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ADRENERGIC RECEPTORS IN THE HEART[1]

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Introduction

Catecholamines, acting through alpha- and beta-adrenergic receptors, modulate a variety of physiological responses in the heart. Most importantly catecholamines increase the rate and force of cardiac contraction. These actions occur mainly as a consequence of the binding of the endogenous substances norepinephrine and epinephrine to specific adrenergic receptors on the plasma membrane of cells in the heart. Whereas the effects of sympathetic nervous stimulation on the heart have been examined for many years (reviewed in 57), only recently has it become possible to measure directly the properties of the receptors for catecholamines in the heart. Here we discuss a few selected areas of active research where radioligand binding techinques have been applied to the study of adrenergic receptors in the heart.

Direct Demonstration of Adrenergic Receptors

Beta-adrenergic receptors were first identified in the dog heart using [^{3}H]DHA in 1975 (3). [^{3}]DHA binding sites had the characteristics expected of interaction with beta-adrenergic receptors. For example, the sites recognized catecholamines with the appropriate stereospecific potency series. Subsequently, beta-adrenergic receptors have been identified and characterized in the hearts of many other species including rat, frog, cat, chick embryo, rabbit, guinea pig, and mouse (5, 10, 14, 16, 17, 25, 27, 28, 37, 45,

[1]Abbreviations: [^{3}H] DHA, (–)[^{3}H]dihydroalprenolol; [^{3}H]DHE, [^{3}H]dihydroergocryptine; SHR, spontaneously hypertensive rats.

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49, 65, 67, 70). Radioligand binding has been used to measure directly the affinity of a variety of drugs for beta receptors. Also, regulation of the number of beta receptors in the heart by various influences has been extensively examined. For example, the effects of development and ageing (7, 9, 14, 56), ischemia (51), guanine nucleotides (29, 66), and altered thyroid state (reviewed in 32) have been extensively investigated.

In the hearts of several species there also appear to be alpha-adrenergic receptors that induce contractile responses (reviewed in 57). Alpha-adrenergic receptors have been demonstrated in the heart with [^{3}H]DHE in rat (24, 59, 62, 69), rabbit (58), and fetal lamb (15). Also, [^{3}H]WB4101 (55, 75), and [^{3}H]prazosin (35) have been used to label alpha receptors in the heart.

Beta-Adrenergic Receptor Subtypes in the Heart

Ahlquist initially distinguished alpha- and beta-adrenergic receptors in 1948 (2). Subsequently, in 1967, Lands et al (39) found two subtypes of beta receptors termed beta$_1$ and beta$_2$. Beta$_1$ and beta$_2$ receptors were operationally defined by their relative affinities for epinephrine and norepinephrine: Beta$_1$ receptors have approximately equal affinity for epinephrine and norepinephrine, whereas beta$_2$ receptors have a higher affinity for epinephrine than for norepinephrine. A wide variety of drugs are now available with different affinities for one or the other beta receptor subtype [for recent review see (53)].

Recent interest has been focused on the beta-adrenergic receptor subtypes mediating inotropic and chronotropic responses in the heart, particularly in light of the clinical implications. Both beta$_1$ and beta$_2$ receptors are found in the hearts of certain species. A number of studies have found differences in the chronotropic and inotropic effects of series of catecholamines (e.g. 11, 19). Using a number of selective beta-adrenergic agonists and antagonists, Carlsson et al (12) found that the chronotropic effects of these drugs seemed to be mediated by both beta$_1$ and beta$_2$ receptors in the cat heart. Subsequently, these workers found that the atria from dog and human hearts exhibited the same heterogeneous response (1). Carlsson et al have extended their physiological findings in cat heart to indicate that while beta$_1$ receptors are the predominant beta-adrenergic receptor in both cat atrium and ventricle, beta$_2$ receptors have a more significant role in the sinus node than in the ventricle (13). O'Donnell & Wanstall (52) also found that both beta$_1$ and beta$_2$ receptors mediated chronotropic responses in isolated cat atria but that chronotropic responses in isolated guinea pig atria were apparently mediated exclusively by beta$_1$ receptors. The study of Dreyer & Offermeier suggests differences in the beta-adrenergic receptors mediating chronotropic and inotropic responses in guinea-pig atria (19). Thus the cat atrium might serve as a good model for the human situation, whereas the guinea-

pig atrium might be a better tissue for examining the potency of novel agonists and antagonists at $beta_1$-adrenergic receptors since chronotropic responses do not seem to involve functionally significant $beta_2$ receptors (52). Some workers have speculated that the $beta_1$ receptors respond to norepinephrine released from nerve terminals in the mammalian heart whereas the $beta_2$ receptors are activated by circulating epinephrine from the adrenal medulla. Interestingly, in the amphibian heart where epinephrine rather than norepinephrine is the neurotransmitter, the predominant receptor mediating atrial contraction is a $beta_2$ receptor (61).

Stimulated by such observations, extensive efforts have been made to determine quantitatively the number of $beta_1$ and $beta_2$ receptors in the heart. No radioligands presently available label exclusively $beta_1$ or $beta_2$ receptors; indeed, the two most commonly used beta-adrenergic receptor ligands, [^{3}H]DHA and [^{125}I]iodohydroxybenzylpindolol, do not distinguish between $beta_1$ and $beta_2$ receptors. Therefore in order to distinguish $beta_1$ from $beta_2$ receptors in the heart, several groups have used unlabelled drugs shown to have different affinities at $beta_1$ and $beta_2$ receptors in intact tissues. Competition curves of these drugs with the radioligand are constructed. The relative proportion of $beta_1$ and $beta_2$ receptors can then be determined by a number of analytical techniques. The affinities of the $beta_1$ and $beta_2$ receptors for the selective drugs may be calculated. Using a graphical method based on a modified Scatchard plot, Nahorski and collaborators (8) found that while rat lung contained both $beta_1$ and $beta_2$ receptors, rat heart contained predominantly, if not exclusively, $beta_1$ receptors. Minneman, Molinoff and their co-workers have investigated beta receptors in the hearts of several species in a similar fashion and analyzed their data using a computer-assisted iterative technique (47). A third approach for delineating receptor subtypes (25, 30) involves the computer modeling of untransformed binding data from competition curves using a nonlinear least-squares curve-fitting technique. In our view, the latter approach is the most appropriate; the relative merits of these techniques have been discussed elsewhere (41, 48). Using the curve-modelling technique Hancock et al (25) found that rat ventricle contains exclusively $beta_1$ receptors whereas frog ventricle contains predominantly $beta_2$ receptors as well as ~20% $beta_1$ receptors. Minneman et al (47) suggested that rat heart contains 83% $beta_1$ and 17% $beta_2$ receptors, although they did not demonstrate that the data were statistically better fit by two receptor subtypes than by $beta_1$ receptors exclusively. These studies (46) provide useful information about directly determined affinities of a variety of selective drugs for $beta_1$ and $beta_2$ receptors. The heart contains a variety of cell types. Since fibroblast-like cells from the heart grown in culture contain $beta_2$ receptors (40), results of such studies from cardiac homogenates must

be interpreted with caution. By these techniques it has also been shown that the atria from cats and guinea pigs contain both $beta_1$ and $beta_2$ receptors whereas the ventricles contain essentially only $beta_1$ receptors (28). These results are in good general agreement with the physiological findings discussed above.

Regulation of Adrenergic Receptors by Catecholamines

Exposure of a wide variety of cells to agonist drugs or hormones for a period of time leads to a state of decreased responsiveness to further stimulation by that agonist. This diminished responsiveness, occurring as a result of prolonged exposure to elevated concentrations of, for example, catecholamines, has been referred to as desensitization, tachyphylaxis, or refractoriness. Desensitization of beta-adrenergic receptor stimulation of adenylate cyclase has been characterized extensively in a number of model systems —e.g. frog erythrocytes (50, 68) and cultured cells (60, 63).

The mechanisms by which catecholamines may produce desensitization of beta-adrenergic receptors coupled to adenylate cyclase are multiple and complex. The phenomenon of desensitization is quite general; however, the molecular mechanisms by which this occurs may vary considerably from one cell type to another. Radioligand binding techniques have demonstrated that alterations in the beta-adrenergic receptor itself may contribute to at least some forms of catecholamine-induced refractoriness. However, other forms of desensitization appear to involve lesions occurring mainly or perhaps exclusively distal to the receptors. Intracellular cAMP concentration is modulated by its synthesis by adenylate cyclase and degradation by phosphodiesterases. Either of these processes may be modified in a desensitized cell. The activation of adenylate cyclase by catecholamines is dependent on the functioning of beta-adrenergic receptors, adenylate cyclase molecules, and guanine nucleotide regulatory components which appear to be essential intermediates between beta receptor stimulation and adenylate cyclase activation. Modification of one or more of these components may be the mechanism for desensitization in a particular cell. The role of cyclic nucleotides in the heart has recently been reviewed (20).

The results of direct examination and characterization of some of these components for the desensitized beta-adrenergic receptor-adenylate cyclase system in the heart have recently been reported. Chronic injection of rats with isoproterenol leads to a hypertrophied heart that exhibits a decreased magnitude and sensitivity of contractile response to in vitro stimulation by isoproterenol. Tse et al (64) extensively investigated the mechanisms by which this change occurred. Desensitization of the heart to isoproterenol was associated with a reduction in both sensitivity and maximal response

of adenylate cyclase to activation by isoproterenol. A decrease in the number of beta-adrenergic receptors in the desensitized hearts was also noted. The beta receptors were measured with [^{3}H]DHA whose affinity for the receptors was unchanged after desensitization. Thus it appears that loss in beta-adrenergic receptors with isoproterenol injection plays a major role in desensitization. However, there was also a loss in NaF-stimulated adenylate cycle in the desensitized hearts which might suggest a lesion(s) in either the catalytic unit of adenylate cyclase or the guanine nucleotide regulatory component. The latter possibility may be reflected in the apparent decrease in affinity of the remaining receptors for isoproterenol (64). It has been suggested that high-affinity agonist binding reflects interaction of the beta receptor with the guanine nucleotide regulatory component (44). There appears to be an impaired ability of agonists to bind with high affinity to the beta receptors in this model of desensitization in the heart; this phenomenon has been extensively characterized in desensitized frog erythrocytes (36). Quite different findings have been reported in another model of cardiac desensitization. March et al (45) exposed chick embryo ventricles to 1 μm isoproterenol for 30 min; this led to a diminution in the subsequent inotropic response to isoproterenol. There was no loss, however, in beta-adrenergic receptor number measured with [^{3}H]DHA; also NaF-stimulated adenylate cyclase was unchanged in the densensitized hearts. The major defect appeared to be diminished maximal stimulation by isoproterenol of adenylate cyclase in membranes derived from the desensitized hearts. This was interpreted to indicate an uncoupling of the beta-adrenergic receptor —adenylate cyclase complex, though there was apparently no loss in the beta receptors's affinity for isoproterenol assessed in competition curves with [^{3}H]DHA. There are thus major phenomenological differences in the results of these two important studies. These could reflect differences in the species used (rat vs chick embryo); compare, for example, the mechanism of desensitization in frog erythrocytes (68) with that in turkey red cells (31). Also, the time point at which the mechanisms of desensitization were evaluated could be an important variable. In the astrocytoma cell line studied by Harden et al (26), a functional uncoupling of the beta-adrenergic receptors occurs prior to the loss of beta receptors from the membranes.

These models of desensitization may be relevant to changes that occur in the heart with various forms of hypertension (4). For example, the hearts of spontaneously hypertensive rats (SHR) show diminished sensitivity to the inotropic and chronotropic effects of isoproterenol (38). Limas & Limas (42) reported a diminished number of beta-adrenergic receptors (without change in affinity for [^{3}H]DHA) in SHR compared with normal controls. Interestingly, the diminished number of beta-adrenergic receptors was al-

most fully evident by 5 weeks of age, even though the rise in blood pressure was quite modest at that point. The authors conjectured that these early changes in beta receptors might reflect increased sympathetic drive in these animals (33, 34). However, Bhalla et al found similar numbers of beta-adrenergic receptors in SHR and controls with a reduced affinity of isoproterenol for the receptors in SHR (9). Woodcock et al (71–73) reported that in 3 different models of hypertension (one-kidney Goldblatt, desoxycorticosterone acetate/salt, and SHR) there were similar reductions in cardiac beta-adrenergic receptors. Adenylate cyclase activities were measured in cardiac membranes from the former two models of hypertension. It was found that maximal isoproterenol-activated adenylate cyclase was dimished whereas NaF-stimulated activities were not diminished compared to controls (72). The authors also reported decreased alpha-adrenergic receptor numbers in the hearts of hypertensive animals, whereas there was no change in the number of beta receptors in the lungs or kidneys of these animals (73).

The receptor changes in these models of hypertension in the rat are reminiscent of those in isoproterenol-induced desensitization of the heart (64) described above. Whether the desensitization seen in hypertension is induced by elevated catecholamines, increased sympathetic tone or transmitter release (18), or some other mechanism is unclear. While there seems to be some agreement that hypertension causes a reduced beta-adrenergic receptor number in the heart, one other study (21) found no change in receptor number.

Since catecholamines can desensitize beta-adrenergic receptors, it is possible that the presence of endogenous catecholamines modulates the number and/or properties of beta-adrenergic receptors in the normal heart. Indeed, several models have been developed wherein the heart is depleted of catecholamines leading to hypersensitivity. Increased responsiveness of adenylate cyclase to catecholamines is seen in rat hearts denervated by 6-hydroxydopamine or depleted of catecholamines by reserpine (54). Also, increased sensitivity is observed in the hearts of SHR after withdrawal of propranolol treatment (38). Treatment of rats with propranolol for two weeks was found to lead to an increase in beta-adrenergic receptor number in the heart (22). However, another study found no change (6). Whether the number of beta receptors in the human heart increases after propranolol treatment is not known. Also, denervation of the rat heart with guanethidine (23) or 6-hydroxydopamine (74) is associated with an increased number of beta-adrenergic receptors. Furthermore, depletion of catecholamines in the heart as a consequence of hypertrophy induced by aortic constriction apparently led to an increase in beta-adrenergic receptor number (43), though the relationship of this finding to the decrease in the number of

beta-adrenergic receptors seen in certain models of hypertension is unclear. While beta-adrenergic receptors may increase in the heart, the relationship of these findings to the putative "propranolol withdrawal syndrome" in man is unknown.

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