

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

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:

$$Y = \frac{N}{k' + kN} - M \quad (1.1)$$

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:

$$\rho_A = \frac{[A]}{[A] + K_A} \quad (1.2)$$

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 ($\text{Response}_A = \rho_A \cdot E_{\max}$):

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

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:

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

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

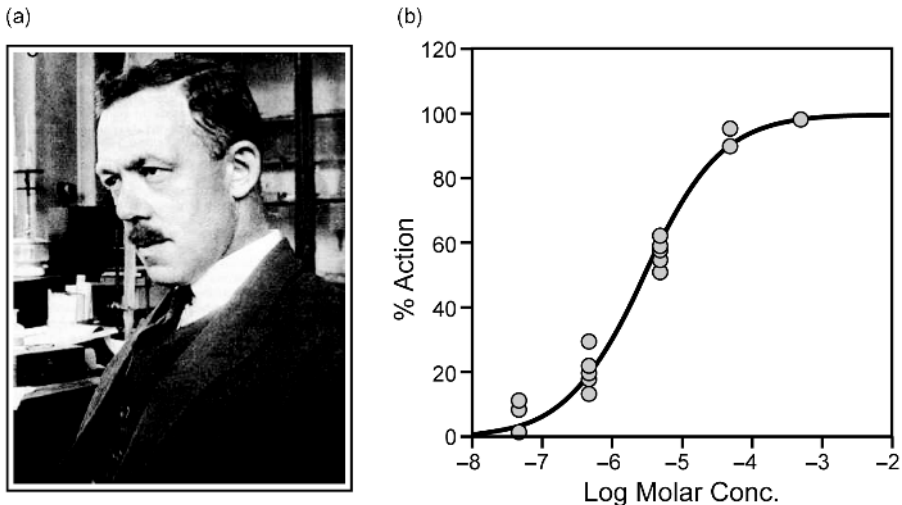


Figure 1.1 (a) Alfred J. Clark (1885–1941), Professor of Pharmacology at University College London and later Chairman of Pharmacology in Edinburgh. Clark applied chemical laws to biological phenomena and is regarded as the father of receptor pharmacology. (b) Clark’s data showing responses of frog heart 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 μ M, and is approximately maximum at 500 μ M.

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:

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

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:

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

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)

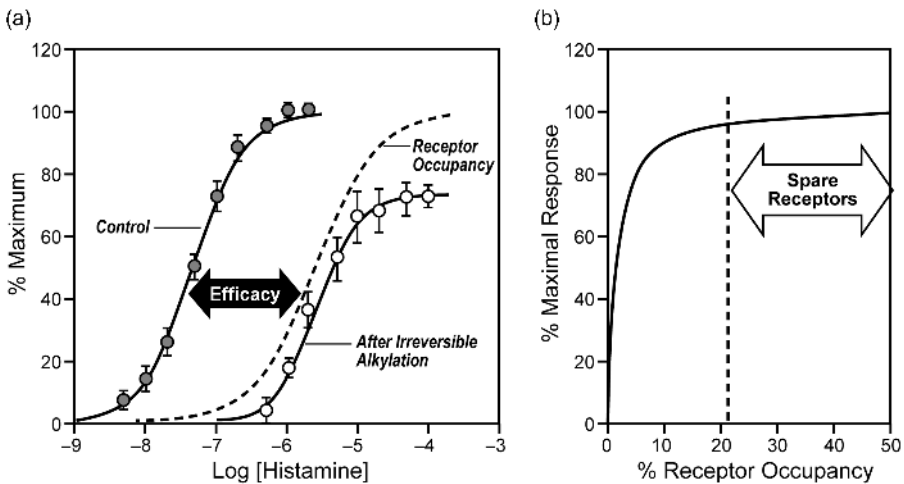


Figure 1.2 Response of guinea pig ileum to contraction by histamine and the relationship between histamine receptor occupancy and tissue response. (a) Contractile responses of guinea pig ileum to histamine. Ordinates: percent maximal contraction to histamine (solid lines) or calculated receptor occupancy 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.

could be used by the tissue to control sensitivity to the hormone or neurotransmitter. Experimental evidence to support the existence of spare receptors came with the use of receptor alkylating agents (specifically, β -haloalkylamines) which could irreversibly block portions of the receptor population in any given tissue [9, 10]. It was observed that irreversible removal of large portions of receptor populations rendered tissues less sensitive to agonists but that these agonists still were capable of producing the maximal tissue response. This experimentally defined the shape of stimulus–response relationships as postulated by Stephenson; an example of the process used to do so is shown in Fig. 1.2. These data formed the concepts leading to the present model universally used to depict agonist response in tissues, namely the operational model.

1.4. THE OPERATIONAL MODEL OF DRUG ACTION

While Stephenson's treatment of drug receptor-mediated response can be used to fit data to molecular models, it still utilized efficacy essentially as a fitting parameter tailored to make experimental data fit the model. This arbitrary nature of efficacy led Black and Leff to postulate a new model of drug action based on observed effects of drugs in tissues; they called this approach the operational model of drug action [12]. This model is based on the premise that the efficacy term emerges from an experimentally observed behavior of pharmacological systems, specifically the saturable relationship between receptor stimulation and observed response. An example of the shape of such a relationship is shown in Fig. 1.2b. The hyperbolic shape of this relationship forms the basic premise of this model; the ligand occupied receptor [AR] activates the cellular stimulus–response cascade with a general equilibrium dissociation constant denoted K_E (this is the concentration of [AR] complex producing 50% maximal response):

$$\frac{\text{Response}}{E_{\max}} = \frac{[\text{AR}]}{[\text{AR}] + K_E} \quad (1.7)$$

The more efficient is the process from production of [AR] to response, the smaller is K_E . Substituting mass action for the production of [AR] yields the equation for the operational model:

$$\text{Response} = \frac{[\text{A}] \cdot [\text{R}_t] \cdot E_{\max}}{[\text{A}]([\text{R}_t] + K_E) + K_A \cdot K_E} \quad (1.8)$$

The constant used to characterize the propensity of a given system and a given agonist to yield response is the ratio $[\text{R}_t]/K_E$; this is denoted τ . Substituting for τ yields the working equation for the operational model:

$$\text{Response} = \frac{[A] \cdot \tau \cdot E_{\max}}{[A](\tau + 1) + K_A} \quad (1.9)$$

It can be seen that tissue response is now a function of biologically related quantities, namely the receptor density $[R_t]$ and K_E , concentration of occupied (activated) receptor available for interaction with the cellular machinery mediating tissue response.

At this point, it should be pointed out that the unknown nature of the biochemical reactions linking receptor occupancy and tissue response is not an impediment to the system-independent measure of drug activity. This is because of the null method. Thus, when comparisons of agonists are made in the same tissue at equal levels of response, then the impact of the biochemical cascade translating receptor occupancy and tissue response is removed since their effects are the same for both agonists. Under these circumstances, ratios of receptor affinities (i.e., K_A) and/or ratios of efficacies (ratio of τ values) become unique identifiers of the particular agonist–receptor pairs. Up to this point, only agonism has been discussed, but receptor theory also has provided a number of models to describe the antagonism of agonist response. It is worth considering these before discussion of 7TM receptor mechanisms.

1.5. RECEPTOR ANTAGONISM

There are two major mechanisms of receptor antagonism: orthosteric, whereby the antagonist and agonist compete for the same binding site on the receptor, and allosteric, whereby each has their own binding site on the receptor and the interaction between them takes place through a conformational change in the receptor protein. It is important to differentiate these since these respective antagonist types have different behaviors in pharmacological and physiological systems.

All equations for orthosteric molecular interaction can be derived from the integral of the differential equation describing the receptor occupancy by an antagonist with time ($\partial\rho_B/\text{dt}$) as a function of time and the competition between agonist $[A]$ and antagonist $[B]$ for receptors [13]:

$$\partial\rho_B/\text{dt} = k_2 \frac{[B]}{K_B} \left((1 - \rho_B) - \frac{(1 - \rho_B)[A]/K_A}{[A]/K_A + 1} \right) - \rho_B \quad (1.10)$$

where ρ_b is the equilibrium receptor occupancy by antagonist and K_A and K_B are the equilibrium dissociation constants of the agonist and antagonist receptor complexes, respectively. Upon integration this yields:

$$\rho_{AB} = ([A]/K_A / ([A]/K_A + 1)) \cdot (1 - (\vartheta(1 - e^{-k} 2^{\Phi t}) + \rho_B e^{-k} 2^{\Phi t})) \quad (1.11)$$

where:

$$\vartheta = [B]/K_B / ([B]/K_B + [A]/K_A + 1) \quad (1.12)$$

$$\rho_B = [B]/K_B / ([B]/K_B + 1) \quad (1.13)$$

$$\Phi = ([B]/K_B + [A]/K_A + 1) / ([A]/K_A + 1) \quad (1.14)$$

For accurate estimation of K_B (1/affinity of the antagonist), there must be enough time elapsed in the experiment for the agonist to re-equilibrate with the antagonist-bound receptors. If there is sufficient time for this to occur (the dissociation rate of the antagonist is rapid such that the agonist can attain correct receptor occupancy) and $\text{time}/k_2 > 10$, then Equation 1.11 for receptor occupancy by agonist (ρ_A) reduces to the familiar, and much simpler, equation for simple competitive antagonism presented by Gaddum [14]:

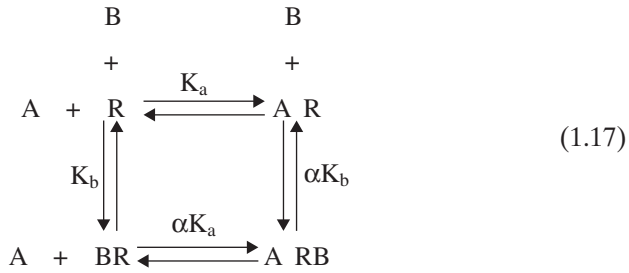
$$\rho_A = \frac{[A]/K_A}{[A]/K_A + [B]/K_B + 1} \quad (1.15)$$

This equation predicts the well-known shift to the right of agonist dose–response curves with no diminution of maxima produced by competitive antagonists (note that agonist (ρ_A) will be complete ($\rho_A \rightarrow 1$) when $[A] \gg [B]$). On the other hand, if the antagonist has a slow offset, there may not be sufficient time for re-equilibration during the experiments and noncompetitive antagonism may result. Under these circumstances, $\text{time}/k_2 < 0.01$ and Equation 1.11 reduces to the Gaddum equation for noncompetitive antagonism:

$$\rho_A = \frac{[A]/K_A}{[A]/K_A (1 + [B]/K_B) + [B]/K_B + 1} \quad (1.16)$$

Under these conditions, Equation 1.16 predicts that the presence of the antagonist ($[B] \neq 0$) essentially precludes complete receptor occupancy by the agonist (ρ_A always < 1 with nonzero values of $[B]$). This can produce dose–response curves with depressed maxima. It can be seen that competitive (surmountable) and noncompetitive (insurmountable) are only kinetic extremes of the same mechanism of drug action (orthosteric binding of antagonist to the agonist binding site).

The other major mechanism for drug-induced receptor blockade is through allosteric interaction whereby the agonist and antagonist bind to their own sites on the receptor, and the interaction between them occurs through a conformational change in the receptor:

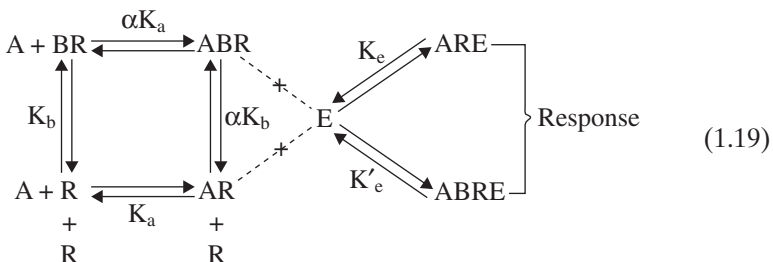


The effect of the modulator on the affinity of the receptor for ligand A is quantified by a factor α (designated the cooperativity constant—the use of this symbol should be differentiated from its use denoting intrinsic activity by Ariens); the affinities of ligands A and B for the receptor are K_a and K_b , respectively. Under these circumstances, the effect of an allosteric modulator on the binding of ligand A is given by [15]:

$$\rho_A = \frac{[A]/K_A (1 + \alpha[B]/K_B)}{[A]/K_A (1 + \alpha[B]/K_B) + [B]/K_B + 1} \tag{1.18}$$

Equation 1.18 defines the changes in affinity of the receptor for A when the modulator B is bound; these can be positive (i.e., increase affinity when $\alpha > 1$) to yield potentiation of binding or negative (decrease affinity when $\alpha < 1$) to yield antagonism. Unlike orthosteric antagonism, binding is not precluded by a negative allosteric modulator but rather, the affinity of the receptor is reset to a different level. Also, since allosteric effect is mediated by binding of the modulator at a separate site, it is saturable; that is, when the allosteric sites are completely bound by modulator, the effect reaches a maximal limit.

The other major delineation between orthosteric and allosteric effect is that allosteric effects can modulate agonist affinity and efficacy separately. This is because modulators essentially stabilize a new conformation of the receptor. To describe the effect on agonist efficacy, an extended model of allosteric modulation of receptors is required. Thus, the Ehlert allosteric model [15] (scheme 18) is linked to the Black and Leff operational model [12] to yield the following [16–18]:



The model for this is a melding of Equations 1.9 and 1.18 to yield [16–18]:

$$\text{Response} = \frac{[A]/K_A \tau (1 + \alpha\beta[B]/K_B) E_{\max}}{[A]/K_A (1 + \alpha[B]/K_B + \tau(1 + \alpha\beta[B]/K_B)) + [B]/K_B + 1} \quad (1.20)$$

In this model, the agonist and modulator-bound receptor complex has the potential ability to signal; therefore, β refers to the change in the efficacy of the agonist when the receptor is bound by modulator ($\beta = \tau'/\tau$ where τ' is the efficacy of the agonist when the modulator is bound to the receptor). This permits the model to predict a range of separate effects on affinity and efficacy of the agonist.

While orthosteric effects are preemptive (once the antagonist binds, no effect can be produced by the agonist) whereby there is never a species of protein with both agonist and antagonist bound, allosteric effects are permissive. This latter property means that there are protein species with agonist and antagonist co-binding simultaneously. Under these circumstances, the antagonist (actually more specifically, allosteric modulator) can change the receptor reactivity toward the agonist in a number of ways, that is, ranging from increased to decreased affinity, increased to decreased efficacy. Moreover, these effects can be probe dependent, that is, be different for different agonists [19]. This can lead to interesting effects such as that seen with the N-methyl-D-aspartate (NMDA) receptor antagonist ifenprodil [20]. This drug reduces the efficacy but increases the affinity of the receptor for NMDA; under these circumstances, ifenprodil potency, as an antagonist, increases with increasing concentrations of NMDA; that is, the antagonism increases as the system is more highly driven.

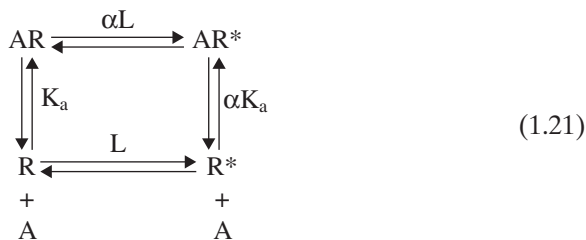
These models of receptor function can be used to characterize agonist and antagonist action using null methods with no knowledge of receptor structure or biochemistry required; that is, the receptor can be viewed as an operational on–off switch. While this was imperative in the early years of receptor theory where no mechanism-based knowledge concerning receptor function was available, the last 20 years of technological advancement has furnished a wealth of information about the structure and function of receptors. This has added greatly to the understanding of drug and receptor function. It is worth considering how knowledge of receptor structure and biochemical function has added to the models used to describe and predict drug action.

1.6. SPECIFIC MODELS OF GPCRS (7TM RECEPTORS)

Improvement in biochemical and structural techniques has provided the capability to characterize receptors and, more importantly, the other cellular bodies with whom they interact. There are two fundamental discoveries that led to our current model of receptor function, namely receptor allostereism and

membrane protein translocation (the “mobile receptor hypothesis” [21]). Allosterism was first described for ion channels [22, 23] and enzymes [24] and was subsequently applied to receptors [25–27]. The application of the idea that ligands can specifically bias protein conformation through selective binding (conformational selection [28]) gives a molecular basis for the concept of agonist efficacy (and actually, all allosteric modulation of receptors).

In this scheme, drugs bind to a limited number of preexisting receptor conformations and stabilize those through binding (at the expense of other conformations according to Le Chatelier’s principle of an equilibrium responding to perturbation). Selective affinity of the ligand for specific receptor active states creates a bias in the collection of receptor conformations, that is, a ligand with higher affinity for one of the conformations will enrich it through selective binding. Scheme 21, shown below, shows how selective affinity can produce receptor activation: shown are two receptor conformations R and R* controlled by an equilibrium dissociation constant L where $L = [R^*]/[R]$:



The scheme above shows a ligand A with an affinity (defined as the equilibrium association constant $K_a = k_1/k_2$) of K_a for receptor state R and αK_a for receptor state R*. The factor α denotes the differential affinity of the agonist for R*; that is, $\alpha = 10$ denotes a 10-fold greater affinity of the ligand for the R* state. Therefore, α (selective affinity) confers the ability of the ligand to alter the equilibrium between R and R*. This can be seen by calculating the amount of R* (both as R* and AR*) present in the system in the absence of A and in the presence of A. The equilibrium expression for $([R^*] + [AR^*])/[R_{tot}]$ where $[R_{tot}]$ is the total receptor concentration given by the conservation equation $[R_{tot}] = [R] + [AR] + [R^*] + [AR^*]$ is:

$$\rho = \frac{L(1 + \alpha[A]/K_A)}{[A]/K_A(1 + \alpha L) + 1 + L} \quad (1.22)$$

In the absence of agonist ($[A] = 0$), $\rho_0 = L/(1 + L)$ while in the presence of a maximal concentration of ligand (saturating the receptors; $[A] \rightarrow \infty$) $\rho_\infty = (\alpha(1 + L))/(1 + \alpha L)$. Therefore, the effect of the ligand on the proportion of the R* state is given by the ratio ρ_∞/ρ_0 . This ratio is given by:

$$\frac{\rho_\infty}{\rho_0} = \frac{\alpha(1 + L)}{1 + \alpha L} \quad (1.23)$$

Equation 23 predicts that if the ligand has an equal affinity for both the R and R* states ($\alpha = 1$) then ρ_{∞}/ρ_0 will equal unity, and no change in the proportion of R* will result from maximal ligand binding. However, if $\alpha > 1$, then the presence of the conformationally selective ligand will cause the ratio ρ_{∞}/ρ_0 to be >1 , and the R* state will be enriched by the presence of the ligand. R* is a completely new conformation of the receptor which could be more or less sensitive to the endogenous agonist or could signal in its own right (be an agonist). The fact that 7TM receptors are designed to be allosteric in nature (bind small molecule hormones or neurotransmitters in one region of the receptor to cause a change in shape to affect a protein-protein interaction in another part of the receptor) may make 7TM receptors especially prone to allosteric effects by other small molecules.

The other major idea that changed the way in which we view 7TM receptors is the discovery that they float in the lipid membrane and can associate with other membrane-bound proteins to become different species [21]. This confers two special properties on receptors that provide for maximal signal control. The first is that the relative stoichiometries of receptors and interactants can be used by the cell for fine-tuning of signal magnitude and cell sensitivity. The second is that it allows the receptor to function in a much more complicated mode than a simple on-off switch. In a hard-wired mode whereby the activated receptor is mandatorily linked to a single response element, receptor activation is binary in that excitation either is or is not imparted to the cell. In a floating disconnected mode, the receptor can link to a range of couplers giving it the capability to discern which response element it activates in response to which initial stimulus it receives. In short, it becomes a microprocessor.

1.7. THE RECEPTOR AS MICROPROCESSOR: TERNARY COMPLEX MODELS

The floating receptor hypothesis resulted in the prototype model of 7TM receptor action, namely the ternary complex model (first published by DeLean and colleagues [29]). This describes a receptor that, when activated by an agonist, moves laterally in the cell membrane to physically couple to a trimeric subunit referred to as a G protein. Supporting this model were data showing that physical complexes between receptors and G proteins could be isolated after addition of agonist to receptor systems (i.e., References 30, 31). The prevalence of this mechanism (receptor protein that recognize external ligands and transmit signals to cellular GTPase heterotrimers called G proteins to elicit response) led to the pervasive name for 7TM receptors from that period as G protein-coupled receptors (GPCRs).

Receptor behavior that was inconsistent with the ternary complex model was observed nine years after its description; this was the impetus for the publication of the extended ternary complex model [32]. Specifically, Costa and Herz [33] noted that a peptide opioid receptor antagonist selectively

reduced the basal level of NG108 cells containing μ opioid receptors and that this behavior was due to the reversal of elevated basal tissue response due to receptors that spontaneously formed an active state. There was no provision for spontaneous non-ligand-dependent activation of receptors in the original ternary complex model; thus, a new model had to be devised: this was the extended ternary complex model.

The extended ternary complex model describes a receptor that can exist in two states, active ($[R_a]$) and inactive ($[R_i]$), named for their ability to activate G proteins $[G]$ that coexist according to an allosteric constant unique for the receptor type (denoted $L = [R_a]/[R_i]$)—see Fig. 1.3a). The affinity of the ligand for $[R_i]$ is denoted K_a (equilibrium association constant); the ligand has a differential affinity for $[R_a]$ of αK_a . The unbound receptor has an affinity for G protein of K_g ; ligands can confer a different affinity of the receptor for G protein denoted γK_g . This model describes response production (elevated concentrations of $[R_a]$ and $[AR_a]$) as a fraction of total receptor species (denoted ρ) as:

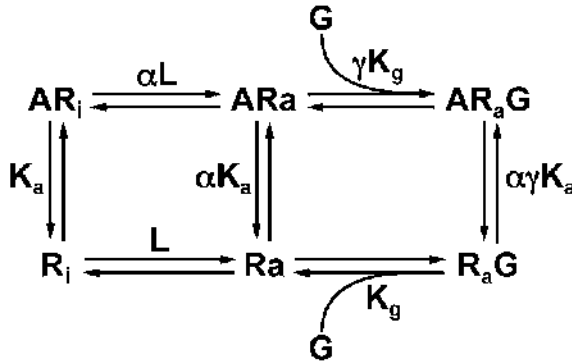
$$\rho = \frac{L[G]/K_G(1 + \alpha\gamma[A]/K_A)}{[A]/K_A(1 + \alpha L(1 + \gamma[G]/K_G)) + L(1 + [G]/K_G) + 1} \quad (1.24)$$

where K_A and K_G are equilibrium dissociation constants (reciprocals of association constants). Figure 1.3b shows the effects of changing α on dose–response curves of a system with existing constitutive activity (shown as an elevated basal response which can be reduced by ligands with $\alpha < 1$). Such ligands are referred to as inverse agonists. Formally identical effects are observed with changes in γ values.

The extended ternary complex model gives a vectorial quality to efficacy. As discussed above, efficacy can be described as a selective affinity of the ligand for various receptor states. Thus, $\alpha > 1$ leads to positive agonism while $\alpha < 1$ results in inverse agonism. This model has been referred to as a “two-state” model, probably because of the two unliganded species $[R_i]$ and $[R_a]$. However, this is a misnomer since the model actually describes infinite receptor states when the receptor is ligand bound; that is, the magnitude of γ confers a unique affinity of the receptor for G proteins when the receptor is ligand bound. Under these circumstances, every value of γ defines a new ligand-bound receptor state.

A theoretical shortcoming of the extended ternary complex model is the fact that it allows only the activated receptor to form complexes with G protein. Thermodynamically, there is no reason a priori that all receptor species (active and inactive) cannot bind to G proteins; when this is added to the extended ternary complex model, a cubic model results where the receptors form the species $[ARiG]$ as well as $[ARaG]$. The resulting model is known as the cubic ternary complex model [34–36]; this is a more rigorous and thermodynamically correct model, but it is more difficult to use since there are a greater number of parameters that cannot be independently estimated.

(a)



(b)

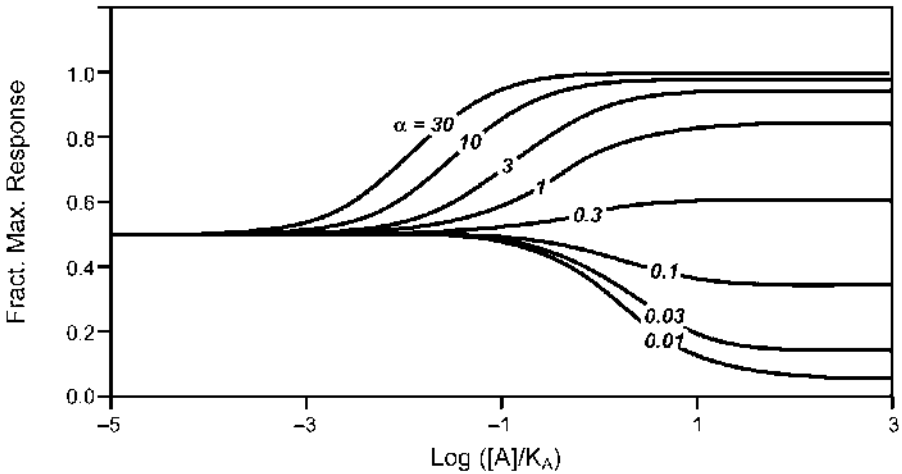


Figure 1.3 The extended ternary complex model for 7TM receptor function [32]. (a) Schematic diagram showing a receptor that can exist in an inactive $[R_i]$ and active $[R_a]$ state; both states can interact with a ligand A and the active state interacts with G protein $[G]$. A variant is the cubic ternary complex model where the inactive receptor can also interact with G protein [34–36]. (b) Effects of ligands with varying efficacy (α values) producing the response-yielding species $[AR_aG]$ according to Equation 1.24. This simulation shows a system with constitutive activity (basal effect = 0.5) and the effect of positive ($\alpha > 1$) and inverse ($\alpha < 1$) agonists.

At this point, the existing models, while being able to accommodate complex receptor function (vide infra), still basically considered receptors as on–off switches. However, the floating nature of the receptor and the ability to form complexes with multiple membrane-bound proteins as well as biochemical studies of receptors produced evidence that multiple interaction of receptors with cytosolic proteins in the cell membrane exist (i.e., see Fig. 1.4). For example, the thyrotropin receptor has the capability of coupling to all four

7TM Receptor Coupling Pleiotropy

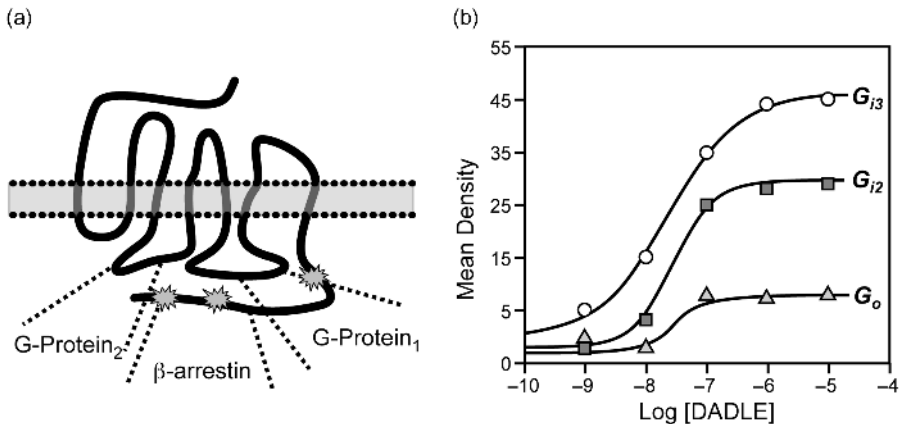


Figure 1.4 Pleiotropic coupling for 7TM receptors. (a) Various regions of the cytosolic loops of receptors can bind to various cellular coupling proteins (i.e., G proteins, β-arrestin). Gray areas represent sites for receptor phosphorylation. (b) Coupling of three G proteins to opioid receptors; ordinates are density measurements reflecting amount of ternary complex (receptor, G protein, and opioid agonist D-Ala²,D-Leu⁵ enkephalin [DADLE]). Data redrawn from Reference 37.

major G protein families (a total of 10 G protein subunits) [38]. In general, the use of recombinant receptor systems made clear that most receptors could couple to multiple G proteins [39–44]; it is not entirely clear to what extent these multiple couplings are artifacts of recombinant overexpression or physiological relevant fine-tuning of receptor signaling.

The preceding discussion has focused on receptor coupling that results in activation of G proteins. Historically, the canonical view of GPCR signaling cascades places receptor activation in a queue preceding receptor phosphorylation, binding of phosphorylated receptor to arrestin-family adapters, uncoupling from G proteins [45], and internalization of those complexes to the endocytotic compartment [46]. The binding to β-arrestin was thought primarily to serve the function of turning off the G protein signal. A major recent development in receptor pharmacology is the discovery of the ability of GPCRs to signal directly through the β-arrestin pathway to activate extracellular signal-regulated kinases (ERKs) through the formation of cytosolic “receptosomes” [47, 48]. Under these circumstances, β-arrestins can be considered to act as multifunctional adapters and scaffolds enabling the recruitment of signaling molecules (i.e., ERK) and other assemblies in an activation-dependent manner [49, 50]. Thus, receptors are now known to produce activation of ERK1/2 pathways via G protein-dependent or G protein-independent pathways [51–53]. G protein versus receptosome signaling differs both from

its cellular origins and temporal relationship to ligand stimulation. For example, parathyroid hormone (PTH) produces G protein-dependent and G protein-independent stimulation of ERK1/2. While the G protein component is transient, a temporally distinct long-lasting stimulation is produced by direct stimulation of β -arrestin by the activated receptor. Interestingly, different chemical analogues of PTH have been shown to selectively activate these separate signaling pathways. Thus, while [Trp¹]PTHrp-(1–36) selectively produces G protein-mediated ERK1/2 stimulation, [D-Trp¹², Tyr³⁴]PTH-(7–34) selectively produces β -arrestin-dependent, and G protein-independent, stimulation of ERK1/2 [54].

1.8. RECEPTORS AS BASIC DRUG RECOGNITION UNITS

In lieu of direct biochemical characterization, historically, receptor classification was based on the observed system-independent measurement of agonist potency ratios (PRs) and antagonist equilibrium dissociation constants. This approach depends on the concept that the receptor is the single discerning unit for agonism. Under these circumstances, the affinity and efficacy terms in Equation 1.9 (namely K_A and τ respectively) refer to the specific interaction of a given agonist for a given receptor (irrespective of which cell type response is mediated). Under these circumstances, the magnitude of the relative ratio of potency (PR) for two full agonists (A and B) is a unique identifier of the agonists and receptor type since it is independent of all tissue-based response elements:

$$PR_{AB} = \frac{K_{AA} \cdot (\tau_B + 1)}{K_{AB} \cdot (\tau_A + 1)} \quad (1.25)$$

Deviation of such PR estimates were considered to be presumptive evidence of differences in the receptor (as the minimal recognition unit). The emphasis of classical pharmacology was recognition of chemicals since the response systems usually came as an intact unit (i.e., isolated tissues). However, the independent nature of the receptor and response elements (a “floating” receptor interacting with different free G proteins and other cytosolic proteins) adds another element of recognition, namely the recognition of the response element after agonist binding. The impact of this factor became clear with the use of recombinant receptor systems where the relative stoichiometry of these elements could be varied. In addition, technological advances furnished the means to selectively observe the individual components of cellular response, thereby allowing the measurement of changes in distinct receptor-coupled pathways to agonist activation. These types of systems furnished experimental data that was totally inconsistent with the previously described assumptions concerning PRs of agonists. Specifically, it was observed that the

potency of agonists for a single receptor differed when different response cascades, mediated by that same receptor, were measured. For example, for pituitary adenylate cyclase-activating polypeptide (PACAP) receptors, which pleiotropically mediate changes in cyclic adenosine monophosphate (AMP) and inositol phosphate 3 (IP₃), two PACAP peptide fragments (PACAP₁₋₂₇ and PACAP₁₋₃₈) produced elevated cyclic AMP and IP₃ in cells. However, the relative potency of these two agonists for these pathways was reversed [55]. Thus, the relative efficacy of PACAP₁₋₂₇ for cyclic AMP elevation is higher than that for PACAP₁₋₃₈ but lower for elevation of IP₃. In historical terms, this would have implied that the responses to the two agonists were mediated by different receptors. Since only one receptor type was transfected into the cell, this was not an option requiring consideration of alternative ideas. It should be noted that relative ratios of potency need not be reversed to denote functional selectivity since the actual numerical value of the PR accurately depends on relative affinity and efficacy of the agonist for the receptor recognition unit.

A tacit assumption in the historical view of agonist PRs is the idea that all agonists produce a uniform receptor-active state, that is, they all flip the receptor switch in an identical manner. However, there is an abundance of evidence to show that proteins spontaneously produce a myriad of conformations in response to thermal energy [56–59]. In accordance to the concept of conformational selection [28], ligands interact with these collections of conformations (termed “ensembles”) and stabilize subsets of them through selectively high affinity [60, 61]. If there were a collection of conformations, then some may have greater affinity for some response elements than others. Under these circumstances, the minimal recognition unit, with respect to the response elements of the cell, would not be the receptor per se but rather the receptor-active state (i.e., ligand-stabilized subset of receptor conformations made after ligand binding). If the active state were the minimal recognition unit, then the experimental results indicating differing PRs for different response pathways can be accommodated. Such differences require that different ligands stabilize different ensembles of receptors to produce different macro-active states interacting with the cell.

The varying PRs observed for PACAP analogs led to a modified model of receptor stimulus whereby stimulus could “traffic” to different portions of a cellular stimulus–response cascade [62]. There has been a large body of evidence since that time to verify this mechanism with many ligands (see References 63–67 for reviews of specific papers), suggesting that many ligands produce different receptor-active states. This is, in fact, consistent with experimental observations. There is another large body of data from a number of experimental approaches that confirm that ligands can stabilize different receptor conformations [68–72]. This idea completes the general notion of 7TM receptors as microprocessors. Thus, a range of transient receptor conformations can be stabilized by different ligands to form varying predominant conformations that then interact with a range of independent cytosolic reactants to produce cellular response.

1.9. RECEPTOR STRUCTURE

In parallel experiments conducted over the past decades, studies on 7TM receptor structure have contributed to the models of function. In particular, point mutation studies have elucidated the various separate regions that interact with different ligands and different response coupling elements. The notion of receptor allostery has attained prominence now that functional, as opposed to binding, high-throughput screens are increasingly being used for new drug discovery. It can be argued that the early use of radioligand binding assays in screening has biased systems to the detection of orthosteric ligands, leaving an impression that allosteric molecules for 7TM receptor are comparably rare [73]. However, 7TM receptors are nature's prototype allosteric protein binding small hormones and neurotransmitters in one region of the protein and changing shape to produce a change in a protein-protein interaction in another part of the protein. Theoretically, it would be predicted that many small molecules would function as drugs in an allosteric manner. As functional high-throughput screens are implemented, a corresponding increase in the number of allosteric modulators are being discovered.

Allosteric molecules can produce immensely powerful receptor-mediated effects. For example, biochemical [74, 75] and structural [76] studies of the chemokine (C-C) motif receptor 5 (CCR5) receptor show a number of small molecule allosteric modulators that can block the interaction of enormous proteins (CCR5 and gp120, the HIV viral coat protein both over 120 Da). These ideas, taken together with the notion that ligands stabilize preferred conformations of receptors to affect response, lead to the notion that the complete surface of the receptor may be considered a potential drug binding active site. With this in mind, structural definition of binding pockets may not be as relevant to 7TM receptors as it is for enzymes.

1.10. FUTURE CONSIDERATIONS

Present concepts of 7TM receptor function suggest a broad range of chemical interventions for possible therapeutic utility. Receptors are now known to have an intrinsic activity (spontaneous formation of active states) that probably is part of the normal sampling of tertiary structures in conformational space. This makes them active control points for cells to limit and increase external signals and internal needs. A useful way to interrogate possible chemical probing of such a system is through the Receptor Probability model described by Onaran and Costa [60, 61]. This model makes no assumptions as to the conformation of the receptor, nor its pharmacological function, but rather describes the probability that a given ligand will produce a conformational bias in the ensemble that may or may not subsequently have biological effect. This opens the concept of efficacy away from a linear idea whereby ligands must produce a set number of effects (i.e., activation, desensitization,

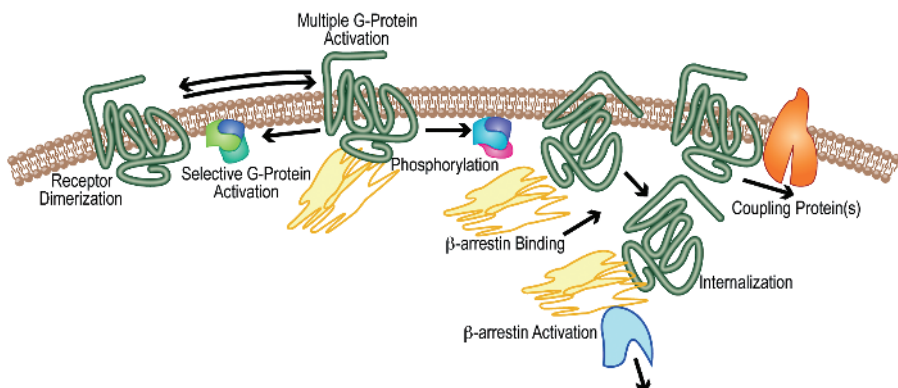


Figure 1.5 Some interactions of 7 transmembrane receptors with cellular components to generate phenotypes of efficacy. Cellular response does not automatically indicate direct interactions of the ligand-bound receptor with some of these processes (i.e., not a universal indicator of molecular efficacy) and ligands can have mixed efficacies, that is, be an inverse agonist at one G protein subtype and a positive agonist at another. This scheme also highlights the potential for cellular signaling through non-G protein pathways (i.e., β -arrestin).

internalization) in order. With the probability model, efficacy can be “collateral” [77] in that ligands can produce subsets of activities. For example, there are a number of antagonists for many receptor types that actively produce receptor internalization without producing receptor activation [78; see also Reference 65].

In general, a rich array of behaviors can be ascribed to 7TM receptors (Fig. 1.5). For example, fine-tuning of external control may be achieved by receptors in the formation of hetero- or homodimers (or higher-order oligomers) or association with other membrane proteins such as receptor activity-modifying proteins (RAMPs). In fact, it is an unfortunate misnomer to refer to 7TM receptors as GPCRs as they are known to couple to a vast other array of signaling proteins in the cell [79–83]. Under these circumstances, 7TM receptors can be considered to be viable active moieties in cells, the activity of which can be altered by agonists, inverse agonists, antagonists, and agents that otherwise alter their disposition (i.e., actively internalize receptors).

Another general idea to emerge is the labile nature of receptors in response to binding. In light of thermodynamic concepts relating to spontaneous formation of receptor conformations, binding should be considered an active, rather than a passive, phenomenon; that is, binding of any ligand to the receptor will change the overall makeup of the conformational ensemble (i.e., according to Eq. 1.23). Simulations of the effects of binding to a random collection of protein conformations show a correlation between affinity and “efficacy,” the latter being defined as the ability to change the conformation of the receptor

ensemble [84]. This can be made more evident by examination of equations describing collections of protein conformations. Thus, consider a system containing a root inactive receptor conformation R and multiple other conformations labeled R_i where $i = 1$ to n all controlled by various allosteric constants (denoted L_i). Under these circumstances, the fraction of receptors not in the R state under basal conditions is given by:

$$\rho_{\text{nonR}} = \frac{\sum_{i=1}^n L_i}{\left(1 + \sum_{i=1}^n L_i\right)} \quad (1.26)$$

With affinity of A for R_i as K (equilibrium dissociation constant of ligand-receptor complex is K^{-1}) and affinity of A for each state K_i as $\Psi_i^{-1}K$, the fraction of receptors not in the R state in the presence of a saturating concentration of ligand is given by [66]:

$$\rho_{\text{nonR}} = \frac{\sum_{i=1}^n L_i + [A]/K \sum_{i=1}^n \Psi_i L_i}{[A]/K \left(1 + \sum_{i=1}^n \Psi_i L_i\right) + \left(1 + \sum_{i=1}^n L_i\right)} \quad (1.27)$$

It can be seen that no change in the fraction of receptors different from the R state will occur in the presence of the ligand only if the affinities of the ligand for every state is the same (equal to K , i.e., $\Psi_{i \text{ to } n} = 1$). If the ligand has differential affinity for any of the states R_i , then it will alter the fractional makeup of the ensemble and produce different relative quantities of the specific conformations. Thus, a receptor system, at any given instant, provides a ligand with a choice of conformations to which it can bind; in effect, the ligand enters a “conformational cafeteria.” The ligand, in turn, will bind to proportions of states commensurate with the respective affinities it has for each state. However, as these conformations are interconvertible, the proportions of the states will change according to the selective affinities they have for the ligand; that is, the ligand becomes a stabilizing influence toward certain preferred states for which it has the highest affinity. This is an extension of the effects described for two states but highlights the probability of changes in ensembles with increasing conformational states; that is, the more states there are, the higher the probability that a given ligand with macroaffinity for the receptor will alter the relative quantities of the various conformations upon binding.

The rich range of behaviors observed for 7TM receptors, intrinsic or in response to chemicals or cellular reactants, makes these proteins versatile and complex control points for physiological function. This, in turn, gives pharmacologists and medicinal chemists vast opportunity to exploit these complex control units through selective binding to specific tertiary conformations. The complexity of this overall system is both advantageous (high potential) and disadvantageous (difficulty in assessing value). It may be that introduction of

new selective ligands into the most complex system of all (patients in the clinic) will be the step that discerns value from extraneous complexity.

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