A Simple Non-parametric Procedure for Rapid Estimation of Scatchard Parameters

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A simple non-parametric procedure for the calculation of Scatchard parameters from radioligand binding experiments by evaluating total binding curves is described. It is based on the mathematical theory describing any number n of ligands reacting with any number m of binding sites developed by Feldman (1972). Additionally, the level of nonspecific binding is regarded as an unknown parameter taking into account the precisely additive nature of specific and nonspecific binding as reported by Hulme et al. (1978). The numerical solution of the resulting equation for the parameters mentioned was performed as derived by Hart (1965).

In this communication the "n by m" model of Feldman (1972) has been applied for the case $n = 1$ and $m = 1$, and the calculation procedure proposed has been used for the evaluation of Scatchard analysis of ^{3}H-dihydroalprenolol (^{3}H-DHA) binding in the cerebral cortex of rat brain.

Binding assay

Adult rats (three months old) were killed by decapitation, the brains removed, placed on ice and the cerebral cortices quickly dissected. The tissue was homogenised in ice-cold 50 mmol/l Tris-HCl, pH 8.0 (about 0.3 mg protein/ml), centrifuged at 45 000 g for 20 min. The resulting pellet was washed, centrifuged and the final resuspension of the membrane fraction was stored at $-20\,^\circ$C not longer than one week. After thawing of samples beta-adrenergic receptors were assayed by the ^{3}H-dihydroalprenolol (^{3}H-DHA) binding method (Bylund 1978). Briefly, 900 μl of tissue supension was incubated with ^{3}H-DHA (specific activity 1628 GBq-/mmol, Radiochemical Centre Amersham, England) ranging in concentration from 0.09 to 4.7 nmol/l in the assay at 25 °C for 30 min in a final volume of 1 ml. Three aliquots were incubated with and three without 100 μmol/l L-isoproterenol-D-bitartrate as a specific displacer. The samples were filtered under vacuum through glass-fibre filters type NK VIII (VEB Feinpapierfabrik Neu-Kaliss, G.D.R.), the filters were rinsed with 8 ml ice-cold buffer, placed in scintillation vials and the retained radioactivity determined by liquid scintillation counting. Specific binding of ^{3}H-DHA was obtained as the difference in the radioactivity of the filters in the presence and absence of L-isoproterenol in the sample, respectively.

Calculation procedure

Applying the "*n* by *m*" model of Feldman (1972) for the case $n = 1$ and $m = 1$ (one ligand reacting with one binding site) and taking into consideration the portion of nonspecific binding b_u with respect to the total binding b_t in a manner as discussed by Hulme et al. (1978) following equation (describing the concentration dependence of radioligand binding) has resulted:

$$\frac{b_t}{f} = \frac{K_A b_{max}}{1 + K_A f} + X_2 \tag{I}$$

where f is the free ligand concentration at equilibrium, K_A designates the association equilibrium constant, b_{max} denotes the maximum binding capacity, and X_2 represents the portion of nonspecific binding equal to b_u/f. The solution of equation (I) for the parameters K_A, b_{max}, and X_2 is analogous to the derivations described by Hart (1965) for the more general case. Therefore, only the resulting equations will be given here only. Using the notations

$$X_1 = K_A b_{max} \text{ and } b_t/f = Y$$

and additionally applying multiple linear regression analysis, following linear equation system (II) should be solved first:

$$\begin{aligned}
ap + b/f/ - c/b_t/ &= /Y/ \\
a/f/ + b/f^2/ - c/fb_t/ &= /Yf/ \\
a/b_t/ + b/fb_t/ - c/b_t^2/ &= /Yb_t/
\end{aligned} \tag{II}$$

where p denotes the number of experimental pairs of values (b_t, f), and the brackets $/Q/$ should represent the summation operator

$$\sum_{i=1}^{p} Q_i.$$

The resulting solutions a, b, and c of the equation system (II) hold following relations:

$$\begin{aligned}
K_A &= c \\
K_A X_2 &= b \\
X_1 + X_2 &= a
\end{aligned} \tag{III}$$

Thus the quantities K_A, b_{max}, and X_2 of equation (I) can be calculated. The extension of this procedure to the case $n = 1$ and $m = 2$ (one ligand reacting with two different binding sites) is straightforward and should not be explicitly presented here.

Statistics of multiple linear regression
(with two independent variables)

Transforming experimental data (Y, f, b_t) into the standard-deviation form:

$$\bar{Y}=\frac{/Y/}{p}, \quad \bar{f}=\frac{/f/}{p}, \quad \bar{b}_t=\frac{/b_t/}{p},$$

$$s_{dY}=\sqrt{\frac{/Y-\bar{Y}/}{p}}, \quad s_{df}=\sqrt{\frac{/f-\bar{f}/}{p}}, \quad s_{db_t}=\sqrt{\frac{/b_t-\bar{b}_t/}{p}},$$

the solutions of eqn. (II) become very easy as derived elsewhere (Cohen and Cohen 1975):

$$b=\beta_1\frac{s_{dY}}{s_{df}}, \quad c=-\beta_2\frac{s_{dY}}{s_{db_t}}, \quad a=\bar{Y}-b\bar{f}+c\bar{b}_t$$

with

$$\beta_1=\frac{r_{Yf}-r_{Yb_t}r_{fb_t}}{1-r_{fb_t}^2}, \quad \beta_2=\frac{r_{Yb_t}-r_{Yf}r_{fb_t}}{1-r_{fb_t}^2}$$

(β_1, β_2, standardized partial regression coefficients) and

$$r_{Yf}=\frac{/(Y-\bar{Y})\,(f-\bar{f})/}{\sqrt{/(Y-\bar{Y})^2/}\,\sqrt{/(f-\bar{f})^2/}}.$$

The other standardized product-moment correlation coefficients r_{Yb_t} and r_{fb_t} follow analogously.

The multiple correlation coefficient ψ is then obtained as

$$\psi=\sqrt{\beta_1 r_{Yf}+\beta_2 r_{Yb_t}}$$

and the standard errors SE of the partial regression coefficients readily converted in original units can be computed as follows:

$$SE_b=\frac{s_{dY}}{s_{df}}\sqrt{\frac{r_{Yf}\,(1-\psi^2)}{p-3}}, \quad SE_c=\frac{s_{dY}}{s_{db_t}}\sqrt{\frac{r_{Yb_t}\,(1-\psi^2)}{p-3}}.$$

Further statistical analysis of the regression (F-or t-test) can now be performed as described elsewhere (Cohen and Cohen 1975; Overall and Klett 1972).

The approach derived in this paper was applied for the estimation of Scatchard parameters (K_A, b_{max}) from ^{3}H-DHA binding experiments. A comparison of the Scatchard parameters evaluated by taking into account experimentally determined nonspecific binding with those calculated from the total binding alone shows a good

Table 1. Comparison of Scatchard parameters (K_A, b_{max}) and nonspecific binding (X_2) obtained by different methods of evaluation of ^{3}H-DHA binding experiments as described in the text

Parameter	Experimental data*	Calculated data**
K_D/nmol l^{-1}	0.66 ± 0.05	0.74 ± 0.03
b_{max}/fmol	44.4 ± 4.5	50.4 ± 8.6
X_2	11.2 ± 0.4	10.3 ± 1.0

* data obtained taking into account experimentally assayed nonspecific binding
** data calculated from total binding values alone as described in the text. Experimental data are the results of weighted least squares linear regression (weight = 1/sample variance). Calculated data are the results of weighted multiple linear regression analysis taking into account nine experimental binding values (b_i, f) within a ligand concentration range of 0.09 to 4.7 nmol/l in the assay. For details of the experimental methods and calculation procedure see text. All the errors are standard deviations. K_D denotes the equilibrium dissociation constant ($= 1/K_A$), b_{max} designates maximum receptor number. The multiple linear correlation coefficient was calculated to $\psi = 0.996$.

agreement (Table 1). The non-parametric procedure proposed in this paper is simple and little time consuming even without the use of an extensive computer technique. A programmable pocket calculator should be sufficient for solving the linear equation system (II). This approach provides a rapid verification of experimentally determined nonspecific binding curves. Otherwise, it could provide quick estimates to begin parametric non-linear regression analysis for the parameters K_A and b_{max}, applying, e.g. the more accurate versatile computerized approach "LIGAND" developed by Munson and Rodbard (1980). The advantages and problems of parametric and non-parametric methods have been well discussed by Cornish-Bowden and Eisenthal (1974).

An extension of this procedure to more complex binding models is possible and straightforward as described above, it is, however, considerably more time consuming.

The aim of this communication was to submit a quick and little time-consuming method for the estimation of binding parameters.

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