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A comparison of linear and nonlinear parameter estimates in drug receptor quantitation

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Many quantitative estimates of drug receptor number (B_{max}) and affinity (K_d, dissociation constant) in radioreceptor experiments are derived from linear and nonlinear transformations of the general hyperbolic binding curve, analogous to kinetic constants derived from the transformation of a Michaelis-Menton plot of initial velocity of reaction, against concentration of substrate (Dowd & Riggs, 1965):-

$$\text{Amount Bound} = \frac{B_{max} \cdot K_a \cdot F_c}{(1 + K_a \cdot F_c)}$$

(B_{max} = total number of binding sites, K_a = association constant, F_c = free concentration)

In the absence of experimental error, transformations of the hyperbolic curve based on the law of mass action, such as the Scatchard, double reciprocal and direct linear plots provide good estimates of the binding parameters, B_{max} and K_d (Scatchard, 1949; Cornish-Bowden & Eisenthal, 1974; Madsen & Robertson, 1974). However, when data is subjected to random error of the nature and magnitude encountered in radioligand binding studies, discrepancies are apparent between parameter estimates obtained using different transformations. Theoretically, the most accurate, simple and unbiased way to fit experimental data to such a model is by iterative, computer assisted, nonlinear regression providing a direct least squares estimate of the parameters (Batchelor, 1977).

Parameter estimates of B_{max} and K_d were set at 200×10^{-15} mols and 3.0 nM respectively, and a theoretical saturation binding curve constructed. This was subjected to random noise of 0–10% and B_{max} and K_a calculated by Gauss-Newton iterations, providing a least squares fit, utilising an IBM360 computer and University of California BMDP3R nonlinear regression programme. The results obtained were compared with those from conventional transformations of the curve (Table 1).

Table 1

Data treatment	B_{max} $\times 10^{-15}$ *mols*	95% *Confidence limits*	K_d *nM*	95% *Confidence limits*
Nonlinear regression	199	186–211	2.83	2.52–3.23
Double reciprocal	173	150–203	1.49	1.49–2.16
Scatchard plot	198	184–210	2.93	2.53–3.30
Direct linear plot	196	170–226	3.1	0.4–4.6

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