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COMMENTARY

CLASSIFICATION AND QUANTITATION OF β-ADRENERGIC RECEPTOR SUBTYPES*

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The existence of distinct β-adrenergic receptor subtypes was first postulated by Lands *et al.* [1, 2]. These investigators found that physiological responses that were mediated through activation of β-adrenergic receptors could be subdivided into two major categories, termed β_1 and β_2. This classification was based on the rank order of potency of a series of drugs in eliciting biological responses. Many investigators have subsequently obtained physiological evidence for the existence of β-adrenergic receptors with different pharmacological properties in a variety of tissues [3–5].

β_1-Adrenergic receptors have approximately the same affinity for epinephrine and norepinephrine. These receptors are found mainly in heart, adipose tissue and brain. β_2-Adrenergic receptors have a greater affinity for epinephrine than for norepinephrine and are found in lung and liver and in both smooth and skeletal muscle. However, due to problems such as restricted access of drugs to receptors and selective uptake and degradation of drugs by tissues, it has been difficult to determine the exact pharmacological specificity of β_1 and β_2 receptors. A wide range of pharmacological specificities has, in fact, been observed for β-adrenergic receptors in different tissue preparations [3–7], prompting the suggestion that there may be a virtually limitless spectrum of subtypes of β-adrenergic receptors [4]. Variations in the observed pharmacological specificity of a receptor in a particular organ may, however, be due to pharmacokinetic factors other than differences in the receptor itself. For example, the apparent selectivity of certain drugs for tracheal β-adrenergic receptors as compared to vascular β-adrenergic receptors was shown to be due to differences in the extra-neuronal uptake of these compounds [8]. In addition, the β-adrenergic receptors in a particular organ may not all be of a single subtype [9, 10], and variations in the pharmacological specificity of different tissue preparations may be due to varying ratios of receptor subtypes. These considerations have necessitated the development of methods for directly quantitating β-adrenergic receptors *in vitro*, using tissue homogenates.

β-Adrenergic receptors linked to adenylate cyclase

In most tissues, occupation of β-adrenergic receptors by agonists leads to an increase in adenylate cyclase activity. Under appropriate conditions this effect can often be observed following homogenization of the tissue. Since homogenization greatly reduces complications due to selective uptake and unequal access of drug to the receptor, several studies have examined the pharmacological specificity of β-adrenergic receptors linked to adenylate cyclase in membranes prepared from homogenates of a variety of tissues [11–16]. The β_2 selective agonists salbutamol and soterenol have been shown to activate adenylate cyclase in membranes prepared from the lung but not from cardiac tissue [11] or fat cells [12]. The β_1 selective antagonists practolol, metoprolol and para-oxprenolol block β-adrenergic receptor stimulated adenylate cyclase activity with a greater potency in heart [12–14] and adipose tissue [12, 13] than in liver [12, 13], trachea [13] or lung [12, 16]. These studies provide additional evidence for differences in the pharmacological specificity of β-adrenergic receptors in different tissues. In addition, these studies demonstrate that both β_1 and β_2 receptors can activate adenylate cyclase.

Examination of β-adrenergic receptors by monitoring radioligand binding

It is now possible to measure β-adrenergic receptors by directly monitoring the specific binding of radiolabeled high affinity antagonists to tissue fragments [17–20]. The two most widely used ligands are [^{3}H]dihydroalprenolol (DHA; $K_D \sim 1$ nM) and [^{125}I]iodohydroxybenzylpindolol (IHYP; $K_D \sim$ 50 pM). The binding of these compounds to membrane fractions from a wide range of tissues has been shown to satisfy the criteria expected for binding to β-adrenergic receptors. Binding is saturable, reversible and stereospecific, and displays the pharmacological specificity expected of binding to β-adrenergic receptors [17–21].

Experiments employing radioligands have a number of advantages over studies carried out *in situ* with perfused organs. The assays are done in tissue homogenates so that problems of access of drugs to their binding sites are minimized. Since the event being examined is the initial interaction of the ligand with the receptor, effects of the drug distal to the receptor (such as effects on the catalytic moiety of adenylate cyclase or on cyclic AMP-dependent protein kinase) are eliminated. In addition, compensatory effects mediated through other receptors or cellular processes do not occur. Since the reaction being monitored is a simple bimolecular interaction, it can be expected to display certain quantitative

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characteristics (rates of association and dissociation, equilibrium affinity constants) which can be experimentally determined. Furthermore, quantitative comparisons of the affinities of various drugs for receptors in different tissues is relatively straightforward in studies carried out with *in vitro* binding assays. Finally, the simplicity and technical ease with which experiments are performed can result in accurate data which is highly reproducible between experiments. Thus, radioligand binding assays can be very useful in determining the classification of receptor subtypes. On the other hand, it is important to recognize that most organs contain a variety of cell types. For example, the mammalian brain contains glial cells and blood vessels as well as a variety of types of neurons. It is likely that β-adrenergic receptors are associated with each of these cell types. It is also possible that some tissues contain both β_1- and β_2-adrenergic receptors and that both receptor subtypes can mediate the same physiological response [9, 10].

Use of radioligands to study receptor subtypes

Results obtained with binding assays have generally supported results obtained with perfused organs or studies of adenylate cyclase activity. Thus, the pharmacological specificity of tissues such as fat cells, heart and cerebral cortex appears to parallel that of β_1-adrenergic receptors, while lung, liver and cerebellum appear to contain a preponderance of β_2-adrenergic receptors.

Several groups of investigators have attempted to use receptor binding assays to classify β-adrenergic receptor subtypes [22–34]. All of the methods so far in use involve the same fundamental technique; that is, an examination of the characteristics of displacement of a radioligand by a drug which shows *in vitro* selectivity for β_1 or β_2 receptors. Although this approach can be very useful, there are several basic conditions which must be satisfied and several conceptual and methodological pitfalls which must be avoided if one is to develop an accurate method for quantitating β-adrenergic receptor subtypes. The major goal of this commentary is to define the assumptions that must be verified to validate the use of radioligand binding techniques to study receptor subtypes. The discussion will be oriented to a consideration of β-adrenergic receptor subtypes, but the principles may be directly applicable to consideration of subtypes of other neurotransmitter receptors.

Criteria for classification of β-adrenergic receptor subtypes

The fundamental criterion that must be fulfilled in the delineation of receptor subtypes is that *the properties of the receptor must be conserved*. This means that the pharmacological specificity of a particular receptor subtype must be identical in every tissue that is examined. For example, the properties of a β_1 receptor in the heart must be the same as the properties of a β_1 receptor in the brain, in adipose tissue, or even in a tissue that contains a preponderance of β_2-adrenergic receptors, such as the lung or cerebellum. If the pharmacological properties of a particular receptor subtype were to depend on the tissue in which the receptor is found, then clearly receptors should not be classified by examining their pharmacological properties. Fortunately, the ready availability of a variety of drugs makes it possible to rigorously define the pharmacological properties of particular β-adrenergic receptor subtypes.

When radioligand binding assays are used to distinguish β_1 and β_2 receptors, the inhibition of radioligand binding by drugs showing selectivity for β_1- and β_2-adrenergic receptors is measured. These data are then analyzed either directly [27] or after transformation to a modified Scatchard or Hofstee plot [23–26, 29–34], either graphically [23–26] or by computer [27, 29–34]. The analysis provides evidence as to the presence of one or more than one type of receptor in the preparation. If there are two apparent receptor subtypes, the relative proportion and affinity of each site for each drug can be calculated.

Several basic requirements must be met in the use of radioligand binding assays to study receptors. First, the interactions of the drugs with the receptor must be reversible and competitive. Second, the percentage of radioligand bound must be small so that the gradual displacement of the radioligand from its binding site does not change the concentration of free radioligand. If these requirements are met, then the actual equilibrium dissociation constant (K_D) of the displacing drug can be calculated by correcting for the concentration of radioligand by the method of Cheng and Prusoff [35]. This equation has been shown to be experimentally valid for the inhibition by various drugs of IHYP binding to membranes prepared from rat lung.* In this tissue it has been shown that the apparent K_D value of a drug for displacing IHYP binding, as calculated by the method of Cheng and Prusoff [35], is indeed independent of the concentration of the radioligand.*

There are several basic assumptions involved in the use of ligand binding assays to study β-adrenergic receptor subtypes. The fundamental assumptions in all of the published techniques are identical and are listed in Table 1. First and foremost is the assumption that there are only two subtypes of the receptor. Given the intrinsic limitation of the quality of data that can be obtained, none of the techniques for data analysis can distinguish between more than two or three classes of sites. Evidence relating to this assumption is discussed below. The second assumption is that the interactions of drugs (both agonists and antagonists) with each receptor subtype must

Table 1. Fundamental assumptions underlying available methods for measuring β-adrenergic receptor subtypes with radioligand binding assays

(1) There are only two β-adrenergic receptor subtypes in the tissues being examined.

(2) The interaction of agonists and antagonists with each receptor subtype follows simple Michaelis–Menten kinetics with Hill coefficients of 1.0.

(3) The radioligand has the same affinity for both receptor subtypes.

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follow simple Michaelis–Menten kinetics and display no evidence of positive or negative cooperativity. Available techniques do not distinguish complex negatively cooperative behavior from multiple receptor sites. The final assumption is that the radioligand has the same affinity for both receptor subtypes. This assumption can be easily examined by performing a Scatchard analysis of saturation isotherms of the radioligand binding. If the Scatchard analysis is linear and the apparent K_D value does not differ significantly between tissues with different populations of receptor, then it is reasonable to assume that the radioligand has a similar affinity for each of the receptor subtypes.

Effects of GTP on agonist interactions with β-adrenergic receptors

The interactions of a large number of antagonists with β-adrenergic receptors in a variety of tissues follow simple Michaelis–Menten kinetics resulting in Hill coefficients of 1.0 and linear Hofstee plots [17–19]. These results are obtained with non-selective antagonists, including propranolol, alprenolol, sotalol and iodohydroxybenzylpindolol. The Hill coefficient of [^{3}H]dihydroalprenolol binding has been reported to be less than 1 even in apparently homogenous tissues such as frog erythrocytes [20]. This finding complicates interpretation of data obtained when [^{3}H]dihydroalprenolol is used to quantitate β-adrenergic receptor subtypes.

The interactions of β-adrenergic receptors with agonists are apparently more complex than are interactions with antagonists. In some tissues, agonists display shallow-displacement curves consistent with apparent negative cooperativity (Hill coefficients of 0.6 to 0.8) [32, 36, 37]. This agonist-specific apparent negative cooperativity disappears in the presence of GTP. Inclusion of GTP in the binding assay also leads to a 4- to 10-fold reduction in the apparent affinities of agonists. It is important to note that Hill coefficients of less than 1.0 are seen in the absence of GTP with both selective and non-selective agonists. This apparent negative cooperativity is not related to and should not be confused with the coexistence of β_1 and β_2 receptors in some tissues. Thus, in the presence of GTP, the interactions of non-selective agonists with the β-adrenergic receptor appear to follow Michaelis–Menten kinetics and Hill coefficients of 1.0 are routinely observed. Tissue preparations used for radioligand binding are usually washed extensively to remove endogenous hormone. Since this washing procedure also removes most of the endogenous GTP [32, 36, 37] it is imperative that GTP be included in binding assays when selective agonists are used to distinguish the receptor subtypes. Since GTP affects both β_1 and β_2 receptors [32], failure to include GTP [23–27] leads to apparent high and low affinity forms of both receptor subtypes for agonists. Thus, tissues which contain only β_1- or only β_2-adrenergic receptors will erroneously be thought to contain two subtypes of receptor. Data obtained with tissues which contain both β_1- and β_2-adrenergic receptors will be essentially uninterpretable since two high affinity forms and two low affinity forms will be present. For example, in the study by Hancock *et al.* [27], GTP was not added to the binding assays when selective agonists were used to try to determine the number and properties of receptor subtypes. These investigators, therefore, reported the existence of both high and low affinity sites for both selective and non-selective agonists [27]. The authors concluded that the rat heart contains only β_1 receptors. However, when experiments are carried out with antagonists or with agonists in the presence of GTP [29], both β_1 and β_2 receptors are clearly seen to be present in rat heart.

Methods of data analysis

To quantitate the relative proportions of the two receptor subtypes and the affinity of each drug for each receptor, a graphic or mathematical analysis of the data is required. Three different methods of data analysis are currently in use. Rugg *et al.* [24] have transformed their data into a modified Scatchard (Hofstee) plot and analyzed it graphically. Minneman *et al.* [29] have also transformed their data into a modified Scatchard (Hofstee) plot and have subjected it to a computer-aided iterative analysis to calculate the proportions of the two receptor subtypes. Hancock *et al.* [27] analyzed their data directly without transformation using a non-linear least squares computerized curve fitting technique. All of these methods have advantages and disadvantages which will be discussed in turn.

The direct graphic analysis of non-linear modified Scatchard (Hofstee) plots used by Rugg *et al.* [24] is attractive in its simplicity. If drugs were available which were entirely selective for β_1 or β_2 receptors, this method would yield accurate results. However, the most selective drugs currently available have only a 20- to 100-fold selectivity. The errors implicit in direct graphic analysis are clearly seen in Fig. 1. This figure shows theoretical data for a drug with a 50-fold selectivity for the two sites. In calculating the data for Fig. 1, the proportion of the two receptor

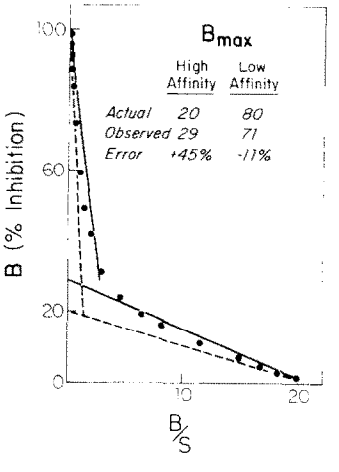

Fig. 1. Computer assisted analysis of Hofstee plots. The data points are those obtained if there are two classes of binding sites with affinities for the competing drug which differ by 50-fold. The ratio of low to high affinity sites is 4 : 1 (80 : 20 per cent). The solid lines represent the lines regressed through the points corresponding to the high and low affinity sites. The dashed lines show the results obtained with a computer assisted analysis.

subtypes was arbitrarily set at 20 per cent high affinity sites and 80 per cent low affinity sites. The solid lines represent the best fit lines as calculated by Rugg *et al.* [24], while the dotted lines correspond to the corrected lines for each of the two components. Direct graphic estimation resulted in a 45 per cent overestimate of the number of high affinity sites and an 11 per cent underestimate of the number of low affinity sites. Since these errors are in opposite directions, the error in the ratio of the concentration of high affinity sites to low affinity sites is even greater. While the correct ratio is 1 : 4, direct graphic analysis resulted in a ratio of 1 : 2.4. It is important to emphasize that the data in Fig. 1 assumed that the ratio of the affinities of the competing drug for the two subtypes was 50-fold. Substantially greater errors would result from the use of drugs which show selectivity ratios of only 20- to 30-fold.

A similar modified Scatchard (Hofstee) analysis was developed by Minneman *et al.* [29]. These authors subjected the transformed data to a computerized iterative analysis to correct each component for the contribution of the other component. This method results in an accurate analysis of theoretical data (Fig. 1; [29]) regardless of the degree of selectivity of the competing ligand. The major advantage of this method is that it combines the intuitive simplicity of the Hofstee plot (where the characteristics of the two components can be easily visualized as the asymptotes of the biphasic curve) with a mathematically accurate analysis. This method, however, does have technical limitations related to the quality of the experimental data. The major limitation is that the method relies heavily on data obtained at low concentrations of ligand where only a small amount (less than 20 per cent) of the binding of the radioligand is inhibited. Small errors in the amount bound are propagated to both the x- and y-coordinates. This method also uses an unweighted least squares linear regression analysis which assumes that the magnitude of the error is the same for all of the data points. Since the magnitude of the error actually increases with increasing concentrations of competing ligand, the data analysis would be improved by inclusion of a weighting term to account for the decreased accuracy when a large percentage of the binding of the radioligand is inhibited. It is clear, however, that the ability to resolve a relatively small high-affinity component is heavily dependent on the quality of the experimental data. Regardless of the method of analysis used one must obtain accurate data, particularly at low concentrations of competing drug, in order to obtain an accurate estimate of a small high-affinity component. The practical solution is to maximize the quality of the experimental data by performing multiple replicates in a single experiment and by pooling the data from multiple experiments.

Hancock *et al.* [27] have analyzed displacement curves directly, using a computer aided non-linear least squares curve-fitting technique. Since this method uses untransformed data, errors in the per cent inhibition of binding are not expressed on the abscissa (which in this case is drug concentration). This curve-fitting technique, therefore, results in increased accuracy as compared to the iterative graphic analysis described by Minneman *et al.* [29]. A further advantage of this approach is that the deviation of the observed data from the predicted values is weighted according to the reciprocal of the predicted variance. However, the method of Hancock *et al.* [27] also has certain disadvantages. In this type of analysis, the results of the 'black box' computer analysis must be taken at face value and cannot be intuitively verified. Furthermore, as noted previously, the binding of [^{3}H]dihydroalprenolol shows apparent negative cooperativity in frog erythocytes [20]. This complicates the use of dihydroalprenolol since the K_D value of both subtypes of receptor for the radioligand must be known before calculations can be carried out. In the presence of a Hill coefficient of less than 1.0, apparent rather than true K_D values are determined.

Drugs showing selectivity for β_1 or β_2 receptors in in vitro *binding assays*

A number of drugs have been reported to have different affinities for β_1 and β_2 receptors in radioligand binding assays (Table 2). Some of these drugs are more potent at β_1 receptors (β_1 selective) and some are more potent at β_2 receptors (β_2 selective). Due to clinical considerations, most of these drugs fall into one of two groups. A large number of β_1 selective antagonists have been developed for use in the treatment of angina. Similarly, there are a large number of β_2 selective agonists which are potentially useful in the treatment of bronchial asthma. The observed specificity of these drugs as defined by their ability to inhibit radioligand binding (Table 2) agrees well with their observed specificity *in vivo*. In other words, drugs that are β_1 selective in binding assays are cardioselective *in vivo* and drugs that are β_2 selective in binding assays are bronchial-selective *in vivo*. However, none of the drugs currently available are absolutely selective and most of them have only a 20 to 50 fold difference in affinity for β_1 and β_2 receptors.

A number of other drugs, such as salbutamol and soterenol, which have been shown to be selective in physiological studies are not selective in binding assays [16]. These drugs are agonists at β_2 receptors but antagonists at β_1 receptors, and they have similar affinities for both receptors in radioligand binding assays [16]. Since binding assays do not usually distinguish between agonist and antagonist activities, these agents show selectivity in eliciting a physiological response or in activating adenylate cyclase but show no selectivity in binding assays. Other drugs, including several congeners of methoxamine

Table 2. Drugs with different affinities for β_1 and β_2 receptors in radioligand binding assays

β_1 Selective agonist	β_2 Selective agonists
Norepinephrine	Zinterol (MJ1999)
	Salmefamol
	OPC 2009 (erythroprocaterol)
β_1 Selective antagonists	β_2 Selective antagonist
Practolol	IPS 339
Atenolol	
Metoprolol	
Para-oxprenolol	

(butoxamine and H35/25) which have been shown to possess *in vivo* selectivity for β_2 receptors [38], appear to have identical affinities for β_1 and β_2 receptors in radioligand binding assays [30]. The reason for this discrepancy is not clear; however, it is possible that the *in vivo* selectivity of these drugs is due to factors other than their affinities for the receptor.

Coexistence of β_1 and β_2 receptors in a single tissue

Carlsson *et al.* [9, 10] first suggested that β_1 and β_2 receptors could coexist in a single organ. The evidence now available from radioligand binding studies strongly supports this concept [23–27, 29–34]. In a variety of tissues, the inhibition of radioligand binding by non-selective drugs which have the same affinity for β_1 and β_2 receptors follows simple mass-action principles [24, 29]. However, in these same tissues, inhibition of radioligand binding by β_1 and β_2 selective drugs is more complex. In rat heart, lung, spleen, cerebral cortex, cerebellum, caudate, hippocampus and diencephalon, rabbit lung, and right atria from cat and guinea pig heart, the inhibition of specific IHYP and/or DHA binding by β_1 or β_2 selective drugs results in biphasic Hofstee plots indicative of two binding sites with different affinities for the selective drug [23–26, 29, 30, 39]. These results suggest that both receptor subtypes exist in each tissue. The relative proportions of the two receptor subtypes range from 85 per cent β_1 (rat heart) to 98 per cent β_2 (cerebellum from mature rats).

Biphasic Hofstee plots could reflect negative cooperativity rather than the existence of two distinct receptor subtypes. However, several lines of evidence suggest that this is not the case. In the first place, only drugs known to have different affinities for β_1 and β_2 receptors show curvilinear Hofstee plots [29, 30]. Other drugs which have similar affinities for the two receptors show linear Hofstee plots, indicating the absence of cooperative behavior. Furthermore, all drugs which had been shown to be selective, as assessed by inhibition of ligand binding to membranes enriched with respect to β_1 (rat heart) or β_2 (rat lung) receptors, showed this behavior [29, 39]. Third, in tissues containing only one receptor subtype the interaction of selective and non-selective drugs followed simple Michaelis–Menten kinetics with Hill coefficients of 1.0 [30]. Other data also suggest that the biphasic Hofstee plots observed with selective drugs are due to the presence of two distinct types of receptor. The relative proportion of the two receptor subtypes, as reflected in the apparent curvature of the Hofstee plots, varied markedly during development [34], and the density of β_1 receptors was affected by various pharmacological manipulations. These manipulations had no effect on the density of β_2 receptors [33]. These observations provide further evidence for the coexistence of two independent types of β-adrenergic receptors in the same organ. There are no data presently available which directly address the question of whether the two subtypes of β-adrenergic receptor can coexist in a single cell, or whether the coexistence of these receptors within an organ is due simply to the heterogeneity of cell types within that organ.

Evidence for the existence of only two β-adrenergic receptor subtypes in mammalian tissues

It is impossible to prove that there are only two β-adrenergic receptor subtypes in a given tissue. However, the available evidence strongly supports this conclusion. It is important to note that the properties of each receptor subtype are highly conserved in all mammalian tissues which have been examined. Thus, a β_1 receptor in one tissue has the same pharmacological specificity as does a β_1 receptor in every other tissue [29]. Furthermore, the relative proportions of the two receptor subtypes in a single tissue remain constant regardless of whether a β_1 or β_2 selective agonist or antagonist is used for the determination [29]. The results are thus internally consistent. The existence of a variable amount of a third subtype would be expected to yield discrepancies in terms of K_D values or in terms of the calculated proportion of each subtype. However, it would be difficult to identify a third subtype of β-adrenergic receptor if its properties were very similar to those of β_1- or β_2-adrenergic receptors.

Tissues containing only one receptor subtype

The strongest evidence that there are only two subtypes of β-adrenergic receptor comes from studies of tissues containing only one receptor subtype [30]. In these tissues, the inhibition of specific IHYP binding by β_1 and β_2 selective drugs results in linear Hofstee plots, indicating that there is only a single class of binding sites [30]. In the left ventricle of cat and guinea pig hearts, there appears to be a homogeneous population of β_1 receptors, while rat liver and cat soleus muscle contain homogeneous populations of β_2 receptors [30]. In these tissues it is possible to demonstrate directly that the interaction of each drug (including the selective drugs) with the receptor follows simple Michaelis–Menten kinetics, yielding linear Hofstee plots and Hill coefficients of 1.0. In addition, one can show that the affinities of each drug for each receptor in tissues that contain only one receptor subtype agree well with the affinities calculated from the computer-aided analyses of biphasic Hofstee plots [30]. This evidence strongly suggests that there are only two β-adrenergic receptor subtypes in mammalian tissues.

β-Adrenergic receptors in non-mammalian tissues

As discussed above, there is good evidence that only two subtypes of β-adrenergic receptors exist in mammalian tissues. However, in at least some non-mammalian tissues the kinetic properties and pharmacological specificity of β-adrenergic receptors are distinct from those of mammalian β_1- or β_2-adrenergic receptors. The β-adrenergic receptor of the turkey erythrocyte has been compared to mammalian β_1 and β_2 receptors [28, 40]. Although turkey erythrocytes contain an apparently homogeneous population of β-adrenergic receptors, these receptors have major kinetic and pharmacological differences that clearly distinguish them from mammalian β_1 or β_2 receptors [40]. In addition, characteristics of the β-adrenergic receptors in frog erythrocytes [21] and hearts [27] are very different from those we have observed for mammalian β_1 or β_2 receptors

Table 3. Criteria for a valid *in vitro* assay for β-adrenergic receptor subtypes

(1) The radioligand must bind to both receptor subtypes with the same affinity. Scatchard analysis of saturation isotherms must be linear, and similar K_D values should be observed in tissues containing either mostly β_1 or mostly β_2 receptors.

(2) The binding of nonselective drugs (both agonists and antagonists) should follow simple Michaelis–Menten kinetics, and calculated K_D values should be similar in tissues containing either mostly β_1 or mostly β_2 receptors.

(3) Selective drugs should be shown to have *different* affinities for inhibiting radioligand binding in tissues containing either mostly β_1 or mostly β_2 receptors. It is desirable to use both agonists and antagonists as well as both β_1 selective and β_2 selective drugs.

(4) In tissues containing both receptor subtypes, *all* of the selective drugs should show biphasic Hofstee plots for the inhibition of radioligand binding, while *none* of the nonselective drugs should show this behavior.

(5) Using appropriate methods for dissection of the two component Hofstee plots:

(a) The affinity of each selective drug should be the same for a particular receptor in each tissue examined.
(b) The relative proportion of β_1 to β_2 receptors should be independent of the particular selective drug used.

(6) Tissues containing apparently pure populations of each receptor subtype should be sought. In these tissues, all drugs (both selective and nonselective) should show linear Hofstee plots for the inhibition of radioligand binding.

(7) The affinity of each of the selective drugs for the receptor in tissues containing only one receptor subtype should be the same as in tissues containing both receptor subtypes.

[30]. Therefore, extrapolation of studies on β-adrenergic receptors in non-mammalian tissues to those in mammals should be approached with caution.

Summary

There is now convincing evidence that many mammalian organs contain both β_1- and β_2-adrenergic receptors and that the relative proportion of the two subtypes varies widely in different species and organs. The coexistence of β_1- and β_2-adrenergic receptors may explain many of the differences in relative drug potencies that have been described in studies with intact organs. Several methods have been developed recently which permit a quantitative determination of the density and properties of β_1- and β_2-adrenergic receptors in tissues which contain both receptor subtypes. The results obtained suggest that there are only two subtypes of β-adrenergic receptors in mammalian organs [30]. The two subtypes develop independently in regions of the rat brain [34], and the relative distribution varies widely in different organs [29]. Furthermore, the relative densities of β_1- and β_2-adrenergic receptors are independently affected by various pharmacological manipulations [33].

The existence of independently regulated β-adrenergic receptor subtypes makes it imperative to assess separately the effect of a given physiological or pharmacological manipulation on the receptor subtypes present in a tissue. The methods which are now available to study β-adrenergic receptor subtypes each have strengths and weaknesses and must be used with caution (see Table 3). In any case, it is likely that application of methods similar to those now available to study β_1- and β_2-adrenergic receptors will be useful in quantitative studies of subtypes of other neurotransmitter and hormone receptors.

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