This Review is the first in a thematic series on **Novel Aspects of Cardiovascular G Protein-Coupled Receptor Signaling: Implications for New Therapeutics**, which includes the following articles:

- Introduction to the Series
- Biased Ligands for Better Cardiovascular Drugs: Dissecting GPCR Pharmacology
- G-Protein–Dependent and –Independent Signaling Pathways and their Impact on Cardiac Function
- Compartmentalization of β-Adrenergic Signals in Cardiomyocytes
- G-Protein-Coupled Receptor Kinases (GRK) as Therapeutic Targets in Cardiovascular Disease
- Regulators of G-Protein Signaling in the Heart and Their Potential as Therapeutic Targets

**Howard Rockman, Guest Editor**

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### Introduction to the Series on Novel Aspects of Cardiovascular G-Protein-Coupled Receptor Signaling

Howard A. Rockman, Robert J. Lefkowitz

The initial concept of drugs acting on receptors is generally credited to John Newport Langley (1852–1925) and his pioneering work on the antagonistic effects of atropine,1 and to Paul Ehrlich (1854–1915), who coined the word “receptor.”2 It was in 1905 that Langley (together with the much forgotten Thomas Renton Elliott) proposed that a “receptive substance” was the site of action of chemical mediators liberated by nerve stimulation.3 At approximately the same time, Ehrlich in his studies on immunity postulated that a drug could have a therapeutic effect only if it “unites with certain chemical groupings in the protoplasm of cells” and called such cell groupings “poison receptors” or just “receptors.”2

In experiments performed in the early 1900s, the English pharmacologist and neurophysiologist Sir Henry Hallett Dale (1875–1968) identified the muscarinic and cholinergic actions of acetylcholine and subsequently shared with Otto Loewi the Nobel Prize in 1936 for discoveries relating to the physiological effect of Ergot preparations, the concept evolved that there were two classes of adrenotropic receptors: those whose action results in excitation and those whose action results in inhibition of the effector cells.5,3

This concept of two classes of adrenotropic receptors was further clarified by Raymond Ahlquist at the Medical College of Georgia, when in 1948 he published his work showing that it is an oversimplification to classify adrenotropic receptors as either excitatory or inhibitory.6 By studying the relative potencies of six sympathomimetic amines on several isolated mammalian preparations, Ahlquist6 postulated that their action on postsynaptic sites was mediated by two types of adrenergic receptors, which he called α and β. It was based on the conceptual framework of receptor subtypes proposed by Ahlquist that Sir James Black set out on a quest to develop a drug that would diminish the myocardial demand for oxygen as a treatment for angina pectoris. In the mid 1960s, together with the chemist John Stephenson, they synthesized and screened numerous competitive antagonists of catecholamines in an in vitro bioassay system of guinea pig cardiac...
papillary muscle to measure strength of contraction. Applying analytic modeling of hormone receptor systems based on the application of the law of mass action, they developed mathematical models to describe parameters of affinity (binding) and efficacy (response-generating).7 By generating dose-response curves for a number of compounds, Black and Stephenson could demonstrate the rightward displacement by a competitive antagonist, which led to the identification of the β-blocker, propranolol.7

Contemporaneously, in the late 1950s and early 1960s, Earl Sutherland used biochemical techniques to show that an intermediate substance was required for the action of epinephrine, which he termed “the second messenger.”7 The newly identified substance proved to be the adenine nucleotide cAMP, and the enzyme generating this substance was adenylyl cyclase but still failed to generate cAMP in response to hormone stimulation.10 Gilman et al10 showed that these mutant cells lacked the transducer function, which they called nucleotide regulatory proteins or N proteins.9 Rodbell proposed a three-step model in which “N” proteins (now called G proteins) serve as a primary switch to mediate agonist receptor interactions, a process he termed signal transduction.9 Building on the work of Sutherland and Rodbell, Gilman in the late 1970s used a mutant clonal S49 lymphoma cell line (cyc-), which despite its name contained adenylyl cyclase but still failed to generate cAMP in response to hormone stimulation.10 Gilman et al10 showed that these mutant cells lacked the transducer function, which they subsequently purified as a GTP-binding protein.

Skepticism About Receptors
Despite the fact that the concept of receptor as a site of hormone action was introduced more than a century ago, there was considerable skepticism over the years as to whether such receptors actually existed as a physical entities. In 1943, Dale was not convinced that adrenaline receptors existed and stated “it is a mere statement of fact to say that the action of adrenaline picks out certain such effector-cells and leaves others unaffected; it is a simple deduction that the affected cells have a special affinity of some kind for adrenaline; but I doubt whether the attribution to such cells of ‘adrenaline-receptors’ does more than restate this deduction in another form.”11 Moreover, Sutherland believed “… in most and perhaps all tissues the beta receptor and adenylyl cyclase are the same.”12 In Alquist’s original 1948 article, he states “the adrenotropic receptors are those hypothetical structures or systems located in, on or near the muscle or gland cells affected by epinephrine.” In 1973 he goes on to state “this would be true if I were so presumptuous as to believe that α and β receptors really did exist. There are those that think so and even propose to describe their intimate structure. To me they are an abstract concept conceived to explain observed responses of tissues produced by chemicals of various structure.”13

Adrenergic Receptors Exist as Physical Entities
It was clear that to definitively prove the existence of receptors as membrane molecules, new methods would need to be developed so that their properties no longer needed to be inferred from downstream signaling events. Applying the technique of (−)−[3H] alpenolol radioligand binding to frog erythrocyte membranes, high-affinity β2 adrenergic receptors (also known as β2 adrenoceptor) were identified and were shown to be coupled to adenylyl cyclase.14 Affinity chromatography methods were developed, which led to the purification and cloning of the first G-protein-coupled receptor (GPCR), the β2-adrenergic receptor,15 and then the subsequent cloning of the enzyme involved in β-adrenergic receptor desensitization, the β-adrenergic receptor kinase.16 In the course of purifying β-adrenergic receptor kinase, it was discovered that the ability for the enzyme to desensitize the β2-adrenergic receptor was lost with increased enzyme purification. This suggested that a cofactor was necessary for desensitization and led to the discovery of β-arrestin.17 Although the molecular mechanisms of activation and deactivation of ligand-stimulated GPCR signaling was thought to be solved, recent discoveries have led to a new appreciation of the complexity and elegance of the GPCR transduction system.18

The Quest for Receptor Specificity
Pharmacologists have always quested for ever-greater specificity in drug action, in other words, to maximize the desired effects and minimize the untoward or undesired effects. In essence, side effects are a misnomer in that they are often on-target effects of a drug, just not the ones that are desired. For example, take the β2-agonists in the treatment of asthma. Bronchodilation and tachycardia are both on-target efficacies for a β2-agonist; however, bronchodilation is desired and tachycardia is undesired. In the 1960s, the discovery of receptor subtypes allowed the pharmacological distinction of the α and β adrenergic receptors and heralded the era of receptor subtype specificity of drug action. With the development of ligand-binding methods in the 1970s and cloning methods in the 1980s and 1990s, it was possible to develop high-affinity ligands for many GPCR subtypes. Each of these developments in pharmacology allowed for greater specificity of action in drug development. Now, the discovery of “biased agonism,” the concept that a single receptor can stimulate multiple signaling efficacies, has led to the next wave in drug development. Developing high-affinity ligands that stimulate only a select subset of signaling pathways for one receptor subtype should allow one to enhance desirable efficacies while limiting the untoward efficacies of a therapeutic drug.

It is with this background of discoveries of the past 100 years in receptor pharmacology that we introduce this review series on GPCR. Highlighted are current understanding of how different ligands can selectively activate a subset of receptor-mediated signaling pathways (DeWire and Violin); the role of β-arrestin-dependent signaling in cardiac function (Tilley); how GRK represent a therapeutic target for cardiovascular disease (Belmonte and Blaxall); the dynamic nature of β-adrenergic receptors in specialized cellular compartments (Xiang); and how molecules that bind and regulate G-proteins known as regulators of G-protein signaling may also be important therapeutic targets (Zhang and Mende).
hope this series will be thought-provoking and will lead the reader to view GPCR signaling in a new light.

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References

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