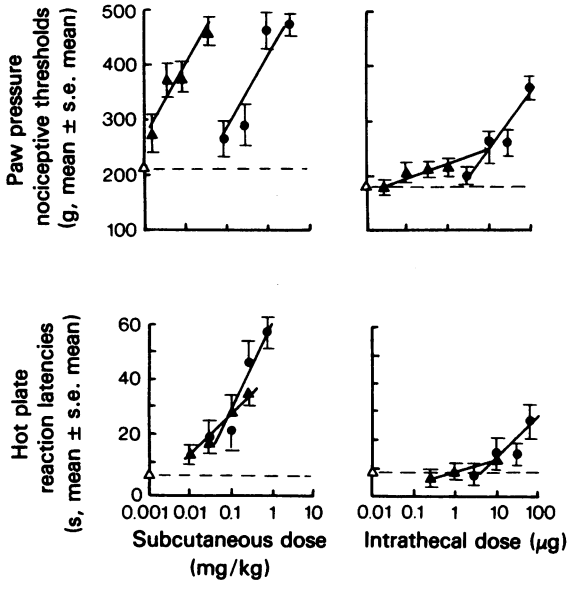

Figure 1 Effects of morphine (●) and buprehorphine (▲) given intrathecally or subcutaneously in the hot-plate (55°C) and paw pressure tests in the rat.