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Quantitative Resolution of Beta-Adrenergic Receptor Subtypes by Selective Ligand Binding: Application of a Computerized Model Fitting Technique

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SUMMARY


Frog myocardium appears to possess both beta1 and beta2 receptors, based on the potency order of several adrenergic agonists to compete for [3H]dihydroalprenolol binding. Selective beta blocking agents are able to distinguish two receptor subtypes in frog myocardium, but only one site in rat ventricle. Computer modeling using a PDP 11/45 indicates that all rat beta receptors are beta1, whereas only 15%-25% of frog ventricular beta receptors are of the beta1 subtype. Computerized curve fitting can provide a more accurate estimate of receptor parameters than currently available graphical methods of analysis.

INTRODUCTION

The use of radiolabeled ligands has facilitated the study of various properties of the beta-adrenergic receptors in many tissues (1). One of the characteristics of beta receptors investigated by this method has been the distinction between beta1 and beta2 receptors originally proposed from physiological observations by Lands et al. (2). For example, the adenylate cyclase-coupled beta-adrenergic receptor of the frog erythrocyte appears to possess binding properties of the beta2 type (3), whereas the rat heart demonstrates binding affinities for ligands predicted for beta1 receptors (4).

Until recently, it has been believed that individual tissues contain only one of the beta receptor subtypes. However, pharmacological studies by Caisson et al. (5) demonstrated a mixture of beta1 and beta2 receptors in kitten, but not rat heart. Similar physiological techniques have indicated that frog myocardium might contain a small beta1 component in addition to a predominant population of beta2 receptors (6).

Using radiolabeled ligand techniques, Barnett et al. (7) recently demonstrated a mixture of 25% beta1 and 75% beta2 receptors in rat lung, but rat heart studies demonstrated only one class of sites. A graphical method derived from the classical Scatchard data analysis ("pseudo-Scatchard") was used to estimate the relative proportions of receptor subtypes in these ligand binding experiments.

In the present study, we have applied a computerized model fitting technique to analyze the ligand binding data and determine the relative proportions of beta1 and beta2 receptors in frog and rat myocardium. The results of this analysis indicate that frog myocardium contains a small beta1 component, whereas rat heart contains only beta2 receptors.

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In the present application, for each curve the number "m" of ligands is two, ligand 1 being the labeled ligand (DHA) and ligand 2 being the competitor. The number "n" of classes of binding sites is set to either 1 or 2, but could be larger. A Scatchard transformation of data (not shown) from saturation studies resulted in a straight line, indicative of high affinity binding of DHA with equal affinity for all receptors. Secondly, in the presence of a competitive ligand (e.g., propranolol) there was an apparent decrease in the affinity of DHA for the receptors without any change in the maximum amount of DHA bound, as predicted for true competitive binding. DHA appears to be non-selective for either beta1 or beta2 receptor subtypes and the same value was assigned to its two affinity constants, $K_{11}$ and $K_{12}$, for sites 1 and 2, respectively.

The deviations of the observed points from the predicted values were weighted according to the reciprocal of the predicted variance (13). The data were repeatedly fit using the model for one, two, or more classes of binding sites. The model providing the best fit was chosen on the basis of the lowest value of mean squares of residuals. The computer programs provided the best estimates (with their standard error) for the affinity constants of each ligand and the concentration of receptors in each subtype. All computations were performed using an interactive program in PL/1 using a PDP 11/45.
FIG. 1. Competition curves for specific DHA binding to frog ventricular membranes by beta-adrenergic agonists. The ordinate indicates the percent of maximal specific DHA binding, 0.0221 nM. (Specific binding is defined as the difference between binding in the absence of any competing ligand and binding in the presence of 10 M propranolol.) The abscissa is the molar concentration of various agonists. The lines are computer modeled best fits. The symbols indicate the means of actual data points for 2 (hydroxybenzylisoproterenol, ✔), 11 (isoproterenol, U), 9 (epinephrine, A), and 10 (norepinephrine, ✔) separate experiments with each agonist.

FIG. 2. Competition curves for specific DHA binding to rat ventricular membranes by beta-adrenergic agonists. The ordinate indicates the percent of maximal specific DHA binding, 0.0083 nM. The abscissa is the molar concentration of various agonists. The symbols indicate the means of actual data points derived from two separate experiments with each agonist (hydroxybenzylisoproterenol, ✔, isoproterenol, U, epinephrine, A, norepinephrine, ✔). The experimental data obtained in the frog ventricle were best fit with a model in which 15%-25% of the receptors are beta1 and 75%-85% are beta2. The relative affinities of the antagonists for these receptor types and the standard error of the mean of these estimates were also derived by the program (Table 1). Propranolol had equal affinity for both beta1 and beta2 receptors, whereas practolol and butoxamine had affinities for the two frog receptor subtypes that differed by nearly two orders of magnitude. In contrast, data obtained in the rat could be fit most optimally by a model with only one binding site for both DHA and the competitor. Table 1 lists the dissociation constants.
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