CONTENTS

ARTHUR A. HANCOCK, ANDRE L. DELEAN, AND ROBERT J. LEFKOWITZ. Quantitative Resolution of Beta-Adrenergic Receptor Subtypes by Selective Ligand Binding: Application of a Computerized Model Fitting Technique. 1

MICHAEL R. WESSELS, DEBRA MULLIKIN, AND ROBERT J. LEFKOWITZ. Selective Alteration in High Affinity Agonist Binding: A Mechanism of Beta-Adrenergic Receptor Desensitization. 10

KENNETH P. MINNEMAN, LINDA R. HEGSTRAND, AND PERRY B. MOLINOFF. The Pharmacological Specificity of Beta-1 and Beta-2 Adrenergic Receptors in Rat Heart and Lung in Vitro. 21

KENNETH P. MINNEMAN, LINDA R. HEGSTRAND, AND PERRY B. MOLINOFF. Simultaneous Determination of Beta-1 and Beta-2 Adrenergic Receptors in Tissues Containing Both Receptor Subtypes. 34

DAVID C. U'PRICHARD, W. DIETRICH BECHTEL, BRUNO M. ROUOT, AND SOLOMON H. SNYDER. Multiple Apparent Alpha-Noradrenergic Receptor Binding Sites in Rat Brain: Effect of 6-Hydroxydopamine. 47

BIE SHUNG TSAI AND ROBERT J. LEFKOWITZ. Agonist-Specific Effects of Guanine Nucleotides on Alpha-Adrenergic Receptors in Human Platelets. 61

IAN CREES, TED B. USDIN, AND SOLOMON H. SNYDER. Dopamine Receptor Binding Regulated by Guanine Nucleotides. 69

MARK I. HOLCK, BERNARD H. MARKS, AND CYNTHIA A. WILBERDING. Characterization of Alpha-Adrenergic Receptors in Guinea-Pig Vas Deferens by [3H]Dihydroergocryptine Binding. 77

KENNETH P. MINNEMAN, LINDA R. HEGSTRAND, AND PERRY B. MOLINOFF. Multiple Opiate Receptors: Different Regional Distribution in the Brain and Differential Binding of Opiates and Opioid Peptides. 91

C. E. DUNLAP III, F. M. LESLIE, M. RADO, AND B. M. COX. Ascorbate Destruction of Opiate Stereospecific Binding in Guinea-Pig Brain Homogenate. 105

ATSUNOBU YODA AND SHIZUKO YODA. Suppression by Sodium, Potassium or Nucleotides of Binding between Cardiac Steroid (Digoxigenin) and Sodium- and Potassium-Dependent Adenosine Triphosphatase Formed in the Presence of Magnesium and Phosphate. 120

LLOYD H. MICHAEL, ARNOLD SCHWARTZ, AND EARL T. WALLICK. Nature of the Transport Adenosine Triphosphatase-Digitalis Complex: XIV. Inotropy and Cardiac Glycoside Interaction with Na*, K*-ATPase of Isolated Cat Papillary Muscles. 135

VIVIANE MAES, JOHAN HOEBEKE, ANTOINE VERCRUYSSE, AND LOUIS KANAREK. Optical Studies into the Nature of the High Affinity Binding Site of Human Serum Albumin for Phenylbutazone. 147

DAVID A. JOHNSON, ROGER COOKE, AND HORACE H. LOH. Effect of Opiate Agonists and Antagonists on Lipid Bilayer Fluidity. 154

S. A. SIMON, T. J. MCINTOSH, P. B. BENNETT, AND B. B. SHRIVASTAV. Interaction of Halothane with Lipid Bilayers. 163

JACK Y. VANDERHOEK AND MAURICE B. FEINSTEIN. Local Anesthetics, Chlorpro-
Quantitative Resolution of Beta-Adrenergic Receptor Subtypes by Selective Ligand Binding: Application of a Computerized Model Fitting Technique

ARTHUR A. HANCOCK, ANDRE L. DELEAN, AND ROBERT J. LEFKOWITZ

Howard Hughes Medical Institute Laboratory, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

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 method to resolve beta-adrenergic receptor subtypes in frog and rat myocardium.
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.

RESULTS

Binding of DHA to membrane vesicles from both frog and rat ventricle demonstrated appropriate stereoselectivity and a high affinity for DHA. The $K_D$'s obtained from Scatchard analysis (data not shown) were 3.6 nM in the frog and 2.6 nM in the rat. The reciprocal of the $K_D$'s were utilized as the affinity constants of DHA in subsequent computer analyses. The maximum number of binding sites for DHA was approximately 100 fmol/mg protein in frog ventricle and 35 fmol/mg protein in the rat.

Displacement curves of agonist competition against approximately 3 nM DHA revealed a different pattern for the two species. Figure 1 illustrates the ability of four adrenergic agonists to compete for DHA binding in the frog ventricle. The pattern observed is similar to that found with beta2 systems such as the frog erythrocyte (3). Estimates of the EC50's from dose response curves indicated a potency ratio for hydroxybenzylisoproterenol: isoproterenol: epinephrine: norepinephrine of 1200:70:7:1. By contrast the pattern observed in the rat ventricle was as expected for beta1 receptor binding (Fig. 2), i.e., the relative agonist potencies of hydroxybenzylisoproterenol: isoproterenol: epinephrine: norepinephrine were 30:25:1.7:1.

The ability of three beta-blocking agents to compete for DHA binding to membrane vesicles was also different in the two species. Figure 3 illustrates dose-response competition curves for approximately 3 nM DHA binding in frog ventricle. The potency order of propranolol: butoxamine: practolol was 2000:6.7:1. In the rat myocardium, butoxamine and practolol were approximately equipotent, and approximately 1000 fold weaker than propranolol (Fig. 4). In the experiments using rat heart the three antagonist dose-response curves appeared to differ only in the potency of the competitors, i.e., all three curves were parallel (Fig. 4). In the frog heart, however, the displacement of DHA by butoxamine and practolol is more complex than that obtained with propranolol. Dose response curves (Fig. 3) indicate a second component with butoxamine and practolol. In order to analyze the interaction of beta-receptor agents with DHA binding sites a computer modeling system was developed. Figures 1 through 4 illustrate the ability of the model to fit the experimental observations. The lines indicate the best fit from the computer model, whereas the symbols represent the actual data points. Predicted values for ligand affinities and proportions of beta1 and beta2 receptors obtained from agonist and antagonist competition curves are
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 obtained using this model.
I

-io

l0 7 106 iO

Concentration Antagonist (M)

FIG. 3. Competition curves for specific DHA binding to frog ventricular membranes by beta-adrenergic antagonists. The ordinate indicates the percent of maximal specific DHA binding, 0.0239 nM. The abscissa is the molar concentration of various antagonists. The symbols indicate the means of actual data points for 3 (butoxamine, A) to 6 (propranolol, \( \beta \), practolol, U) separate experiments with each antagonist.

COMPUTER RESOLUTION OF MYOCARDIAL BETA1 AND BETA2 RECEPTORS

The constants of the three antagonists for the apparently homogeneous beta1 receptors in the rat ventricle. Agonist competition curves (Figs. 1 and 2) for both frog and rat heart data were fit with a model involving at least two apparent classes of binding sites. Table 2 lists the relative proportions and dissociation constants of agonists for receptor subtypes. Also included in Table 2 are the agonist potency ratios for the beta receptor subtypes. A small fraction of frog beta receptors appears to possess the potency order expected of beta1 sites, whereas in the remainder, hydroxybenzylisoproterenol, a potent beta2-selective agonist, is 28-fold more potent than isoproterenol, and epinephrine is 15-fold more potent than norepinephrine, as expected for beta2 receptors. Two classes of beta receptors were apparent in rat heart experiments, both having similar potency orders for agonists, conforming to beta1 ex-