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CARDIAC ADRENERGIC RECEPTORS

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ABSTRACT

Recently developed pharmacological and biochemical techniques have brought new insights about the structure, function, and regulation of β-adrenergic receptors. This chapter focuses on the cardiac β-adrenergic receptor and the possible clinical and physiological implications of this new information.

INTRODUCTION

Epinephrine and norepinephrine acting through β-adrenergic receptors produce both positive inotropic and chronotropic effects in the heart. Two characteristics of hormone- and drug-responsive tissues are the very low concentrations of these agents that are effective in evoking a biologic response and the structural specificity of the agent producing the response. These characteristics, namely high affinity and structural specificity, led to the concept of specific cellular receptors for catecholamines.

In 1948 Ahlquist first suggested that there were two types of adrenergic receptors, which he termed α and β (1). These were defined, using physiologic parameters, by their relative responsiveness to a series of sympathetic amines. The α-adrenergic receptor was characterized by a potency series of epinephrine > norepinephrine $\gg$ isoproterenol; for the β-adrenergic receptor the potency series was isoproterenol > epinephrine > norepinephrine. Lands et al (2) in 1967 provided evidence for two subtypes of β-adrenergic receptors termed β_1 and β_2. These were defined by their relative affinities for epinephrine and norepinephrine. β_1-Adrenergic receptors such as those in cardiac and adipose tissue display approximately

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equal affinity for epinephrine and norepinephrine. In contrast, β_2-adrenergic receptors such as those in bronchial and vascular smooth muscle have considerably greater affinity for epinephrine than norepinephrine (2). Agonist occupation of either β_1- or β_2-adrenergic receptors results in activation of the membrane-bound enzyme adenylate cyclase with consequent increased intracellular levels of cyclic AMP. This increased concentration of cAMP activates cAMP-dependent protein kinases that initiate a physiologic response.

α-Adrenergic receptors can also be classified into two subtypes termed α_1 and α_2 using both anatomical and pharmacological criteria (3). The α_1 receptors are the classical postsynaptic α receptors mediating smooth muscle contraction. α_2-Adrenergic receptors are found in several locations including presynaptic nerve terminals, where they mediate feedback inhibition of norepinephrine release, and on human platelets, where they mediate epinephrine-induced aggregation. The α_1- and α_2-adrenergic receptors can be distinguished pharmacologically by their relative affinities for various agonists and antagonists (4). For example, prazosin usually demonstrates a 1,000- to 10,000-fold selectivity for α_1-adrenergic receptors while yohimbine demonstrates a selectivity for α_2-adrenergic receptors. Since "presynaptic" α_2-adrenergic receptors can be found in non-neural tissues such as human platelets and adipose tissue, the pharmacologic classification has largely replaced the anatomical classification. The α_1 and α_2 receptors appear to mediate physiologic responses by different mechanisms. The α_2 receptors have been found to inhibit adenylate cyclase in a variety of tissues such as the human platelet where decreased levels of cyclic AMP are associated with platelet aggregation (5). In contrast, α_1 receptors appear not to interact with adenylate cyclase but may be associated with alterations in cellular calcium fluxes (5).

CARDIAC β-ADRENERGIC RECEPTORS

Cardiac β-adrenergic receptors were first directly identified in canine heart by radioligand binding techniques in 1975 (6). Subsequently, cardiac β-adrenergic receptors have been identified in a variety of nonmammalian and mammalian hearts, including the human heart (7). Radioligand binding assays of β-adrenergic receptors are similar in principle to the radioimmunoassay of hormone concentrations. The basic requirements for performing β-adrenergic receptor studies are a radiolabeled, biologically active drug (radioligand) of high affinity, a membrane preparation containing β-adrenergic receptors, and a reproducible method for separating ligand bound to the receptor from that which is free in solution. After incubating the membrane preparation with the radioligand, the bound and

free ligand can be separated by vacuum filtration using glass fiber filters (8). As might be anticipated, there is some nonspecific absorption of radioligand to the membranes as well as to the filter. In order to correct for this nonreceptor binding, membranes can be incubated with the radioligand in the presence of saturating concentrations of a specific β-adrenergic drug such as isoproterenol and the amount of radioligand bound determined. The amount of radioligand bound under these conditions represents "nonspecific" binding. By subtracting the nonspecific binding from the total binding (that observed in the absence of competitor), the "specific" binding is determined. With the use of radiolabeled β antagonists such as $(-)[^{3}H]$dihydroalprenolol, $(\pm)[^{125}I]$iodohydroxybenzylpindolol, or $[^{125}I]$iodocyanopindolol, it is possible to quantitate both β-adrenergic receptor number and antagonist affinity by constructing saturation curves. This is accomplished by adding increasing concentrations of radioligand to a constant amount of membranes and measuring the specific binding at each concentration of radioligand. The data can then be analyzed by either Scatchard analysis (8) or computer modeling techniques to determine receptor number and antagonist affinity (9).

β-ADRENERGIC RECEPTOR SUBTYPES

In 1972 Carlsson et al (10) provided pharmacologic evidence that both β_1- and β_2-adrenergic receptors were present and functional in the cat heart. They found that while β_1-adrenergic receptors are the predominant β-adrenergic receptor in both cat atrium and ventricle, β_2-adrenergic receptors have a more significant role in the sinus node than in the ventricle (10). Similar findings have now been demonstrated in both the human and canine heart (7). With the realization that cardiac tissues from a variety of species contain mixed populations of the two subtypes of β-adrenergic receptors, radioligand binding techniques were developed to assess the relative proportion of β_1- and β_2-adrenergic receptors present in the membrane preparation. Over the past ten years a number of β-adrenergic receptor subtype-selective antagonists and agonists have been developed that are 5- to 200-fold β_1 or β_2 subtype selective.

The use of these subtype-selective agents in radioligand binding experiments enables researchers to quantify the relative proportions of β_1- and β_2-adrenergic receptors. Currently the most widely applied technique is the construction of competition binding curves employing nonsubtype-selective radioligands versus subtype-selective competitors (9). In systems where two subtypes are present, competition curves obtained with such subtype-selective ligands are shallow or biphasic in character, while curves obtained with nonsubtype-selective antagonists in competition with

nonsubtype-selective radioligands are steep and uniphasic (see Figure 1). The complex nature of the competition curves of subtype selective antagonists relates to the differential affinity of these agents for the two receptor subtypes. Since the radioligand has equal affinity for both subtypes the competition of selective drugs essentially produces two overlapping curves whose summation is responsible for the two components observed in the competition curves (see Figure 1). In order to analyze such competition curves, a mathematical method of analysis must be applied to quantitate the relative proportions of the two receptor subtypes and the affinity of the competitor for each receptor. A technique now widely applied is the

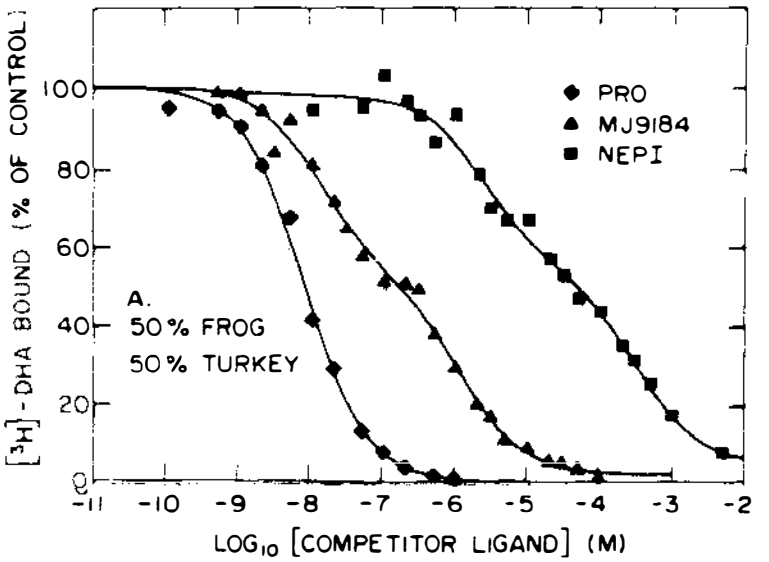

Figure 1 Competition curves for [^{3}H]DHA and β-adrenergic ligands for a membrane mixture containing equal proportions of β_1-adrenergic (turkey erythrocyte) and β_2-adrenergic receptors (frog erythrocytes). The data represent competition curves for propranolol (PRO) (a nonsubtype-selective antagonist); zinterol (MJ9184), a β_2 subtype-selective partial agonist, and norepinephrine (NEPI), a β_1 subtype-selective agonist. All binding curves were performed in the presence of guanine nucleotides to prevent formation of the agonist-specific high affinity state. It is apparent that the nonsubtype-selective antagonist propranolol cannot distinguish between the two receptor subtypes and hence produces a steep uniphasic curve. MJ9184 and NEPI can differentiate between the two subtypes and thus produce shallow biphasic curves that can be resolved into two components. The computer modeling techniques estimate that there are $54.1 \pm 1.5\%$ β_1-adrenergic receptors and $45.9 \pm 1.5\%$ β_2-adrenergic receptors. This is in close agreement with the actual values of 55.5% β_1 (turkey) and 44.5% β_2 (frog) adrenergic receptors determined from actual measurements of the receptor subtype densities added to the mixture (9). Data are from Reference 9.

analysis of untransformed binding data using a nonlinear least-squares computer modeling method based on mass action principles (9).

An example of this type of analysis in cardiac tissue is demonstrated in Figure 2. Membranes prepared from normal human left ventricle are incubated with the nonselective radioligand [^{125}I]iodocyanopindolol and increasing concentrations of a β_1 subtype-selective antagonist, atenolol, or the β_2 subtype-selective antagonist, zinterol, are added. Competition curves are then constructed using the experimentally determined data points and computer modeling techniques to determine the most appropriate curve to fit the data points (9, 11). Analysis reveals that the human left ventricle contains $86 \pm 1\%$ β_1-adrcnergic receptors and $14 \pm 1\%$ β_2-adrenergic receptors (11). Similar analysis of human right atrial membranes reveals that the atrium contains $74 \pm 6\%$ β_1-adrenergic receptors and $26 \pm 6\%$ β_2-adrenergic receptors (11). These results, i.e. that the heart contains a mixture of β_1- and β_2-adrenergic receptors with a higher proportion of β_2-adrenergic receptors in the right atrium, confirm the pharmacological data mentioned previously.

These findings have obvious clinical implications. For example, if a truly

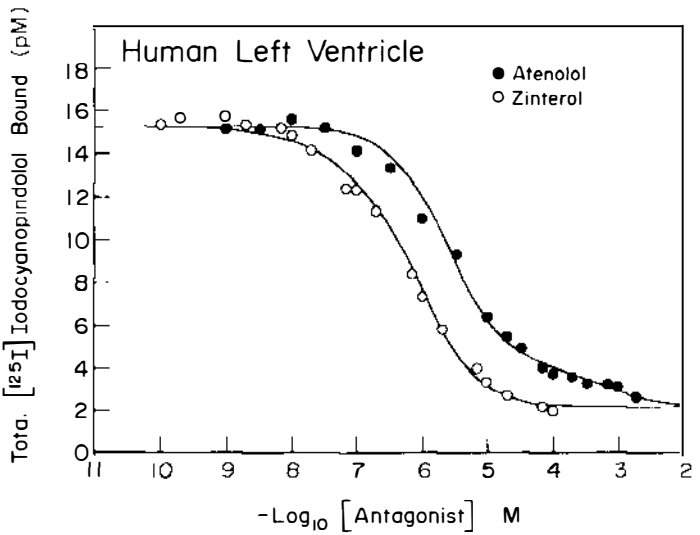

Figure 2 Competition curves for atenolol (β_1-selective antagonist) and zinterol (β_2-selective partial agonist) with [^{125}I]iodocyanopindolol in membranes derived from human left ventricular tissue. The data points are experimentally determined while the curves are computer generated (11). Computer modeling reveals that the proportion of β_1-adrenergic receptors is $86 \pm 1\%$ and that of the β_2-adrenergic receptor is $14 \pm 1\%$. These techniques provide a powerful method to estimate the relative proportions of β_1 and β_2 adrenergic receptors in tissues containing a mixture of both subtypes (11).

subtype-selective agent can be developed then one may be able to selectively alter chronotropy or inotropy as the clinical situation warrants. Dobutamine is a positive inotropic drug that comes close to fulfilling the criteria for a subtype-selective agent. If dobutamine (a β_1 selective agonist) is infused intravenously in man in doses equiionotropic to isoproterenol (a nonsubtype-selective agonist), the isoproterenol produces a much greater increase in heart rate than does dobutamine (12). This is consistent with the notion that β_2 receptors are involved with sinus node function while contractility is regulated primarily by β_1 receptors.

AGONIST-RECEPTOR INTERACTIONS

β-Adrenergic antagonists appear to inhibit catecholamine action simply by occupying the β-adrenergic receptor and thus denying agonists access to the receptor. It might therefore be expected that agonists would interact with β receptors in a different manner than antagonists since agonists promote physiologic changes and antagonists do not. Radioligand binding studies have provided insights into the differences between agonist and antagonist receptor interactions. It is now well established that guanine nucleotides such as GTP decrease the affinity of agonists but not antagonists for the receptor (13). This effect should not be surprising since it has been known for more than ten years that hormone-sensitive adenylate cyclase systems require GTP for effective enzyme activation (14).

Insight into how agonists, β-adrenergic receptors, and guanine nucleotides interact to promote activation of adenylate cyclase has been provided by radioligand binding studies in which agonists compete for occupancy of the receptor with a radioligand. It has become apparent that agonists but not antagonists can induce or stabilize a high affinity form of the β-adrenergic receptor. Thus, in the presence of a β-adrenergic agonist the β-adrenergic receptors exist in a dynamic equilibrium consisting of two affinity states, one of high affinity for agonists and one of lower affinity. Radioligand binding studies have demonstrated that the relative ability of an agonist to promote the formation of this affinity state is directly related to the intrinsic activity or efficacy of the agonist for activation of adenylate cyclase (13).

In addition to being required for activation of adenylate cyclase, guanine nucleotides also appear to perturb the agonist-specific dynamic equilibrium of high and low affinity states such that they convert all the receptors into the low affinity state (13). Experimentally this can be demonstrated by constructing agonist competition curves in the presence and absence of guanine nucleotides (Figure 3*a*). In the absence of GTP the agonist competition curve is shallow or biphasic, indicative of the ability of agonist

to differentiate between the two affinity states. By computer modeling techniques similar to those mentioned above, the affinity of agonists for each affinity state as well as the relative proportions can be determined (9, 13). In the presence of Gpp(NH)p (the nonhydrolyzable form of GTP) the agonist competition curve is shifted to the right (lower affinity) and steepens. This curve is now consistent with a single lower affinity state of the receptor indistinguishable from the low affinity state of the receptor observed in the absence of Gpp(NH)p. Thus, GTP or its analog simply shifts the dynamic equilibrium of high and low affinity states to only low affinity state receptors. Recently it was demonstrated that the high affinity state of the β-adrenergic receptor is composed of a ternary complex consisting of hormone, receptor, and a guanine nucleotide regulatory protein (15). The guanine nucleotide regulatory protein (N) is a membrane-bound protein that mediates the effects of guanine nucleotides and is directly responsible for activating the catalytic unit of adenylate cyclase (14, 15).

A current model incorporating these concepts into an overall scheme for the mechanisms involved in adenylate cyclase activation is shown in Figure

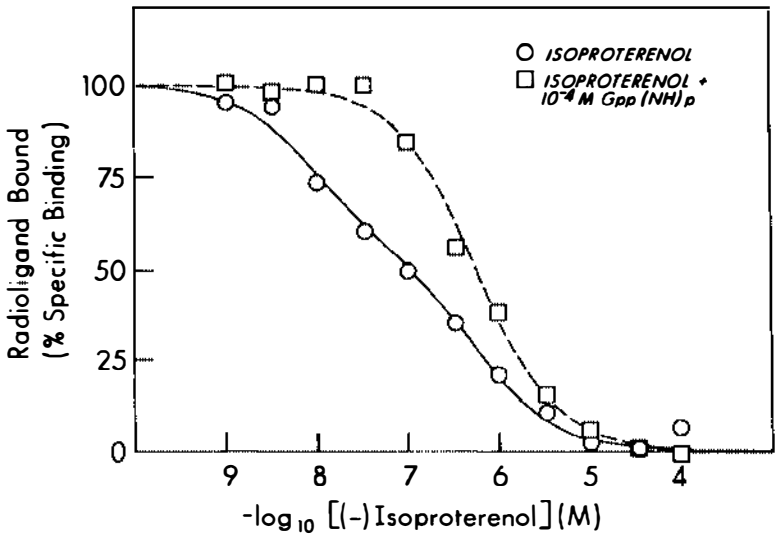

Figure 3a Isoproterenol competition curves in the presence and absence of guanine nucleotides [Gpp(NH)p] in rat heart membranes. Isoproterenol at the indicated concentrations competed for occupancy of the β-adrenergic receptor with [^{3}H]DHA (2 nM). The membrane preparation as well as the radioligand binding conditions have recently been described (17). The data points were experimentally determined while the fitted curves were computer generated (9).

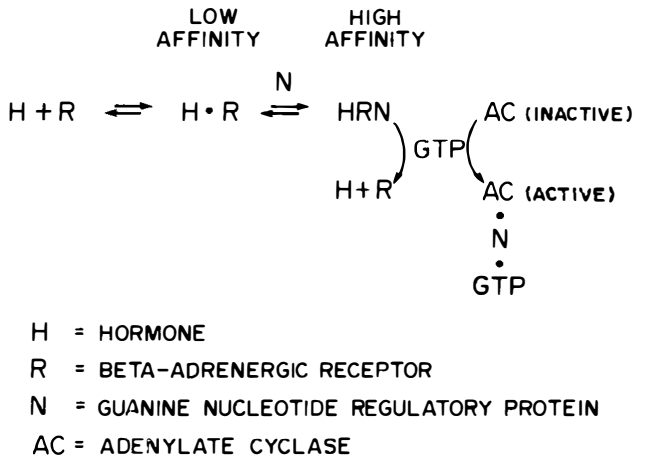

Figure 3b Schematic model for the activation of adenylate cyclase by catecholamines.

3*b*. When an agonist interacts with a β-adrenergic receptor, it induces a change in the receptor (presumably conformational) that promotes the stabilization of a "ternary complex" consisting of hormone, receptor, and N protein (HRN). The formation of this complex is associated with the loss of tightly bound GDP from the N protein, which is then replaced by GTP (the guanine nucleotide required for activation). The association of GTP with the ternary complex destabilizes it so that the receptor reverts to its low affinity form. The N-GTP complex can then interact with the catalytic unit of adenylate cyclase thereby activating it to generate cyclic AMP. This activation is terminated by a GTPase (associated with the N protein) that cleaves the terminal phosphate of GTP converting it to GDP, which does not promote activation (16).

The ability of agonists to promote the association of H, R, and N to form the ternary complex, the essential intermediate in activation of adenylate cyclase, has been referred to as the "coupling" process. The more "tightly coupled" a system, the more effective an agonist is in producing a biochemical or physiologic response. As we illustrate below, recent findings suggest that the formation of the HRN complex, i.e. the coupling process, as delineated by radioligand binding and computer modeling, is modulated by a variety of pathophysiologic conditions.

PHYSIOLOGIC REGULATION

With the advent of radioligand binding techniques and the ability to quantitate β-adrenergic receptors, it became evident that β-adrenergic

receptors were not static entities but were dynamically regulated by a variety of pathophysiologic states. Receptor number appeared to increase (up-regulate) or decrease (down-regulate) in response to various hormonal perturbations. Examples of such regulation in cardiac tissue are demonstrated in Table 1.

Although changes in receptor number are important, it is no longer adequate simply to describe receptor number changes. As mentioned earlier regulation of tissue sensitivity to catecholamines may be controlled at various steps of the transmembrane signaling process. The three major components of the β-adrenergic receptor–adenylate cyclase system, namely the β-adrenergic receptor, N protein, and the enzyme adenylate cyclase, can all be regulated in terms of both quantitative and qualitative changes. These alterations all potentially perturb the coupling process of the system and thus change the efficacy with which agonists produce a physiologic effect.

Two conditions known to decrease the ability of β agonists to activate adenylate cyclase are hypothyroidism and desensitization or tachyphylaxis due to prolonged exposure of tissue to catecholamines (7). Depending on the model system studied, regulation of receptor number or receptor coupling or both may be the major point(s) of control (7, 13). Uncoupling of β-adrenergic receptors from the N protein is apparent as a rightward shift in the agonist competition curve to lower affinity. Computer modeling techniques permit the quantification of such alterations and suggest that the change in overall affinity is due to the decreased ability to form the high affinity ternary complex (HRN) of receptor and N protein manifested by a decreased affinity of agonists for the high affinity state of the receptor (13). This suggests that the uncoupling observed is due to impaired ability of R and N to interact in an effective manner (13). The exact mechanisms responsible for an impaired ability of R and N to interact remain unknown and could be due to alterations in R, in N, or in the membrane milieu in which they normally interact.

Evidence is beginning to accumulate to suggest that enhanced coupling can also occur (17). Examples of alterations in the coupling process in cardiac tissue are also demonstrated in Table 1.

To date, only a single study of the physiologic regulation of β-adrenergic receptors in the human heart has been published (18). In this report left ventricular muscle from normally functioning hearts was compared to tissue from hearts in end-stage congestive heart failure. A 50% decrease in the β-adrenergic receptor density was found concomitant with a 45% decrease in maximal isoproterenol-stimulated adenylate cyclase activity. The fact that fluoride-stimulated and histamine-stimulated adenylate cyclase activities were unaltered suggests that the alteration in isoproterenol stimulation was in fact a catecholamine-specific effect and not a

Table 1 Regulation of the cardiac β-adrenergic receptor–adenylate cyclase system[a]

Species	Intervention	Receptor number	Receptor coupling[b]	Adenylate cyclase[c]
Rat	hyperthyroidism	increased	increased	increased
Rat	hypothyroidism	decreased	no change	decreased
Rat	adrenalectomy	no change/increased	increased	decreased
Rat	HBP (Goldblatt)	decreased	N.D.	N.D.
Rat	HBP (DOCA-salt)	decreased	N.D.	decreased
Rat	HBP (SHR)	no change/decreased	decreased	decreased
Rat	6OH dopamine	increased	N.D.	N.D.
Rat	propranolol	increased/no change	N.D.	N.D.
Rat	guanethidine	decreased	N.D.	N.D.
Rat	ethanol treatment	decreased	N.D.	N.D.
Rat	ethanol withdrawal	increased	N.D.	N.D.
Rat	diabetes	decreased	N.D.	N.D.
Rat	isoproterenol administration	decreased	no change	decreased
Guinea pig	CHF	increased	N.D.	N.D.
Dog	ischemia	increased	N.D.	increased
Chick embryo	isoproterenol	no change	N.D.	decreased
Human	CHF	decreased	N.D.	decreased

[a] For individual references, see a recent review (7). Abbreviations are as follows: N.D. = not determined; DOCA = deoxycorticosterone; SHR = spontaneously hypertensive rat; CHF = congestive heart failure.

[b] As determined by agonist competition radioligand binding (see text).

[c] Activity as determined by cAMP generation.

generalized defect in the catalytic unit of adenylate cyclase. The authors suggest that this decreased sensitivity to isoproterenol with consequent decreased catecholamine-induced contractility may be an important factor in congestive heart failure. This interesting conjecture, however, must be kept in perspective. When assessing any end-stage disease it may be difficult to differentiate whether a given finding is responsible for the disease or a consequence of the disease. This is particularly true in congestive heart failure where circulating catecholamine levels are elevated in end-stage disease and this could conceivably induce down-regulation or desensitization. Of course, it is also possible that such a secondary form of receptor regulation might exacerbate the congestive heart failure by diminishing the effectiveness of the normal sympathetic mechanisms that support the failing heart. Further work will be required to understand the role, if any, of perturbations of the β-adrenergic receptor–adenylate cyclase system in the initiation or exacerbation of congestive heart failure.

β-ADRENERGIC RECEPTOR STRUCTURE

A comprehensive understanding of the mechanisms involved in catecholamine action in the heart at the biochemical level requires a knowledge of the structure and molecular properties of the β-adrenergic receptor. There are currently two major approaches to the problem of isolating and characterizing the structure of the β-adrenergic receptor.

The first involves the purification of the glycoprotein containing the β-adrenergic binding site by a variety of biochemical techniques such as affinity chromatography, ion exchange chromatography, and high performance liquid chromatography (7). These techniques have been applied to several model systems including frog and turkey erythrocytes, which contain homogeneous populations of β_2- and β_1-adrenergic receptors, respectively. Shorr et al (19) have demonstrated that the β_2-adrenergic receptor from frog erythrocytes can be solubilized in an active form with digitonin and then purified 55,000-fold, to yield a single glycoprotein with a molecular weight (M_r) of 58,000. This peptide demonstrates all the pharmacologic properties expected of a β_2-adrenergic receptor. Purification of the turkey erythrocyte β_1-adrenergic receptor reveals two peptides with molecular weights of 45,000 and 40,000. Each of these peptides bind adrenergic ligands with β_1 subtype specificity (20). To date, these purification techniques have not been successfully applied to the β-adrenergic receptor from mammalian cardiac tissue.

A second approach to elucidation of the structure of β-adrenergic receptors, namely photoaffinity labeling, has been successfully applied by Stiles et al (21) to the cardiac β-adrenergic receptor from several mam-

malian species including that from the human heart. This technique involves the use of a high affinity β-adrenergic antagonist whose structure has been modified to include a light-sensitive azide side group that upon exposure to ultraviolet light can covalently incorporate into proteins. The molecule is also modified to include a radiolabel in the form of ^{125}iodine; this permits the detection of the protein into which the antagonist has been covalently linked. One such compound, *p*-azido-*m*-[^{125}I]iodobenzylcarazolol ([^{125}I]*p*ABC), has been recently synthesized and characterized (22). Using this compound, Stiles et al (21) demonstrated that the human myocardial β_1-adrenergic receptor binding subunit resides on a peptide of $M_r \approx 62{,}000$. This peptide displays all the pharmacological characteristics expected of a β_1-adrenergic receptor.

Figure 4*a* shows the results obtained with photoaffinity labeling of the

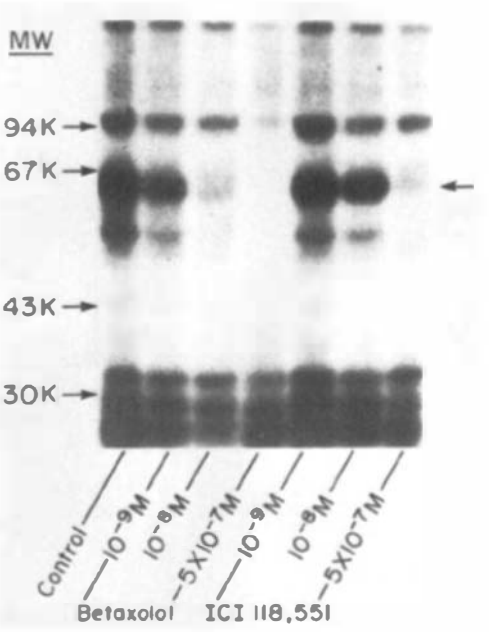

Figure 4a Photoaffinity labeling of human myocardial β-adrenergic receptors. Aliquots of human left ventricular membranes were incubated with *p*-azido-*m*-[^{125}I]iodobenzylcarazolol alone (control) or with the indicated concentration of betaxolol (β_1-selective antagonist) or ICI 118,551 (β_2-selective agent) and photolabeled as recently described (21). The receptors were then solubilized and subjected to SDS-polyacrylamide gel electrophoresis (21). The molecular weight markers (MW) are shown × 1000 (K) and represent the relative mobility of proteins of stated molecular weight. Each lane represents a separate aliquot of labeled membranes. The arrow at the right represents the 62,000-dalton receptor binding subunit. The dark bands on this autoradiograph represent where radioactivity has been incorporated into proteins.

human cardiac β-adrenergic receptor with [^{125}I]*p*ABC in the absence or presence of a competing drug such as betaxolol, a β_1-selective antagonist, or ICI 118,551, a β_2-selective antagonist. Following photoaffinity labeling and solubilization, the peptides are subjected to polyacrylamide gel electrophoresis, a technique that separates proteins based on their molecular weights. The gel can then be exposed to x-ray film and an autoradiograph developed to demonstrate where radioactivity has been incorporated. For comparison, the results obtained with radioligand binding are shown in Figure 4*b* in which betaxolol and ICI 118,551 compete for occupancy of the β-adrenergic receptor with [^{125}I]cyanopindolol, a high affinity, nonsubtype-selective radioligand. The curves demonstrate that the betaxolol is more potent in competing for occupancy of the receptor than is ICI 118,551 as would be anticipated since the human left

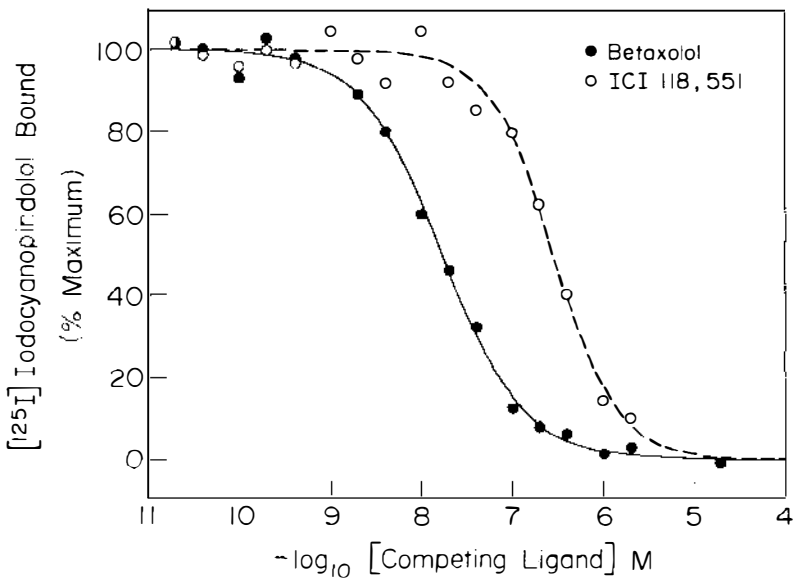

Figure 4b Radioligand binding competition curves of betaxolol (β_1-selective) and ICI 118,551 (β_2-selective) for [^{125}I]iodocyanopindolol ([^{125}I]CYP), a nonsubtype-selective antagonist radioligand. The data points are experimentally determined while the fitted curves are computer generated (9). The maximum [^{125}I]CYP bound represents specific binding as determined by 10^{-4} M isoproterenol. It is apparent that the betaxolol is more potent in competing for occupancy of the receptor than is ICI 118,551 as would be expected for a β_1-selective agent. A comparison of the data from Figures 3*a* and 3*b* reveals that there is an excellent agreement between the ability of these agents to compete for occupancy of the receptor with [^{125}I]CYP and to prevent photoincorporation of [^{125}I]*p*ABC into the receptor protein.

ventricle contains predominantly β_1-adrenergic receptors (11). It can be appreciated that the 62,000-M_r peptide seen in the gel autoradiograph displays the same characteristics demonstrated by the β-adrenergic receptor in membranes as defined by radioligand binding. Thus, occupancy of the β-adrenergic receptor by the competing drug prevents [^{125}I]*p*ABC from gaining access to the β-adrenergic receptor and hence prevents it from being covalently incorporated into the receptor protein structure. Also evident on the autoradiograph is a 55,000-M_r peptide that also displays all the characteristics of a β_1-adrenergic receptor. It is now known that this 55,000-M_r peptide is a proteolytic product of the 62,000-M_r peptide generated during membrane preparation (21). Thus, it is the 62,000-M_r peptide that recognizes and binds catecholamines in the heart and is responsible for initiating the transmembrane signaling process that results in a physiologic response.

This new technique has exciting implications for investigating catecholamine regulation of myocardial function. The technique will permit, for the first time, studies of structural alterations that may occur as the result of various pathophysiologic states such as myocardial ischemia or drug-induced tachyphylaxis. Examples of this type of regulation, i.e. alterations in receptor structure, have recently been demonstrated in certain model systems in which isoproterenol-induced desensitization or tachyphylaxis occurs. These structural alterations in the β-adrenergic receptor are manifested by altered receptor mobility upon gel electrophoresis (23). Whether or not the same type of regulation occurs in mammalian heart remains to be determined.

α-ADRENERGIC RECEPTORS

Much less information is available for α-adrenergic receptors than for β-adrenergic receptors. α-Adrenergic receptors have been directly identified by radioligand binding studies only in rat and guinea pig hearts (24–26). As mentioned above, there are two types of α-adrenergic receptors, termed α_1 and α_2. To date, only α_1-adrenergic receptors have been conclusively demonstrated in cardiac tissue (24). Very few studies have addressed the question of whether or not there is pathophysiologic regulation of α-adrenergic receptors in the heart. α_1-Adrenergic receptor number in the rat heart appears to be regulated by altered thyroid status in a direction opposite to that observed for changes in β-adrenergic receptors. Thus, in hyperthyroidism α_1-adrenergic receptor number appears to decrease while in hypothyroidism the number is increased (26). A single report suggests that α_1-adrenergic receptors may decrease in guinea pig hearts following induction of congestive heart failure (25). The physiologic consequences

of these observations remain unknown. A striking difference between α_1-adrenergic receptors and β-adrenergic receptors in the heart is that α_1-adrenergic receptors appear not to be coupled to adenylate cyclase as are β-adrenergic receptors. The mechanism of action for α_1-adrenergic receptors is most likely related to alterations in calcium-ion fluxes across the membrane or perhaps changes in phosphatidylinositol turnover (5).

CONCLUSIONS

In this brief review, we have attempted to demonstrate how recently developed techniques such as radioligand binding and photoaffinity labeling have provided new insights into the mechanism of action of catecholamines on the heart. It is already clear that membrane-bound adrenergic receptors are dynamically regulated both in terms of number and their "coupling" to the other components of the adenylate cyclase system. These alterations appear to directly influence the physiological effects of catecholamines.

These new methodologies will be useful in developing new α- and β-adrenergic agents including subtype-selective agents for clinical use. In addition, these new experimental tools will provide an approach for investigating how catecholamines interact with the heart at the membrane level in both health and disease.

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