Biochemical Pharmacology, Vol. 36, No. 1, pp. 1–6, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

COMMENTARY

LIFE CYCLES OF CARDIAC α_1- and β-ADRENERGIC RECEPTORS*

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Cell surface receptors for hormones and neurotransmitters are dynamic entities whose expression is altered in a variety of settings. The heart contains both α_1- and β-adrenergic receptors that regulate both rate and force of cardiac contraction, and changes in the number and affinity of these receptors have been found in various physiologic, pharmacologic, and disease settings. Unfortunately, only limited mechanistic information is available to explain such changes in receptor expression. Our thesis in this commentary is that further insights into receptor regulation will require detailed understanding of the "life cycle" and "itinerary" of cardiac adrenergic receptors.

Receptor life cycles

The steady-state level of receptor expression in target cells represents the interplay of cellular processes involved in receptor appearance on, and disappearance from, the cell surface. A number of discrete events contribute to receptor appearance and disappearance. Our knowledge of these events has largely derived from studies of receptors for nutrients [e.g. low density lipoprotein (LDL), transferrin, etc.] and to a lesser extent from studies of nicotinic cholinergic receptors, which are linked to Na^+ entry, or of receptors for hormones such as insulin and epidermal growth factor (EGF), whose mechanisms for transmembrane signaling remain ill-defined (reviewed in Refs. 1–5). It is not yet clear whether other types of drug and neurotransmitter receptors, which transduce signals across membranes via guanine nucleotide binding (G) proteins, will utilize the same cellular mechanisms for receptor appearance and disappearance. However, as a working hypothesis, we assume that similar mechanisms are involved (Fig. 1). Thus, as with other membrane glycoproteins, cell surface receptors are synthesized in the Golgi apparatus and probably initially glycosylated at the time of translation of receptor mRNA. Subsequent processing of the carbohydrate moieties of the glycoproteins occurs during transit or storage in the Golgi. Receptors stored in Golgi vesicles move through the cell's cytoplasm and are ultimately incorporated ("inserted" or "externalized") into the surface membrane. For some classes of receptors this insertion process leads to a random distribution of receptors within the plasma membrane, whereas for others the receptors appear to rapidly congregate in clathrin-rich coated pit regions on the cell surface.

After insertion, receptors can undergo several dif-

* Work in the authors' laboratories is supported by grants from NSF, NIH, American Heart Association, and the Veterans Administration.

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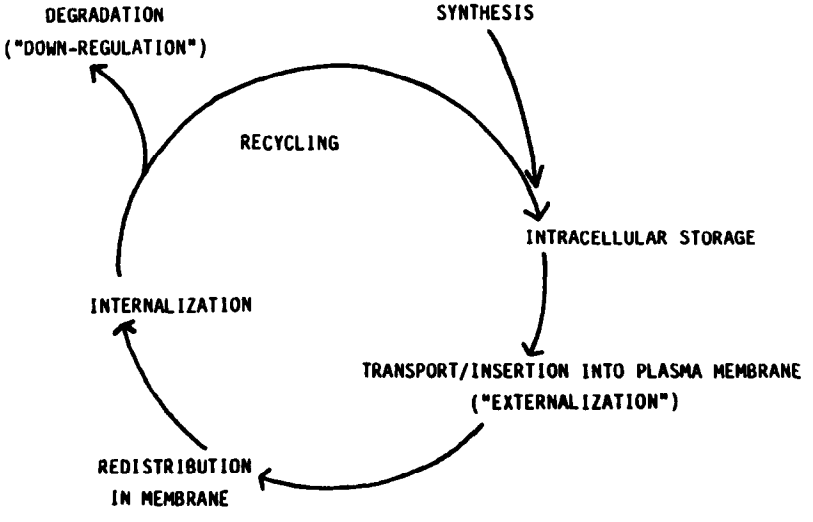

Fig. 1. Life cycle of cell surface receptors.

ferent fates [2–4]: receptors can await interaction with agonists, unoccupied receptors can be internalized back into the target cell, or they can be sloughed from the cell surface. Interaction of agonists with receptors promotes internalization of agonist–receptor complexes. Internalization of receptors is generally thought to occur by pinching off of coated pit regions of the cell surface and subsequent entry of receptors into a distinct class of intracellular vesicles (termed receptosomes or endosomes). These vesicles have several possible itineraries, including intracellular storage, shuttling or recycling to the membrane (perhaps after fusion with Golgi components), and intracellular degradation by lysosomes or other cellular organelles. Receptors inside of cells may exist in an environment in which they are difficult to detect; thus, it may be impossible to use conventional radioligand binding techniques with intact cells to assess properties of intracellular receptors.

Life cycle of β-adrenergic receptors

Less is known about the life cycle of β-adrenergic receptors than is known about receptors for molecules such as transferrin, EGF, or LDL, and even less is known about the life cycle of α-adrenergic compared to β-adrenergic receptors.

Two approaches have been used. One approach is to measure the rates at which receptor binding sites appear to be synthesized and degraded. Optimal methods for such studies in which anti-receptor antibodies are used to examine receptor turnover have not yet been possible for adrenergic receptors. Limited data obtained with other procedures, such as incorporation of heavy amino acids, blockade of protein synthesis, and recovery of receptor binding after irreversible inactivation of receptors, have demonstrated that β-adrenergic receptors typically have a lifetime of several days and that the rates of both synthesis and degradation can be modulated [6–9]. These types of experimental methods have not yet been applied extensively to the heart, although Baker and Pitha injected rats with an irreversible blocking agent, and based on the 200-hr period required for recovery of radioligand binding sites, it would appear that cardiac β-adrenergic receptors are synthesized rather slowly [6]. Occupancy of β-receptors by agonists can lead to a decrease in receptor number; this occurs primarily by enhancement of receptor disappearance and not by suppression of receptor appearance [9–11].

A second approach to examine adrenergic receptor life cycles has been to define "movement" (i.e. change in location) of receptors among various cell fractions. These types of studies have been used primarily to study agonist-promoted desensitization of β-adrenergic receptors (recently reviewed in Refs. 10, 12 and 13). The interactions of agonists with β-adrenergic receptors lead to the rapid activation of adenylate cyclase, the generation of cAMP, subsequent activation of cAMP-dependent protein kinase, and stimulation of various functional responses. Without several minutes, however, β-adrenergic receptors become refractory or desensitized, and cAMP accumulates at a much slower rate. Two methods have been used to demonstrate that desensitized β-adrenergic receptors are located in a cellular environment distinct from the plasma membrane site in which receptors exist under basal conditions. First, the location of the receptor binding sites has been examined after tissues are homogenized and fractionated by differential centrifugation. Desensitized receptors are typically not found in fractions that have plasma membrane markers but instead appear in lighter membrane fractions. The second approach has been to use intact cells and measure the accessibility of the receptors to a variety of ligands. The most useful probe has been the hydrophilic β-adrenergic antagonist CGP-12177, which binds to the vast majority of β-adrenergic receptors on control cells, but to a smaller fraction of receptors on desensitized cells. Desensitized receptors are also inaccessible to catecholamines, although inaccessibility alone is unable to account for decreased functional activity of the receptors [13, 14].

In numerous cell types, agonist treatment promotes the rapid (seconds to minutes) entry of β-receptors into a cellular environment where they are less accessible to certain ligands and where, after tissue homogenization, the receptors appear in light vesicles. This process has been termed "internalization", although sometimes vaguer terms such as "redistribution" or "sequestration" have been used. It is not clear whether the β-receptors are actually internalized into cells and, if so, whether these receptors are internalized by the same pathway used by LDL, EGF and transferrin receptors. Perkins and colleagues [12, 15] have approached this question by comparing the agonist-induced change in β-adrenergic receptors with that of EGF receptors in cultured astrocytoma cells. Exposure to agonist translocates both types of receptors into light vesicles which sediment identically on sucrose gradients; moreover, phenylarsine oxide blocks this process for both types of receptors [15]. These data suggest that β-adrenergic receptors are processed and internalized similarly to receptors for peptide hormones. Other evidence, however, leads to a contrary conclusion.

Placing cells in acid removes peptide hormones from the cell surface without removing intracellular receptors [16, 17]. Such "acid stripping" also occurs for ligands bound to β-adrenergic receptors of S49 lymphoma cells, but unlike results obtained for transferrin receptors of these cells, this "acid stripping" occurs equally for surface and redistributed β-receptors [18]. In this respect, redistribution of β-adrenergic receptors differs from internalization of peptide hormone and nutrient receptors. Moreover, in studies of desensitized β-adrenergic receptors from frog erythrocytes, desensitized receptors were only recovered in light vesicles when the cells were homogenized vigorously; with gentler lysis the receptors migrated with the plasma membrane fractions [19].

The events described above all occur within a few minutes after exposure of β-adrenergic receptors to agonist. Over a longer time course extending to hours or days, other events occur that result in a loss of the total number of detectable β-receptors. This "down-regulation" probably reflects a decrease in the total number of receptors, but may also reflect other events such as covalent modification, which

Table 1. Settings associated with increase or decrease in cardiac β-adrenergic receptor number

Increased receptor number	Decreased receptor number
β-Adrenergic antagonists	β-Adrenergic agonists
Myocardial ischemia	Hypertrophy/Heart failure
Hypertrophy/Heart failure	Pheochromocytoma
Denervation	Hypothyroidism
Hyperthyroidism	

would alter the receptors so that they no longer bind ligands, or sequestration, in which receptors would be located in a compartment to which ligands have no access [13]. The ability of agonists to promote receptor redistribution is probably necessary but may not be sufficient to account for agonist-mediated down-regulation [20].

β-Adrenergic receptors and the heart

Contraction of cardiac muscle is regulated by both neuronally derived and circulatory catecholamines. At β-adrenergic receptors, the resultant activation of adenylate cyclase and the increase in intracellular cyclic AMP alter action potential duration and promote interaction of contractile proteins, perhaps due to changes in calcium availability. Continuous exposure of the myocardium to catecholamines can be associated with a decrease in cardiac inotropic responsiveness as well as an associated decrease (down-regulation) in the number of β-adrenergic receptors (Table 1). Conversely, reduction of β-adrenergic stimulation, either by blockade of receptors with antagonists or by denervation of the heart, leads to an up-regulation of cardiac β-adrenergic receptors (Table 1).

For example, Tse *et al.* [21] injected rats with isoproterenol for 10 days and produced myocardial hypertrophy in association with a decrease in ventricular function, a 33% decrease in number of β-adrenergic receptors, and a decreased ability of isoproterenol to compete for β-adrenergic receptor sites (defined with an antagonist radioligand). The latter result suggests a decreased ability of receptors to form a high-affinity ternary complex between agonist receptors and G_s protein [10, 13]. These data and those obtained in other animal studies are similar to results observed in patients with end stage heart failure [22]. In these patients, the number of β-adrenergic receptors in the left ventricles was decreased by 50% (compared with the number found in normal controls), and this change was associated with about a 50% decrease in isoproterenol-stimulated adenylate cyclase activity and in isoproterenol-induced muscle contraction. These results suggest that circulating catecholamines, which elevate during the development of heart failure, presumably to help maintain inotropic support, may cause a down-regulation of β-adrenergic receptors and a desensitization of the heart to catecholamine action.

One approach in probing mechanisms of regulation of cardiac β-adrenergic receptors is to study isolated cardiomyocytes or cultured cardiac cells. Recent advances with these techniques have led to some mechanistic insights relating the life cycle of adrenergic receptors to the state of cardiac responsiveness to catecholamines. Marsh *et al.* [23] have used cultured ventricular cells from embryonic chick heart and have found that exposure of cardiac β-adrenergic receptors to agonist promotes two phases of receptor regulation: a rapid (<30 min) initial desensitization, which requires intact microfilament function, followed by down-regulation or loss of receptors, which depends on intact microtubules. In addition, Limas and Limas [24] have used cultured cardiac myocytes from rats to examine development and recovery from agonist-induced desensitization, as assessed by [^{3}H]CGP-12177, which, as noted above, selectively detects receptors at the cell surface. As in other cell types exposed to agonist, rat cardiomyocytes show a rapid and reversible loss in [^{3}H]CGP-12177 binding and in β-agonist-promoted cAMP generation; recovery of [^{3}H]CGP-12177 sites upon removal of agonist occurs rapidly and appears to represent a cell energy-dependent recycling of receptors [24]. The Golgi apparatus and microtubules may be involved in this recycling, as suggested by inhibitory effects of monensin (which destroys the pH gradient in vesicles) and colchicine (which blocks microtubule assembly). Data from these cellular studies suggest that cardiac muscle, as a functional unit, might respond to catecholamines in a manner similar to that of other cells and tissues, i.e. by desensitization of certain physiological responses in parallel with a rapid redistribution and a slower down-regulation of β-adrenergic receptors.

We have examined the life cycle of cardiac β-adrenergic receptors using a different approach—assessment of receptors in various subcellular fractions [25]. Using modifications of previously described techniques, we have isolated two fractions of guinea pig left ventricle, a sarcolemma (plasma membrane) fraction and a light (intracellular) fraction that sediments at 130,000 *g* but not 45,000 *g*. When observed by electron microscopy, the light fraction consisted entirely of small vesicles. This fraction contains only a very small amount of activity of 5′-nucleotidase or adenylate cyclase (stimulated by forskolin, GppNHp, or fluoride), and agonists show low affinity, guanine nucleotide-insensitive binding to β-adrenergic receptors. Thus, this light vesicle fraction is probably deficient in the G_s protein and in the catalytic unit of adenylate cyclase and is probably distinct from the sarcolemma. In control animals, we find approximately equal numbers of β-receptors in the sarcolemma and the light vesicle fraction.

We have investigated the distribution of cardiac β-adrenergic receptors in these two fractions in several

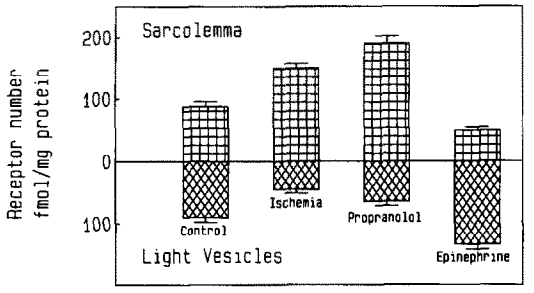

Fig. 2. β-Receptor density (fmoles/mg) defined by [^{125}I]iodocyanopindolol binding in sarcolemma and light vesicle fractions from guinea pig hearts in control animals, animals undergoing 1 hr of ischemia, propranolol treatment (0.15 mg/kg/hr) for 1 week, or epinephrine injection (0.25 mg/kg/i.p.).

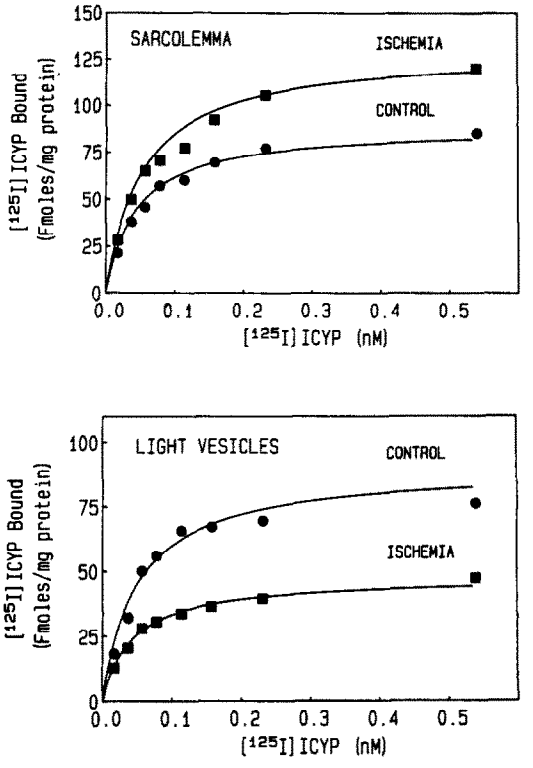

Fig. 3. Specific binding of [^{125}I]iodocyanopindolol, a β-adrenergic receptor radioligand, measured in sarcolemmal and light vesicle fractions of guinea pig hearts after 1 hr of ischemia. The top panel shows that substantially more receptors are present in the sarcolemmal membrane of ischemic cells than in control cells. In contrast, ischemia produces a decrease, compared to controls, in receptor binding sites that can be recovered in light vesicle membranes (bottom panel).

settings: administration of agonists and antagonists and in myocardial ischemia (Fig. 2) [25–27]. Within 45 min after an intraperitoneal injection of epinephrine (0.25 mg/kg) or isoproterenol (0.2 mg/kg), a substantial portion of the surface β-receptors are "internalized" from the sarcolemma to the light vesicle fraction. This is associated with a decrease in isoproterenol-stimulated adenylate cyclase activity in the sarcolemma membranes. Similar data have been obtained by others [28].

In two other settings that we have examined—administration of β-adrenergic antagonists and in myocardial ischemia—we find an opposite change in β-receptor distribution in guinea pig heart, i.e. an "externalization" from light vesicle to sarcolemmal fraction [25, 27]. Guinea pigs infused via subcutaneously implanted osmotic minipumps with propranolol (0.15 mg/kg/hr for 7 days) had a substantial enhancement in β-adrenergic receptor number in sarcolemmal membranes and a depletion of receptors in light vesicle fractions. This receptor redistribution increases the number of surface β-adrenergic receptors that are functional, i.e. that can increase isoproterenol-stimulated adenylate cyclase activity. This redistribution of receptors probably explains previous observations of antagonist-mediated up-regulation of β-adrenergic receptors in the heart and may also explain the "propranolol withdrawal syndrome" in patients in whom the drug is rapidly stopped (e.g. Refs. 25 and 30). We speculate that a continuous shuttling of β-adrenergic receptors occurs between the surface plasma membrane and the intracellular light vesicles. Externalization of β-adrenergic receptors by propranolol is most likely due to a blockade of tonic internalization promoted by endogenous catecholamines. Alternatively, propranolol treatment might decrease synthesis of β-adrenergic receptors (thereby depleting intracellular receptors) or accelerate the translocation of receptors to the surface.

Myocardial ischemia is another setting in which β-adrenergic receptors are reported to be up-regulated [31]. We have developed a model for ischemia in guinea pigs and have observed that, with 30–90 min of ischemia, the number of β-adrenergic receptors in the surface sarcolemma membranes could be reversibly increased about 50% with a concomitant 60% decrease in the light vesicle fraction (Figs. 2 and 3) [25]. These externalized β-adrenergic receptors appear to be functionally active, because isoproterenol-stimulated adenylate cyclase activity was increased. Thus, it appears that myocardial ischemia can cause an externalization of β-adrenergic receptors to the surface, where they may mediate increased catecholamine stimulation.

Since β-adrenergic receptor antagonists, such as propranolol, can prevent or blunt ischemia, we have also tested whether animals treated with propranolol (using the protocol described above) might have blunted externalization of receptors [26] We have found that, after propranolol treatment, ischemia does not alter receptor distribution as it does in control animals. It is possible that propranolol treatment blunts the stimulus for externalization, but more likely it depletes the same intracellular pool of β-adrenergic receptors that can be mobilized by ischemia. These results may help explain a protective role of propranolol treatment in preventing arrhythmias or sudden death associated with myocardial ischemia.

We found recently that treatment of guinea pigs with the β_1-selective antagonist atenolol, as with the non-selective antagonist propranolol, can promote

externalization of β-adrenergic receptors to the surface and can blunt the receptor redistribution that occurs with ischemia [32]. However, pindolol, a β-adrenergic blocking agent that possesses intrinsic sympathomimetic (partial agonist) activity, does not appear to cause an externalization of β-adrenergic receptors and does not prevent the externalization of receptors with subsequent ischemia. This is in agreement with other observations indicating that pindolol treatment does not produce receptor up-regulation or a withdrawal syndrome [33, 34]. The studies with various agonists and antagonists emphasize a key role for intrinsic activity in determining whether internalization or externalization of cardiac β-receptors will occur in response to drug therapy. Moreover, these findings also lend support to the notion that cardiac β-adrenergic receptors in experimental animals and perhaps humans are chronically down-regulated to a certain degree by the tonic stimulation produced by endogenous catecholamines.

α-Adrenergic receptors in the heart

Compared to the extensive literature documenting physiologic and pharmacologic regulation of cardiac β-adrenergic receptors, much less is known about the regulation of α-adrenergic receptors in the heart. Alpha$_1$-adrenergic receptors are the predominant subtype of α-receptor in the myocardium of most species, but their role in cardiac physiology is not yet clearly defined [35]. Recent studies have indicated that these receptors are linked to the hydrolysis of phosphoinositides in cardiac myocytes [36] and that their structure, as assessed by photoaffinity labeling, is similar to α_1-adrenergic receptors in other tissues [37]. Under certain conditions, cardiac α_1-adrenergic receptors can be down-regulated by agonists. For example, rats with norepinephrine-secreting pheochromocytomas have fewer cardiac α_1-adrenergic receptors that do control animals [38]. In hyperthyroidism, the number of cardiac α_1-receptors may also decrease [39]. Other studies have shown that myocardial ischemia and congestive heart failure may be associated with an increase in expression of α_1-adrenergic receptors in crude cardiac membranes [40, 41].

Studies of the regulation of α_1-adrenergic receptors on myocytes have not been undertaken but we recently examined distribution of α_1-adrenergic receptors in subcellular fractions (as defined above) in settings of agonist treatment and myocardial ischemia [27]. We found several notable differences from β-adrenergic receptors. First, under control conditions, the number of α_1-adrenergic receptors present in light vesicles was only 25% of that found in sarcolemmal fractions, whereas a roughly equal number of β-receptors was found in sarcolemmal and light vesicle fractions. Within 1 hr of myocardial ischemia, the number of α_1-adrenergic receptors in the sarcolemma increased about 30% but the number of receptors in the light vesicles did not change. Thus, unlike the ischemia-induced changes in distribution of β-adrenergic receptors, the increase in number of surface α_1-adrenergic receptors in ischemia is not explained by an externalization of receptors from the light vesicle fraction to the sarcolemma. In addition, with agonist (epinephrine) treatment, α_1-adrenergic receptor number was decreased in the sarcolemmal fraction but was not increased in the light vesicle fraction. Thus, the agonist-induced internalization of β-adrenergic receptors from the surface sarcolemma to the light vesicles was not observed for α_1-adrenergic receptors. Therefore, changes in surface α_1-adrenergic receptors appear not to result from entry or exit of α_1-receptors from the pool detected in the light vesicle fraction that we prepare. The exact cellular location of α_1-adrenergic receptors in heart will require further study. Perhaps some of these receptors are sequestered ("cryptic") on the inner surface of the plasma membrane and are relatively inaccessible to ligands, but can be altered by ischemia or by agonist treatment. Such cryptic α_1-adrenergic receptors might provide a pool of receptors that could be recruited under certain conditions.

Conclusions and future directions

Although much less is known about the details of the life cycle for adrenergic receptors than is known for other types of receptors, it seems likely that such life cycles are quite intricate for both α_1- and β-adrenergic receptors in the heart. A more complete understanding of the regulation of adrenergic receptors in various physiological and pathological states demands more than studies that just assess receptor number and affinity; our strong bias is that studies must be designed to probe alterations in the receptor life cycle. Our recent data reviewed above suggest that intracellular redistribution of adrenergic receptors is an important mechanism for modulation of cardiac responsivity to catecholamines. In other preliminary studies, we found that redistribution of adrenergic receptors may also accompany other pathological processes, such as myocardial hypertrophy.

We believe that three approaches will need to be emphasized in future studies. First, understanding the cellular basis of the receptor life cycle in the heart will almost certainly require further studies with cardiomyocytes. Experiments with these cells offer the opportunity to define changes in myocardial cells maintained in carefully controlled environments. However, cardiomyocytes are difficult cells to study, as properties of cells from embryonic or neonatal animals may not accurately reflect those of adult animals, and adult myocytes may not remain viable in culture for the lengthy period required for extensive studies of receptor metabolism. Second, further efforts at subcellular fractionation are likely to be fruitful. Our studies have demonstrated that β-receptors can apparently translocate between the sarcolemma and a light vesicle fraction, but the exact identity of that light fraction is as yet unclear. A number of possibilities—sarcoplasmic reticulum, T tubule, etc.—need to be tested; attempts to localize receptors to specific cellular organelles may prove helpful. Third, new approaches are needed to study adrenergic receptor life cycles. Studies of many other types of receptors have been expedited by the availability of antibodies against the receptor, especially antibodies that recognize an epitope distinct from the ligand binding site. However, high-affinity anti-

bodies against α- and β-adrenergic receptors have not yet been used in studies of the receptor life cycle. In the near future it should also be possible to examine adrenergic receptor expression by measuring receptor-specific mRNA levels using DNA probes [42]. Mechanistic studies of drug and neurotransmitter receptors are rapidly evolving toward more cell biological and molecular biological approaches. The application of these approaches to cardiac adrenergic receptors seems likely to yield important new insights into the physiology and pharmacology of the heart.

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