MECHANISMS OF DISEASE
Franklin H. Epstein, M.D., Editor

MECHANISMS OF MEMBRANE-RECEPTOR REGULATION
Biochemical, Physiological, and Clinical Insights Derived from Studies of the Adrenergic Receptors
Robert J. Lefkowitz, Marc G. Caron, and Gary L. Stiles

VIRTUALLY all hormones and drugs initiate their biologic actions by binding to specific cellular recognition sites, termed receptors. Receptor binding is followed by alterations of cellular metabolic events, such as enzyme activities or ion fluxes, that are ultimately expressed as characteristic physiologic or pharmacologic effects. Although the existence of receptors has been hypothesized for about 100 years, only within the past 15 years have they been directly studied as distinct cellular macromolecules. The development of the remarkably simple technique of radioligand binding opened this new era of investigation. This technique involves using radioactively labeled hormones or drugs, either agonists or antagonists, and directly measuring the binding of the substance to its receptor. To be sure, a variety of pitfalls can confound the approach, but it has now been successfully applied to most of the known major types of receptors. This technique has revolutionized the way in which investigators study the interactions of biologically active substances with their target tissues.

Few substances have more diverse actions than catecholamines such as epinephrine and norepinephrine. These agents, along with synthetic agonists that mimic their actions and antagonists that block their effects, are among the most important therapeutic agents available to physicians. The actions of these drugs are mediated by two major classes of receptors termed α-adrenergic and β-adrenergic receptors. Two subtypes of each receptor, termed α₁ and α₂ and β₁ and β₂, were initially defined on the basis of pharmacologic criteria. Their existence was subsequently confirmed by direct binding studies. The properties of the major subtypes of adrenergic receptors are summarized in Table 1. This information emphasizes the crucial role of the adrenergic receptors in regulating not only the entire cardiovascular system but a variety of metabolic processes as well. Three of the four subtypes of adrenergic receptors are linked to the same biochemical effector — the adenylate cyclase system, which generates the second messenger, 3′,5′-cyclic adenosine monophosphate or cAMP. The β₁ and β₂ receptors stimulate the enzyme, whereas the α₂ receptors inhibit it. α₁ Receptors appear not to be coupled to adenylate cyclase but rather to processes that regulate cellular calcium-ion fluxes.

Their ubiquity and close coupling to a well-defined biochemical effector and the therapeutic importance of the drugs with which they combine have made the adrenergic receptors a subject of intense investigation over the past decade. The development of highly specific radioligands for each of the adrenergic receptors has led to an understanding of the modes by which their function is regulated both physiologically and pathophysiologically. Moreover, these regulatory mechanisms may well be important in the control of tissue sensitivity to drug and hormone action. In this essay recent advances in understanding the basic mechanisms regulating adrenergic receptors are used as models for illustrating general principles concerning the modulation of membrane-receptor function.

HOW ARE RECEPTORS STUDIED?

Several methodologic breakthroughs have provided the impetus for the explosion of new information about membrane receptors over the past decade. The direct ligand-binding technique has been referred to above. It has provided the ability to quantitate the receptors on whole cells, as well as in plasma-membrane fractions from tissues under normal or pathophysiologic circumstances. Such studies have led to the discovery that the number of receptors on cells is not fixed but is very dynamically regulated by a wide variety of circumstances, including diseases and therapeutic interventions. In several of these circumstances the altered receptor concentration appears to contribute to or determine altered tissue sensitivity to hormone or drug action. Table 2 lists some of the situations in which concentrations of α-adrenergic or β-adrenergic receptors are known to vary.

Another application of ligand-binding studies has been in the elucidation of the mechanisms involved in “receptor-effector coupling.” Figure 1 shows the known molecular components of hormone-responsive adenylate cyclase systems. These include the hormone receptor (R) — e.g., the β-adrenergic receptor; the catalytic moiety of the enzyme adenylate cyclase (C), which converts substrate adenosine triphosphate to cAMP; and a coupling protein (N), which is regulated by guanine nucleotides such as guanosine triphosphate, or GTP. Two structurally related forms of this protein exist that couple either stimulatory (e.g., β) or inhibitory (e.g., α₂) receptors to the catalytic moiety. The protein that couples to the stimulatory receptors is designated Ns, and the protein that couples to the inhibitory receptors is termed Ni. The entire system is found in the plasma membrane of the cell.

Figure 2 shows how ligand-binding studies suggested the mechanism by which this coupling process oc-
### Table 1. Subtypes of Adrenergic Receptors (Adapted from Smith et al.2).

<table>
<thead>
<tr>
<th>α Receptors</th>
<th>β Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>E&gt;NE&gt;PE&gt;PE&gt;1</td>
<td>I&gt;E&gt;NE&gt;PE</td>
</tr>
<tr>
<td>Specific antagonists</td>
<td>Specific antagonists</td>
</tr>
<tr>
<td>Prazosin</td>
<td>Propranolol</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>Alpranolol</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>Pindolol</td>
</tr>
<tr>
<td></td>
<td>Metoprolol</td>
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<tr>
<td></td>
<td>Betaxolol</td>
</tr>
<tr>
<td>Physiologic responses</td>
<td>Physiologic responses</td>
</tr>
<tr>
<td>Smooth-muscle contraction in blood vessels and genitourinary tract</td>
<td>Smooth-muscle relaxation in gastrointestinal tract</td>
</tr>
<tr>
<td>Smooth-muscle contraction in selected vascular beds</td>
<td>Stimulus of rate and force of cardiac contraction</td>
</tr>
<tr>
<td>Activation of glycogenolysis (rat liver)</td>
<td>Smooth-muscle relaxation in bronchi, blood vessels, and genitourinary and gastrointestinal tracts</td>
</tr>
<tr>
<td>Inhibition of norepinephrine release from sympathetic-nerve terminals</td>
<td>Facilitation of norepinephrine release</td>
</tr>
<tr>
<td>Inhibition of lipolysis in adipose cells (human, hamster)</td>
<td>Increased glycogenolysis and gluconeogenesis in liver</td>
</tr>
<tr>
<td>Platelet aggregation (human, rabbit)</td>
<td>Increased glycogenolysis in muscle</td>
</tr>
<tr>
<td>Inhibition of renin release from juxtaglomerular cells of the kidney</td>
<td>Increased insulin and glucagon secretion by pancreatic islet cells</td>
</tr>
<tr>
<td>Stimulation of potassium and water secretion by salivary glands</td>
<td>Stimulation of renin release by juxtaglomerular cells</td>
</tr>
<tr>
<td>Inhibition of insulin release by pancreatic islet cells</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>Location</td>
</tr>
<tr>
<td>Postsynaptic</td>
<td>Postsynaptic</td>
</tr>
<tr>
<td>Presynaptic, postsynaptic, and nonsynaptic (e.g., platelets)</td>
<td>Presynaptic and postsynaptic; also present on lymphocytes, and polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>Mechanism</td>
<td>Mechanism</td>
</tr>
<tr>
<td>Alterations of cellular calcium-ion fluxes</td>
<td>Inhibition of adenylate cyclase</td>
</tr>
<tr>
<td>Stimulation of adenylate cyclase</td>
<td>Stimulation of adenylate cyclase</td>
</tr>
</tbody>
</table>

*E denotes epinephrine, NE norepinephrine, I isoproterenol, and PE phenylephrine.

Biochemical studies have subsequently shown that these two forms of the receptor correspond to discrete molecular entities — R (low affinity) and RN (high affinity).8 Since agonists but not antagonists bind to RN with higher affinity, they favor formation of the HRN complex:

\[
H + R + N \rightleftharpoons HR + N \\
\uparrow \quad \uparrow \\
H + RN \rightleftharpoons HRN
\]

The ternary-complex HRN appears to be a required intermediate on the pathway to activation of the enzyme.9,10 GTP binds to this complex, dissociates it to the lower-affinity form of the receptor, and simultaneously activates N (presumably by induction of conformational changes). The activated N–GTP complex then activates the catalytic moiety (C). The entire process is terminated by a GTPase activity on N, which hydrolyzes GTP to guanosine diphosphate, or GDP, thereby deactivating the system.11 As long as hormone or agonist remains present, the entire se-
quence can begin again (Fig. 3). The key element is the transient formation of the high-affinity intermediate-complex HRN, which represents the crucial coupling event.

Two general points about this scheme are worthy of emphasis. The first is the important part that ligand-binding studies have played in elucidating the fundamental mechanisms of receptor-effector coupling in this system. The scheme proposed in Figure 3 appears to apply fairly generally to adenylate cyclase–coupled receptors, including those that stimulate and inhibit enzyme activity.

A second important feature concerns the implications of this scheme for understanding the regulation of receptor function. As noted above, regulation of the concentration of receptors in the plasma membrane constitutes one important mechanism of receptor regulation. Another important mechanism is modulation of the “coupling” of the receptors to their effectors—in this case, the nucleotide regulatory proteins. The coupling step is formation of the HRN complex. Since its formation can be deduced by computer-assisted quantitative analysis of agonist competition curves (cf. Fig. 2B), such curves can be used to assess R–N coupling. These analyses have indicated that a variety of circumstances are associated with altered β-adrenergic-receptor coupling. These include desensitization (see below), changes in thyroid or adrenal status, and pseudohypoparathyroidism. It should be noted that the decreased formation of HRN complexes in pseudohypoparathyroidism is due not to any alteration in the receptors, but rather to a genetic deficiency of the N protein. This deficiency affects the function of many or all of the receptors that stimulate adenylate cyclase (e.g., the β-adrenergic receptor) and not simply the actions of the parathyroid hormone receptor.

Recently developed methods permit the study of the structure of the adrenergic and other plasma-membrane receptors. The two most useful approaches are affinity chromatography and photoaffinity labeling. In affinity chromatography a drug or hormone that combines with a receptor is covalently linked to a solid support such as Sepharose beads, usually by a hydrocarbon side chain. When a detergent-solubilized extract of the cell membrane is passed over such a column, the receptors are adsorbed to the column by means of their binding interaction with the immobilized drug. Other proteins pass through. After appro

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**Table 2. Some Factors Regulating the Number of Adrenergic Receptors in Tissues.**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic agonists</td>
<td>Maturation, aging</td>
</tr>
<tr>
<td>Adrenergic antagonists</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Denervation</td>
<td>Heart failure</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>Cardiac hypertrophy</td>
</tr>
<tr>
<td>Thyroid hormones</td>
<td>Experimental diabetes</td>
</tr>
<tr>
<td>Estrogens</td>
<td>Alcohol withdrawal</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Psychotropic drugs</td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
</tr>
</tbody>
</table>

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HORMONE-SENSITIVE ADENYLATE CYCLASE

\[
\begin{align*}
& H_s \rightarrow R_s \\
& N_s \downarrow \uparrow \rightarrow C \rightarrow N_i \downarrow \uparrow \rightarrow H_i \rightarrow R_i \\
& GTP \downarrow \rightarrow \text{ATP} \rightarrow \text{GDP} \\
& \text{ATP} \rightarrow \text{cAMP} \rightarrow \text{GTP} \\
& \text{GTP} \rightarrow \text{cAMP} \rightarrow \text{GDP}
\end{align*}
\]

**Figure 1. Components of the Hormone-Responsive Adenylate Cyclase System.**

ATP denotes adenosine triphosphate, cAMP cyclic adenosine monophosphate, GDP guanosine diphosphate, and GTP guanosine triphosphate.
let irradiation. The resulting formation of the chemically reactive species (nitrene) leads to covalent incorporation of the drug into the receptor macromolecule. As in other types of approaches to ligand binding, validation of the method is based on the specificity of the receptor. Thus, it is necessary to document that a series of drugs block covalent incorporation of the photoaffinity label into the receptor with potencies comparable to those with which they occupy the receptors. In the case of the adrenergic receptors, it would be expected (for example) that (−)-isomers of agonists and antagonists would be more effective than (+)-isomers in blocking such covalent incorporation into the receptors (Fig. 4). After incorporation, the covalently labeled receptor can be visualized by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS-PAGE) followed by autoradiography.

Figure 4 shows the pattern obtained upon photoaffinity labeling of mammalian β₁-adrenergic and α₁-adrenergic receptors, followed by SDS-PAGE. Although their structures appear to be quite distinct, mammalian α and β receptors each consist of a single glycoprotein of approximately 80,000 daltons and 62,000 to 64,000 daltons, respectively. As discussed below, it appears that the structure of the receptor may itself be subject to dynamic covalent modification, with important functional consequences.

**The Bifunctional Nature of Receptors**

Inherent in the concept of a biologic receptor are two distinct but intimately related functions — binding and activation. Thus, a receptor must first bind biologically active agonist hormones or their functionally inert antagonists. The pharmacologic specificity of a given receptor subtype is determined by the molecular structure of the ligand-binding site. This specificity also determines the relative pharmacologic responses characteristic of each tissue. The second function of a receptor is the activation of a biologic process, which presumably occurs after a conformational change in the receptor is induced by the specific binding of an agonist. This function of receptors is a manifestation of their ability to interact with distinct effector components, such as enzymes (e.g., adenylate cyclase) and ion channels, and to alter their activities in ways that lead to characteristic physiologic responses.

Until recently, it was not possible to document that both these functions resided on a single receptor macromolecule. However, with the advent of techniques for purifying receptor molecules to apparent homogeneity it has become possible, for the first time, to document the bifunctionality of a receptor molecule. This has been accomplished for the β-adrenergic receptor. That the binding protein purified by affinity chromatography in fact contains the β-adrenergic-receptor ligand-binding site can be readily documented through the pure-protein and ligand-binding techniques. Thus, it has been shown that the pure-receptor molecules (of about 64,000 daltons) bind adrenergic ligands with all the appropriate specificity and stereo-
specificity characteristics expected of a true β-adrenergic receptor.³

Documenting the activating function of the receptor, however, has required the development of entirely new approaches (Fig. 5). The ultimate biologic function of β-adrenergic receptors is to confer on the adenylate cyclase system of cells the ability to respond to catecholamines with a typical β-adrenergic specificity. We reasoned, therefore (Fig. 5A), that an assay for the activating function of β-adrenergic receptors might be developed by inserting the receptors, purified from one type of cell, into the membrane of another cell that lacked β-adrenergic receptors of its own but contained the other components of the adenylate cyclase system (i.e., N₄ and C). It was hoped that insertion of the pure receptors into such a cell would establish catecholamine responsiveness. An appropriate acceptor cell is the erythrocyte of the African clawed toad *Xenopus laevis*, which is in fact essentially devoid of β-adrenergic receptors.

According to the procedures developed (Fig. 5B), the purified receptors are first “reconstituted” into artificial lipid vesicles from the detergent (digitonin) in which they were originally solubilized. The vesicles containing the pure receptors are then fused to the *X. laevis* erythrocytes, with polyethylene glycol used as “fusogen.” The fused hybrid cells are lysed, and adenylate cyclase activity is measured in the hybrid membranes. Figure 6 shows the result of such an experiment.²¹ When the xenopus cells are fused to themselves or to lipid vesicles containing no receptor or heat-inactivated receptor, no isoproterenol-responsive adenylate cyclase activity is observed. In contrast, when pure receptor is present in the lipid vesicles, catecholamine-responsive adenylate cyclase is found in the hybrid cells. Moreover, stimulation of the enzyme by isoproterenol is completely blocked by the specific β-adrenergic antagonist propranolol. It should be noted that the endogenous prostaglandin E₁ receptors present in the xenopus erythrocyte membranes function entirely normally after the fusion procedures.

These results are important in several ways. They document that a single receptor molecule is truly bi-functional — i.e., capable of both binding ligands with appropriate specificity and conveying biologic responsiveness on a cell with the same appropriate specificity. Beyond this, however, these results have more general implications. They indicate that an assay is now available for the “activating” function of receptors by reconstitution/fusion methodology. Thus, just as it first became possible to assay the ligand-binding function of the receptors 10 years ago, it is now possible to measure their biologic activating function. This capability should greatly facilitate understanding of the ways in which receptor coupling is regulated at the molecular level. Examples of the uses of this new technique are provided below.

**Physiologic and Clinical Regulation of Adrenergic Receptors**

**Desensitization**

A very general mechanism of cellular adaptation is an attenuation of responsiveness to pharmacologic or hormonal stimulation with time. This phenomenon is referred to as desensitization, tolerance, tachyphylaxis, or refractoriness. Such desensitization phenomena markedly limit the therapeutic efficacy of catecholamines as well as many other agents. Clinical examples of desensitization to catecholamines abound, including the rapid loss of the bronchodilating effects of β-adrenergic agonists administered to patients with asthma; the transient effect of vasoconstrictor α-adrenergic amines administered to patients with hypotension; the waning inotropic effect of β-agonists administered to patients with congestive heart failure; the attenuated response to α-adrenergic constrictor amines used topically as nasal decongest-
ants; and the decreased responsiveness to catecholamines of patients with pheochromocytomas, such that normal resting pulse and blood pressure values may be found at times when circulating catecholamine levels are well above normal.

In experimental systems two broad patterns of desensitization have been delineated. Homologous desensitization refers to a loss of sensitivity only to the class of agonist used to desensitize the tissue. In heterologous desensitization, stimulation by one drug or hormone leads to a broad pattern of unresponsiveness to further stimulation by a variety of other agonists. Obviously, alteration of components beyond the receptor must contribute to heterologous forms of desensitization. However, the receptor is an obvious locus of regulation for homologous desensitization, since the receptor imparts the unique specificity to drug action.

Insights into the molecular mechanisms that underlie such desensitization phenomena have recently come from studies using the newly developed methods for studying adrenergic receptors, described above. These studies have generally involved simple cellular systems, such as mammalian cells in culture, or homogeneous dispersed cell preparations, such as avian and amphibian erythrocytes. All these cells contain the β-adrenergic-receptor–adenylate cyclase system. Moreover, when such cells are exposed for minutes or hours to a β-agonist such as isoproterenol, the adenylate cyclase becomes desensitized to further catecholamine stimulation. Thus, such systems provide useful models for unraveling the biochemical basis for β-adrenergic-receptor desensitization.

Two major pathways leading to such receptor desensitization have already been identified (Fig. 7). The mechanism depicted in Figure 7A has been documented in several cultured cell lines and frog erythrocytes and involves “down regulation,” or loss of receptors from the cell surface. Within minutes of exposure of the cells to isoproterenol the receptors become functionally uncoupled — i.e., incapable of forming the high-affinity HRN complex and therefore of stimulating adenylate cyclase. The biochemical basis for this change is as yet unknown, but cAMP appears not to be involved. Subsequently, the uncoupled receptors are removed from the cell surface and sequestered within the cell in a compartment that is still not well defined. The receptors in this internalized, sequestered compartment are removed from contact with the effector components of the system — i.e., N and C. The sequestered receptors appear structurally intact as assessed by photoaffinity labeling and functionally intact as assessed by fusion with X. laevis erythrocytes. Thus, their functional inadequacy at this stage is apparently due to their physical separation from their normal biochemical effector. When agonist is removed, the receptors recycle to the cell surface and become recoupled to the adenylate cyclase. If agonist-induced desensitization is permitted to continue, then at least in some cells, the internalized receptors are destroyed, presumably by lysosomal proteases. Once this happens, recycling cannot occur and new receptor synthesis is required for restoration of the full complement of surface receptors.

β-Adrenergic-receptor desensitization may also occur without receptor down regulation (Fig. 7B). This sequence of events has been delineated in studies with avian erythrocytes. When such cells are exposed to isoproterenol they become desensitized by a process

![Figure 5. Approaches to Measuring the Biologic Activating Function of the β-Adrenergic Receptor.](image-url)

Panel A shows the characteristics of donor and acceptor cells, and Panel B the reconstitution and fusion of the receptor. PGE1 denotes prostaglandin E1. For explanation, see text.
that involves receptor uncoupling without down regulation and that appears to be mediated largely, if not exclusively, by cAMP. It has been directly demonstrated that in association with desensitization of these cells, the β-adrenergic receptors become phosphorylated. This was shown by labeling the intracellular ATP pool with 32P, (radioactive inorganic phosphate), desensitizing the cells with isoproterenol, purifying the receptors by affinity chromatography, and then performing SDS-PAGE and autoradiography. Current evidence suggests that the receptors may be phosphorylated by cAMP-dependent protein kinase or alternatively by some other kinase, which may in turn be activated by the cAMP-dependent protein kinase.

Phosphorylation of enzymes is a very widespread mechanism for control of their physiologic activities. However, the role of this covalent modification in regulating receptor activity has not previously been known. To assess whether the phosphorylated receptors from the desensitized cells were in fact less active, they were reconstituted and fused with xenopus erythrocytes as described above. In fact, the desensitized and phosphorylated receptors are less able to activate the cyclase after such reconstitution and fusion (Strulovici B, et al.: unpublished data).

Thus, at least two distinct mechanisms appear to be operative in controlling the function of β-adrenergic receptors during desensitization. Either the receptors may be removed from the cell surface (down regulation) and sequestered away from the adenylate cyclase within the cell, or alternatively the functionality of the receptors may be controlled directly by covalent modification such as by phosphorylation. Understanding the cellular and biochemical basis for β-receptor desensitization now opens the way toward attempts to develop rational strategies for circumventing these processes and thus prolonging and augmenting the therapeutic efficacy of catecholamines and other drugs. An important clinical ramification of this new information about agonist-induced receptor alterations leading to desensitization is that whenever possible, such agonist therapy should be as intermittent as possible and the lowest dosage of drug consistent with achieving desired therapeutic goals should be given. This will tend to minimize the development of desensitization to drug effects.

**β-Blockade and the Propranolol-Withdrawal Syndrome**

If treatment with agonists can lead to receptor alterations expressed as desensitization, can antagonist treatment lead to opposite changes? Although this is a controversial area, currently available information suggests that the answer may be yes. When treatment with the β-blocker propranolol is abruptly discontinued in some patients, hypersensitivity to catecholamines becomes clinically evident and unstable angina or myocardial infarction may occur. Hyperresponsiveness to infused catecholamine has been present in some subjects for up to 13 days after propranolol has been withdrawn.

Experiments in animals and human subjects treated with propranolol have documented increases in β-receptor number in various tissues, including circulating leukocytes. Whether these changes in receptor number, or other antagonist-induced changes in β-adrenergic receptors, contribute to the so-called propranolol withdrawal syndrome is a subject for further investigation.

**Thyroid Disease**

Patients with hyperthyroidism have a variety of symptoms suggestive of increased sympathetic-nervous-system stimulation, whereas those with hypothyroidism have a clinical picture that is often quite the opposite. Moreover, many of the signs and symptoms of hyperthyroidism are ameliorated by β-adrenergic blocking agents. These clinical observations appear to be related to the ability of thyroid hormones to regulate the function of adrenergic receptors. As indicated in Table 2 the concentrations of both α-adrenergic and β-adrenergic receptors in tissues are controlled by thyroid hormone (presumably at the level of gene transcription). Moreover, in both hyperthyroidism and hypothyroidism, alterations in receptor coupling to the adenylate cyclase system have been documented. Thus, thyroid hormone control of adrenergic receptors provides a potential explanation for the well-known clinical alterations in adrenergic respon-
siveness that occur in hyperthyroidism and hypothyroidism.

**Ischemic Heart Disease**

Ventricular arrhythmias often develop in patients with myocardial ischemia. The possible relation between alterations in adrenergic receptors and this myocardial irritability has been investigated in several animal models, such as dogs in which myocardial ischemia was produced by ligation of the proximal left anterior descending coronary artery.\(^{37}\) It was found that the number of β-adrenergic receptors, but not that of muscarinic cholinergic receptors, was increased between one and eight hours after occlusion in ischemic as opposed to nonischemic myocardium. This “up regulation” of β-adrenergic receptors was associated with an enhanced ability of isoproterenol to elevate cAMP levels in the myocardium.

By contrast, in cats undergoing experimentally induced ischemia, an increased incidence of ventricular arrhythmias appears to be related to enhanced α-adrenergic responsiveness. In these animals, the number of α-adrenergic rather than of β-adrenergic receptors is increased in ischemic myocardium.\(^{38}\) These species-dependent differences in response to ischemia remain an area for further investigation.

**Congestive Heart Failure**

One of the compensatory mechanisms used by the body to support the failing heart is increased stimulation by the sympathetic nervous system.\(^{39}\) Initially, this stimulation may be transmitted principally via the cardiac sympathetic nerves, which secrete norepinephrine. Subsequently, as failure worsens, more generalized stimulation is provided by adrenomedullary secretion of epinephrine. Ultimately, cardiac stores of catecholamines in subjects with advanced heart failure are markedly depleted. Moreover, it has recently been demonstrated that the density of β-adrenergic receptors in the failed left ventricles of heart-transplant recipients was 50 per cent lower than that in control tissue taken from transplant donors. This reduced receptor density was associated with a reduction in isoproterenol-stimulated muscle contraction and adenylate cyclase activation.\(^{40}\) It is not clear at this point whether the reduced β-adrenergic-receptor number in the failing myocardium is a primary event or is secondary to the “desensitizing” effect of the elevated catecholamine levels to which the tissue is exposed. In either case, however, the decreased β-adrenergic-receptor pool may contribute to limiting the extent to which the sympathetic nervous system or exogenously administered catecholamines can support the failing heart.

**Hypertension**

A number of investigators have used various rat models of hypertension, either induced, as by renalartery constriction, or genetic, as in spontaneous hypertension, to study the possible role of adrenergic receptors in the genesis of hypertension.\(^{23}\) Unfortunately, results to date are somewhat conflicting and are certainly not definitive. In general, reduced concentrations of β-adrenergic receptors in cardiac, vascular, and other tissues have been observed, and in one study a reduction in receptor coupling was found as well.\(^{41}\) Most of these animals had increased sympathetic drive and elevated circulating catecholamines. Again, the primary, as opposed to the secondary, nature of the receptor alterations is not clear at present. One group has speculated that a reduction in vascular β-adrenergic receptors, with no change in α-adrenergic receptors in the face of increased sympathoadrenal drive, could lead to unchecked vasoconstriction and hypertension.\(^{12}\) Although entirely speculative, such

![Figure 7. Desensitization of β-Adrenergic Receptors by Down Regulation and Internalization (A) and by Covalent Modification Such as Phosphorylation (B).](image-url)
hypotheses indicate the way in which direct studies of the adrenergic receptors are refocusing research in the
study of hypertension.

Asthma

A longstanding hypothesis has been that an altered autonomic balance contributes to the pathophysiology of asthma. Indeed, physiologic studies in such patients have suggested decreased β-adrenergic and increased cholinergic and α-adrenergic responsiveness. Over the past five years a large number of studies (reviewed by Stiles et al.23) have addressed the issue of whether the number of β-adrenergic receptors on circulating leukocytes is altered in asthmatic subjects. Although results have been conflicting, the general consensus appears to be that the receptor number is not altered on polymorphonuclear leukocytes unless the patients have been previously treated with a β-agonist bronchodilator. In contrast, the density of receptors on lymphocytes does seem to be reduced.25 The importance of this disparity is not known.

These studies, however, do serve to bring into focus the issue of the use of circulating cells to measure receptor properties (adrenergic or other) in illness. Although the properties of the receptors on these cells may validly reflect those of some other tissue of interest (e.g., heart or lung) there is no a priori reason for this to be so. Thus, all such studies must be interpreted with caution until appropriate validation is provided in each case.

Conclusions

Recent methodologic advances have brought the study of drug receptors such as the adrenergic receptors to the molecular level. These advances have included the development of means to assay the receptors through their binding or activating functions and to purify them and label them with affinity probes. These new approaches have provided the tools for unraveling their molecular structure and the physiologic and pathophysiologic regulation of their function, and have taught us that drugs and hormones have a hitherto entirely unknown class of effects. Thus, in addition to the classical physiologic responses that these agents are known to evoke, they also regulate and modulate the functional properties of their own receptors. They may increase or decrease the number of these receptors, change their efficiency of coupling to biologic effectors, and even alter their structure by stimulating their covalent modification, as by phosphorylation. Perhaps most important of all, the new insights provide a framework for trying to modify these receptor-regulatory phenomena for therapeutic purposes. For example, with increased understanding of the biochemical events that lead to desensitization, it may be possible to develop strategies to interrupt this process while preserving the activating function of drugs. This would ultimately lead to augmented therapeutic efficacy of catecholamines as well as many other drugs. Analogous advances in modifying receptor-regulatory events occurring in illnesses such as those discussed above should also be of therapeutic benefit. Although these research endeavors are as yet in their early stages they hold great promise and represent examples of the direct application of biochemical advances to problems of therapeutics and medicine.

References

SULFHEMOGLOBINEMIA
Clinical and Molecular Aspects
Constance M. Park, M.D., Ph.D., and Ronald L. Nagei, M.D.

SULFHEMOGLOBIN, methemoglobin, and the M hemoglobins must be considered when one is evaluating the cyanotic patient. Sulfhemoglobin is a green-pigmented molecule with a sulfur atom incorporated into the porphyrin ring and a markedly reduced oxygen affinity that makes it ineffective for oxygen transport. It has been associated with drug abuse,[1-7] occupational exposure to sulfur compounds,[8,9] and recently, environmental exposure to polluted air.[10]

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In sulfhemoglobinemia, methemoglobinemia, and the presence of M hemoglobins in the blood, cyanosis is not itself a sign of respiratory insufficiency. Rather, the slate-gray skin color is the result of the spectral characteristics of the abnormal pigments. The actual respiratory status of affected persons is influenced by two factors. In the first place, the decreased oxygen-binding capacity due to nonfunctional hemes can have the effect of an anemia. Secondly, modified hemes can alter the oxygen-transport capacity of normal hemes if they coexist in the same tetramers. These structure-function relationships and their clinical correlates are known for methemoglobin and the M hemoglobins but not for sulfhemoglobin.

In the course of studying a case of drug-induced sulfhemoglobinemia, we have established that at low levels of the abnormal pigment, the percentage of affected hemoglobin is greater than the percentage of sulfuration because the hemoglobin tetramers contain no more than one or two sulfurred hemes. The affected molecules shift toward the unliganded conformation, which reduces the oxygen affinity of their unmodified subunits. This right shift of the partial pressure of oxygen at 50 per cent hemoglobin saturation (p50) ameliorates the effects of the reduced oxygen-binding capacity. For this reason, dyspnea is absent unless the levels of sulfhemoglobin are extraordinarily high. This is in contrast to the case of methemoglobinemia, in which a left shift of p50 impairs oxygen delivery and exacerbates the decreased oxy-