GPCR MOLECULAR PHARMACOLOGY AND DRUG TARGETING

SHIFTING PARADIGMS AND NEW DIRECTIONS

Edited by

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To Josen and Finn, who arrived somewhere in the middle of all of this …

CONTENTS

PREFACE

Although G protein-coupled receptors (GPCRs) have been the subject of study since the early days of pharmacology, our understanding of this large family of receptors continues to evolve. As a result, texts that discuss these receptors must constantly be rewritten and revised as emerging concepts are brought forth. Although many texts focus on GPCRs, none provide an overall look at the molecular pharmacology of this important target class. This book presents an up-to-date review on how scientists are thinking about GPCRs.

Early work includes the lock and key model of Fisher, and "receptive substances" to explain the biological actions of exogenous chemicals on cell "receptors," and Clark's occupancy theory that introduced the idea that the effect of an agonist is proportional to the number of occupied receptors. In the 1960s, the concept of intrinsic activity was introduced to explain the observation that not every agonist of a given receptor induced the same maximum effect. Compounds reaching the maximum were referred to as full agonist and other agonists were named partial agonist. This model was later extended to include drug efficacy and the system-independent concept of intrinsic efficacy. The mathematics applied in the models were relatively simple and allowed calculations to be made on affinity and activity. Looking back, it seems remarkable that most of these concepts were developed when little information on the biochemical nature of receptors or the underlying molecular mechanisms involved in signal transduction were available.

I have been working in the field of GPCRs for about 15 years, a time dwarfed by many of the contributing authors, who are well recognized as being experts in the field. In this time period, many of the basic pharmacology concepts for GPCRs have undergone radical changes. Yet, if one looks back, it seems that years before their general acceptance, there was research to support the paradigm shifts. For example, in 1989 Costa and Herz described antagonists with negative intrinsic activity at wild-type δ-opioid receptors (*PNAS 1989 86: 7321-7325*). Many subsequent studies have confirmed that GPCR proteins can signal in an agonist-independent, constitutive manner, and the concept of inverse agonism is now accepted and included in pharmacology textbooks. But in its early days, the concept of inverse agonism was hotly debated. Thus, the way we think about GPCRs, their basal activity, how they get activated, how they communicate the signal across the cell membrane, how they couple to each other as well as to other receptors and other membrane proteins, how their mutations lead to disease, and the many ways in which

they can be screened for compounds that modulate them are constantly changing.

This textbook will serve as a resource for any scientists (e.g., pharmacologists, chemists, biologists) investigating GPCRs in both academia and industry (pharmaceutical/biotechnology). The book provides a general overview of GPCRs in terms of their biology and pharmacology, as well as recent developments including structure, deorphanization, dimerization, functional selectivity, and accessory proteins. In addition, it presents detailed methods on how to express, manipulate, and measure GPCRs. It is important to recognize that emerging concepts have shaped not only our understanding of GPCR pharmacology but also the drug discovery process itself. Recognition that most of the compounds initially considered to be competitive antagonists were inverse agonists, and studies indicating that the therapeutic outcome of inverse agonists and neutral antagonists can be different, led many companies to initiate drug discovery efforts specifically aimed at identifying inverse agonists. As a result, it is envisaged that this book on the shifting paradigms to GPCR pharmacology will be an essential resource for scientists working to identify compounds that selectively target members of this diverse family.

The book is organized into 17 chapters; the first seven chapters deal with the evolving pharmacology that surrounds this family, including chapters discussing allosteric modulators, receptor dimerization, GPCR deorphanization, G protein activation, and the Frizzled family. The next six chapters deal with methods to screen GPCRs, and include chapters on cell phenotype, "traditional" biochemical techniques, resonance energy transfer, label-free detection, and approaches for ultra-high-throughput screening. The next three chapters present GPCR structures, new ways to express and crystallize the receptors, as well as computational studies such as modeling and rational drug design. The final chapter describes the use of pharmacological chaperones for diseases caused by GPCR mutations.

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CHAPTER 1

The Evolution of Receptors: From On–Off Switches to Microprocessors

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… Try to imagine a pharmacology course that made no mention of receptors …'
—Humphrey Rang, 2006

1.1. INTRODUCTION

The evolution of the receptor concept is traced from the turn of the century where the receptor was an organizational concept to the present day where seven transmembrane (7TM) receptors are considered to be complex control units for cellular function. Drug receptor effect can be quantified without knowledge of molecular mechanism through the operational model of drug action. Alternatively, biochemical knowledge of the receptor as an independent protein unit recognizing both external chemicals and cytosolic protein independently can be used to understand ligand-specific receptor effects and how these might be exploited therapeutically.

1.2. THE RECEPTOR AS AN ON–OFF SWITCH

Historically, the concept of drug receptors began as an abstract idea and continued throughout pharmacology for another 70–80 years as such. Models considering receptors essentially had drugs activate (as a switch) a black box to produce tissue response. It is useful first to consider receptors as pharmacological switches since the theories used to describe drug action in

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these systems lay the foundation for the more mechanistically driven models of receptors in use today. These ideas have formed the basis for quantitative receptor theory on which new information about the biochemistry and structure of receptors has been added. These will then be discussed in terms of specific mechanistic models of 7TM receptor function and how these assist in the quantification of drug effect.

1.3. HISTORICAL BACKGROUND AND CLASSICAL RECEPTOR THEORY

What is considered a "given" today, namely that drugs interact with specific binding sites on the cell membrane called receptors, is an extremely important tenet of pharmacology. As stated by Rang [1], it indeed was "Pharmacology's big idea." The main reason it is such an important cornerstone of pharmacology is that it introduces order into the apparent chaos of physiology. For example, a simple molecule such as epinephrine mediates a myriad of physiological processes. A large subset of these, namely cardiac chronotropy, inotropy, lusitropy, vascular relaxation, lacrimal, pancreatic, and salivary gland secretion, bronchiole, uterine and urinary bladder muscle relaxation, decreased stomach motility, skeletal muscle tremor, and melatonin synthesis are mediated by a small subset of membrane-bound proteins, specifically β_1- and β_2-adrenoceptors. This immediately puts order in the collection of physiological processes in that it gives them a common place to start, namely the interaction of epinephrine with the receptor. This order fits in well with the discipline of medicinal chemistry in that chemists have access to the processes for potential control.

At the turn of the twentieth century, different groups carried out research that caused them to postulate the existence of control points on cells that responded to chemicals, that is, receptors. For example, Paul Ehrlich (1854–1915) carried out studies on agent "606" (salvarsan) for syphilis. His work with dyes and bacteria led him to propose that there are "chemoreceptors" (actually a collection of "amboreceptors," "triceptors," and "polyceptors") on parasites, cancer cells, and microorganisms that could be exploited therapeutically [2]. In Cambridge, John Newport Langley (1852–1926) studied the drug jaborandi (contains the alkaloid pilocarpine) and atropine and concluded that receptors were "switches" that received and generated signals and that these switches could be activated or blocked by specific molecules [2]. However, it is A.J. Clark (1885–1941) who is considered the father of modern receptor pharmacology. Clark was the first to suggest, from studies of acetylcholine and atropine, that a unimolecular interaction occurs between a drug and a "substance on the cell." As he put it [3] …

> … it is impossible to explain the remarkable effects observed except by assuming that drugs unite with receptors of a highly specific pattern.…

In fact, it was Clark who described pharmacological phenomena in chemical terms [3, 4], a concept readily accepted today, but quite heretical in Clark's time. The prevailing concepts guiding physiology at the turn of the century were rooted in homeopathic theories (i.e., a fundamental theory centered on the surface tension of the cell membrane) like the Arndt–Schulz Law and Weber–Fechner Law [2]. A generally accepted statement to describe physiologic phenomena was simply that "certain phenomena occur frequently."

One of Clark's most valuable contributions to pharmacology was the application of mathematical rules to the behavior of biological systems. Thus, the dose–response curve became the common currency of pharmacology, and its judicious use in the work of Clark and others built the framework for what had become known as "receptor theory," namely the application of simple thermodynamic rules to pharmacological systems.

An early example of mathematics applied to the study of receptors was provided by A.V. Hill, a student of Langley. He expressed the time course of contraction of frog rectus abdominus to the agonist nicotine (N) through an equilibrium concentration–response curve of the form:

$$Y = \frac{N}{k' + kN} - M \tag{1.1}$$

where Y is contraction height, M is threshold, and k′, k are constants. While this work predated the routine use of binding isotherms to receptor work considerably, Hill lost interest in the approach, and it was left to Irving Langmuir, a chemist at General Electric Company in the United States, to devise an equation for the quantification of molecules binding to a surface, in particular, chemicals to metal filaments for light bulbs. Thus, the Langmuir adsorption isotherm quantifies the fraction of the substance bound to a surface (the pharmacological counterpart being receptor, denoted ρ_A) by a molecule [A] as:

$$\rho_A = \frac{[A]}{[A] + K_A} \tag{1.2}$$

where K_A is the ratio of what Langmuir referred to as the "rate of evaporation" of the substance away from the surface (pharmacologically, the rate of offset of the molecule from the receptor) divided by the "rate of condensation" of the molecule toward the surface (pharmacologically, the rate of onset toward the receptor). In pharmacological terms, the specific terminology for molecules that produced such activation is "agonist." While mechanistically, this equation is based on thermodynamic principles governing agonist binding to receptors, operationally, it also defines the universally observed relationship between agonists and the pharmacological responses they induce to tissues and cells. Thus, any observed receptor-mediated response in any tissue can be summarized by a form of the Langmuir isotherm where the fractional maximum

given by Equation 1.2 is multiplied by the maximal response observed from the preparation ($\text{Response}_A = \rho_A \cdot E_{max}$):

$$\text{Response}_A = \frac{[A] \cdot E_{max}}{[A] + EC_{50}} \tag{1.3}$$

where EC_{50} refers to the concentration of agonist A that produces half the maximal response to drug A. It should be noted that Equation 1.3 is written for a system demonstrating a Hill coefficient (in honor of A.V. Hill) of unity. If there is cooperativity in the system (either in the binding of the drug to the receptor [*vide infra*] or in the cellular processes that translate drug binding into cellular response), then Equation 1.3 becomes:

$$\text{Response}_A = \frac{[A]^n \cdot E_{max}}{[A]^n + EC_{50}^n} \tag{1.4}$$

where n is the Hill coefficient for the dose–response curve. Figure 1.1 shows data from Clark (effect of acetylcholine on frog heart chronotropy) fit to the Langmuir adsorption isotherm (Eq. 1.3). Irrespective of mechanism, it can be seen that the curve shown in Fig. 1.1 concisely summarizes the data. Thus, the

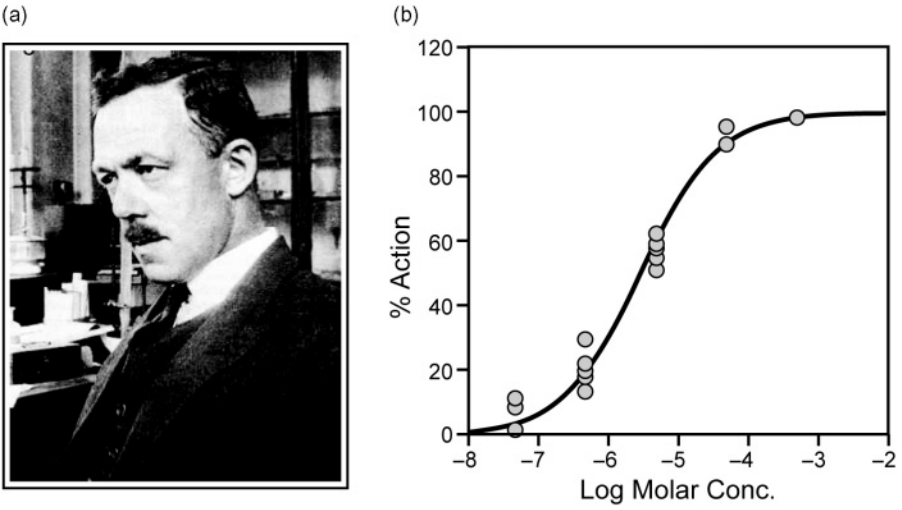

Figure 1.1 (a) Alfred. J. Clark (1885–1941), Professor of Pharmacology at University College London and later Chairman of Pharmacology in Edinburgh. Clark applied chemical laws to biological phenomena and is regarded as the father of receptor pharmacology. (b) Clark's data showing responses of frog heart to acetylcholine. Data points are fit to the Langmuir adsorption isotherm (Eq. 1.3).

16 data points are summarized by a shorthand that states acetylcholine produces a chronotropic effect in the frog heart that begins its effect at 50 nM, is half maximal at 2.8 μM, and is approximately maximum at 500 μM.

The adsorption isotherm furnished an extremely useful method of summarizing and handling dose–response data, but what was still required is a way to relate the observed data to the molecular mechanism of the drugs producing the effect. With no independent estimate of the affinity of the agonist he was using, Clark had to assume a one-to-one correspondence between the molecules of agonist he added to his preparation and the quanta of excitation those molecules gave to the tissue; that is, there was no provision for variance in the "power" of the molecules to induce tissue response. Ariens and Van Rossum, leading a germinal receptor group in Nijmegen, began the process of relating the molecular mechanism of drugs to the observed effects of drugs [5–7]. Thus was introduced the concept of "intrinsic activity," denoted α, as a scaling factor to accommodate the observation that not all agonists produce the maximal response of the preparation. Under these circumstances, Equation 1.4 becomes:

$$\text{Response}_A = \frac{[A]^n \cdot \alpha \cdot E_{max}}{[A]^n + EC_{50}^n} \tag{1.5}$$

where, for $\alpha = 0.5$, this would depict a drug that produced 50% of the tissue maximal response. Intrinsic activity became the first parameter designed to scale observed drug effect with the molecular "power" of an agonist to induce response. While this improved the correspondence between some observed effects of agonists, it was left to R.P. Stephenson, a pharmacologist working in Edinburgh, to extend this process to another level. Stephenson postulated that there was no reason to assume that tissue response was linked to agonist concentration in a linear manner (as was the requirement of the Clark and Ariens treatments). Instead, he postulated the existence of a theoretical parameter he called "stimulus," which is the result of the immediate interaction of the drug with the receptor [8]. This stimulus is imparted to the cell which then processes it in various ways, according to its needs, to yield tissue response. This loosely defined a function (referred to as the stimulus–response function) relating tissue excitation and response. Thus, tissue response was given as:

$$\text{Response}_A = f\left[\frac{[A] \cdot e \cdot E_{max}}{[A] + K_A}\right] \tag{1.6}$$

where e is a term efficacy (used to depict the power of the drug to produce response) and f is the stimulus–response mechanism. The important aspect of Equation 1.6 is that it allows the tissue response to be dissociated from receptor occupancy; experimental data would soon show the importance of that feature of the model.

Stephenson's concept of efficacy was required because of his observation that a series of related alkyltrimethylammonium compounds produced different maximal levels of guinea pig ileal contractions within a similar concentration range [8]. Since the agonist potencies indicated that the compounds had similar affinities for the receptor, Stephenson reasoned that another property of these molecules had to be operative to make them dissimilar in terms of producing muscle contraction; that was the property he termed "efficacy." This concept opened up a completely new way to look at tissue activation through receptors. Specifically, there were no constraints regarding the power of molecules to produce pharmacological response. Technically, powerful agonists could produce maximal tissue response by activating only a portion of the available receptors; the remaining portion would thus be described as being "spare" or not required for the production of maximal response. This offered maximal control to tissue systems since the cellular receptor density (i.e., varying proportions of spare receptors)

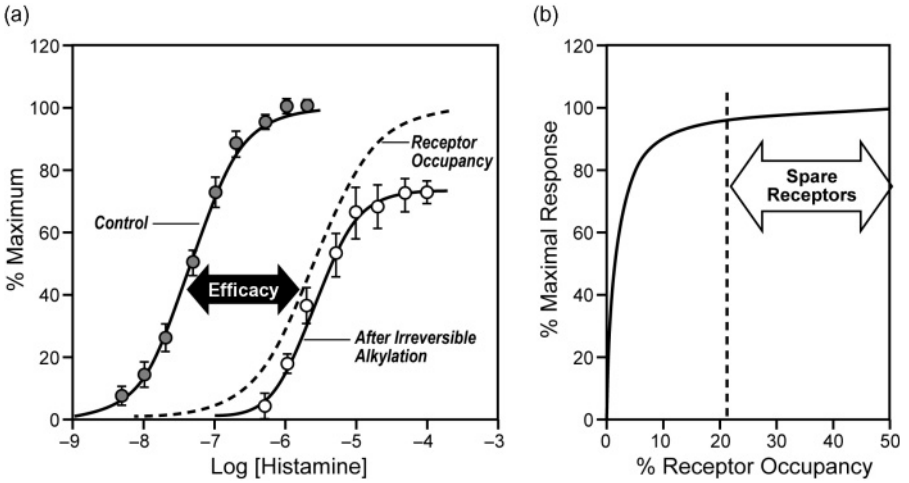

Figure 1.2 Response of guinea pig ileum to contraction by histamine and the relationship between histamine receptor occupancy and tissue response. (a) Contractile responses of guinea pig ileum to histamine. Ordinates: percent maximal contraction to histamine (solid lines) or calculated receptor occupancy from Langmuir adsorption isotherm (Eq. 1.2) for dotted line. Abscissae: logarithm of molar concentrations of histamine. Data shown for control (n = 12) and after alkylation of a portion of the population of histamine receptors with SY-28 (*N*-ethyl-*N*-β-bromoethyl)-1′-naphylmethylamine) (200 nM exposure for 3 min followed by 3 h wash; n = 8). Data from Reference 11. (b) Calculated relationship between histamine receptor occupancy (dotted line in panel A) and control histamine response. This is the experimentally derived stimulus–response relationship between histamine and guinea pig ileum. Note the abscissal axis for panel B does not extend to complete receptor occupancy and that essentially 100% tissue response is obtained with an 18% histamine receptor occupancy.