PHARMACODYNAMICS IN PHARMACOLOGY

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Contents

Summary

This chapter discusses the tools and kinetic models used to quantify drug activity in terms of the system-independent parameters of affinity (the force that binds the drug to the target) and efficacy (the molecular property of the drug that causes stabilization of certain active forms of the receptor thereby generating pharmacological response). The process of response production (agonism) is described in terms of the operational model of drug action. Potency ratios of agonists are discussed as quantifiable measures of agonist activity.

Drug antagonism of response also is discussed in terms of two molecular mechanisms; orthosteric antagonists bind to the same site as the agonist and preclude agonist binding (and therefore response) and allosteric binding whereby the antagonist binds to a different site on the receptor to elicit its effect on the agonist through a conformational change (change in shape) of the receptor. The measurement of the equilibrium dissociation constant of the antagonist-receptor complex is described as the system independent measure of antagonist potency.

1. Introduction

Pharmacology is the science or study of drugs. The word is an amalgam of the Greek *Pharmakos* (medicine or drug) and *logos* (study). In total, this is a broad discipline encompassing different aspects of the use of chemicals to treat and cure disease. **Pharmacodynamics** is the study of the interaction of the drug molecule with the biological target, often referred to generically as the receptor. This forms the basis of Pharmacology in that all therapeutic application of drugs has a common root in pharmacodynamics, i.e. all drug molecules must bind to and interact with receptors to exert an effect.

2. Pharmacological Receptors

In the early years of the history of pharmacology, the concept of the 'receptor' was abstract. The actual physical nature of receptors was not known but rather, they were an abstraction consisting of a means to recognize drugs and read the chemical information encoded in them. Early concepts of receptors likened them to locks and drugs as keys; as stated by Paul Ehrlich '… substances can only be anchored at any particular part of the organism if they fit into the molecule of the recipient complex like a piece of mosaic finds its place in a pattern…'. As technology has progressed, the physical nature of receptors has become clear. Now it is known that 'receptors' can be a number of structures in cells such as enzymes, ion channels, DNA, and structures in the nucleus termed nuclear receptors.

The term receptor also has taken on a more specific meaning, namely families of proteins that reside on the cell membrane (half of the receptor faces out to the extracellular space and half into the cytoplasm). These are highly specific proteins capable of recognizing chemicals such as hormones and neurotransmitters that are present in the extracellular space and transmit signals from these to the cell interior. They do this through changes in protein conformation, i.e. the drug does not enter the cell nor does the receptor change the nature of the drug (as would an enzyme). Perhaps the most versatile class of therapeutic receptors is that of G-Protein Coupled Receptors (GPCRs) so called because they activate small GTPase proteins on the cytosolic side of the cell membrane. These receptors interact with numerous neurotransmitters and hormones as well as different G-proteins. This dual system of recognition gives GPCRs a wide ranging capability to process information.

Selective binding of molecules to receptor proteins is a two way street in that, not only can differences in the structure of the messenger molecules be discerned by receptors, but a reciprocal discernment can take place by receptor in the form of subtypes for the same molecule. For example, the neurotransmitter norepinephrine mediates a plethora of physiological processes but selectivity in the information transmitted by this molecule to systems is produced by the existence of subtypes of receptor for this molecule. Thus, four subtypes of α-adrenoceptor and three subtypes of β-adrenoceptors can differentially read the information coded in norepinephrine to give a texture to signaling for this single molecule. Up until approximately 10 years ago pharmacological production of drug selectivity relied solely upon receptor subtypes.

3. What is Pharmacodynamics?

The principles of pharmacodynamics form the basis for all drug effect, i.e. they describe the association of the drug with the biological target (receptor) and also the mechanisms by which the drug changes the target to evoke a physiological response. There are two basic concepts that describe the action of all drugs; affinity and efficacy. The affinity of the drug describes the molecular forces that keep the drug associated with the target long enough for a biological effect to occur. The efficacy is the intrinsic property of the drug that causes the target to change in response to drug binding. It should be noted that efficacy is a general term often used in clinical medicine to describe the beneficial action of a drug in the therapeutic situation. Thus, a drug can be considered 'efficacious' if it cures the ailment. In the science of pharmacodynamics, efficacy, or more specifically the term 'intrinsic efficacy' has a specific definition, namely that molecular property of the drug that causes the biological target to change its behavior toward the host cell. It will be apparent later why a change in behavior defines intrinsic efficacy and not just excitation of a cellular response. This is because, efficacy has vectorial properties, i.e. it can be positive or negative.

Pharmacodynamics involves use of quantitative tools to measure the affinity and efficacy of drugs; this is an important endeavor since drugs are nearly always tested in surrogate systems for prediction of activity in therapeutic situations. It is very rare that development of drugs has the benefit of testing directly in the therapeutic arena. Therefore, it is very important to develop scales of drug activity that are predictive of activity in all systems, not only the test system. Affinity is a chemical term and can be quantified as a concentration. There is no absolute scale for efficacy; it is measured as a relative quantity.

4. Definitions of Pharmacological Terms

At this point it is useful to define some commonly used pharmacological terms as these associate characteristic behaviors with molecules. The term ***agonist*** refers to a molecule that produces a pharmacological response in a biological system through activation of a pharmacological receptor. The process of chemically-induced response is referred to as ***agonism*** and the molecular property responsible is termed ***efficacy***. The association of a physiological response to agonism is historical since agonism was first studied in systems where the only consequence of drug activation that could be detected was organ response. With increased technologies has come the ability to observe a wide range of receptor behaviors such as internalization, desensitization, homodimer and heterodimer formation etc. Also, the spontaneous production of receptor states that lead to activation also have been detected. In light of these new receptor behaviors, a more encompassing definition of drug efficacy is the property of a molecule that causes the receptor to change its behavior towards its host cell. The term ***antagonism*** describes the binding of a molecule to a pharmacological receptor to render that receptor unable to respond to agonists. Antagonism can be ***surmountable*** such that an excessive concentration of agonist can overcome the antagonism produced by a given concentration of ***antagonist***; this is often, but not necessarily always, the result of competition of the agonist and antagonist for the same binding site on the receptor. Antagonism also can be i***nsurmountable*** in that no amount of agonist is able to overcome the effects of the

antagonist. Antagonism can be ***orthosteric*** whereby the antagonist and agonist compete for the same binding site; under these circumstances, the relative concentrations of the two molecular species and their propensity to bind to the receptor determine the overall pharmacological effect. Another type of antagonist interaction is ***allosteric***. In this mode, both the agonist and antagonist each bind to their own site on the receptor and the interaction between the two occurs through a conformational change in the receptor protein. In this mechanism, the concentrations of the agonist and antagonist as well as their respective propensity to bind to the receptor also determine the overall effect but, in addition, the degree of conformational change to the receptor produced by the antagonist also is relevant. It should be noted that both orthosteric and allosteric antagonism can produce surmountable or insurmountable antagonism of agonist response.

5. Affinities

The mathematical model defining the affinity a drug for a receptor is based on a formulation of the adsorption of molecules onto an inert surface first described by Irving Langmuir. As a chemist for the General Electric Company in the United States, Langmuir was interested in adsorbing chemicals onto metal in the production of filaments for light bulbs and also tubes for radios; he formulated his famous 'adsorption isotherm' in the process. It is worth describing Langmuir's simple reasoning as this tool still forms the basis for the measurement and quantification of drug affinity today. Thus, a molecule A is described which has an intrinsic rate of onset onto the target. In Langmuir's system, the target was the surface of metal but in the context of pharmacodynamics, the target is the binding pocket of a biologically relevant protein such as a receptor. This rate of onset (denoted k_1) is driven by changes in energy, i.e. the energy of the system containing the drug in the receptor binding pocket is lower than the energy of the system with the drug not bound in the pocket. Langmuir called this the 'rate of condensation' of the (in his case) chemical onto the metal surface. However, there also must be defined, what Langmuir described as a 'rate of evaporation' which describes the change in energy when the molecule diffuses away from the surface. In pharmacological terms, this is referred to as the 'rate of offset' of the drug away from the receptor (denoted k_2). When a drug is added to a compartment containing the receptor, then the concentration gradient controls the movement of drug molecules. Initially, the absence of drug in the receptor binding pocket drives the binding reaction toward formation of drug-receptor complexes. However, with time, the bound drug will diffuse out of the binding pocket in accordance with its natural tendency to do so (defined by the energy changes described by the rate of offset). After a period of time, equilibrium will be attained whereby the rate of drug leaving the binding pocket will equal the rate of drug approaching and entering the binding pocket. This ratio of rates (namely k_2 / k_1) determines the amount of drug bound to the receptor at any one instant and this becomes a measure of the avidity of drug binding, i.e. the affinity of the drug for the receptor. This ratio is referred to as the equilibrium dissociation constant (denoted K_{eq}). Langmuir's calculation is as follows:

Fraction of the total area of surface bound by a chemical A is defined as ρ_A.

Fraction of total area left free for further binding of new molecule to the surface = $1-\rho_A$

The amount of drug bound to the surface is the product of the concentration of drug available for binding ([A]), the rate of onset (k_1) and the fraction of surface left free for further binding ($1-\rho_A$):

$$\text{Rate of binding} = d[AR]/dt(+) = [A]k_1(1-\rho_A) \quad (1)$$

where R denotes 'Receptor'. Similarly, the amount of drug diffusing away ('evaporating') from the surface is given by the amount bound (ρ_A) and the intrinsic rate of evaporation of offset k_2:

$$\text{Rate of diffusion off} = d[AR]/dt(-) = \rho_A k_2 \quad (2)$$

At equilibrium, the fractional amount diffusing toward the receptor is equal to the fractional amount diffusing away from the receptor:

$$[A]k_1(1-\rho_A) = \rho_A k_2 \quad (3)$$

Defining K_{eq} as k_2/k_1, this leads to the following relationship for the fractional amount bound (ρ) known as the Langmuir adsorption isotherm:

$$\rho_A = \frac{[A]}{[A]+K_{eq}} \quad (4)$$

It can be seen that when the concentration of the drug is equal to K_{eq}, then $\rho = 0.5$, i.e. the K_{eq} is the concentration of drug that occupies 50% of the available receptor population. Therefore, the magnitude of K_{eq} is inversely proportional to the affinity of the drug for the receptor. For example, consider two drugs one with $K_{eq} = 10^{-8}$ M and another with $K_{eq} = 10^{-6}$ M. The first drug occupies 50% of the receptors at a concentration 1/100 of that required by the second. Clearly the drug with $K_{eq} = 10^{-8}$ has a higher affinity for the receptor than the one with $K_{eq} = 10^{-6}$ M.

Low values for K_{eq} (high affinity) can be the result of either a high rate of onset (high magnitude k_1) or low values for k_2 (slow rate of offset from the receptor). Since the rate of bulk diffusion is a limiting factor in the magnitude of k_1, the value of k_2 usually is the major determinant of the affinity of drugs.

The adsorption isotherm (Eq. (4)) describes the relationship between the concentration of substance A and the amount bound to a given target. The shape of that relationship (ρ_A as a function of varying [A]) is a hyperbolic curve (see Figure 1A). The curve is characterized by a location along the concentration axis (most often identified by a midpoint, the K_{eq} concentration), a slope, and a maximal response. The maximal binding value (denoted B_{max}) is the maximal number of binding sites present in the preparation; ρ_A being the fractional binding, the actual binding values are given by $\rho_A \times B_{max}$. In experimental pharmacology, the location parameter (K_{eq}) and B_{max} are the values most often required to characterize the binding of a given drug to a receptor. It can be seen from Figure 1A that these parameters are difficult to estimate graphically from the depiction of Eq. (4) as drawn. For that reason, a semi-logarithmic form of the adsorption isotherm is used. This more readily estimates the B_{max} (theoretically the value of ρ_A when $[A] \to \infty$) and also the midpoint of the binding curve (K_{eq}). The semi-logarithmic form of Eq. (4) also allows a considerable range of [A] values to be shown to fully characterize a binding reaction. The depiction of a binding curve on a semi-logarithmic scale yields the familiar sigmoidal shape shown in Figure 1B.

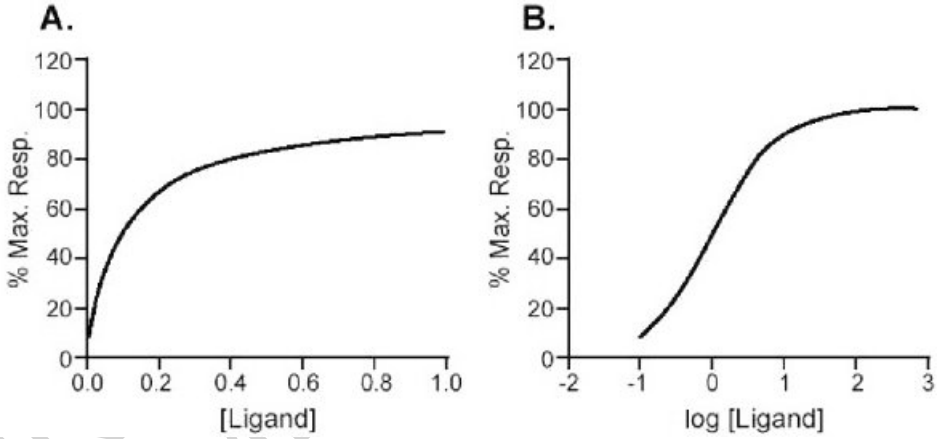

Figure 1: The Langmuir adsorption isotherm. A. Percent ligand bound (ordinates) as a function of the concentration of ligand according to Eq. (4). B. Semi-logarithmic version of the curve in panel A. The adsorption isotherm yields a characteristic sigmoidal curve with a logarithmic abscissa.

Theoretically, measurement of the amount of drug-receptor complex produced by mixing a range of concentrations of drug to a receptor preparation can yield a direct measure of K_{eq}. In some cases, technology has provided systems where this can be done through measurement of the binding of a radioactive drug to a membrane-bound receptor system. Thus, if there is a means to separate radioactive drug bound to the receptor from free solution radioactive drug, then binding experiments can yield affinity. A shortcoming of this approach is the fact that the affinity of only radioactive drugs can be obtained in this way. However, interference with the binding of a radioactive drug by any drug that binds to the same receptor can also yield a direct

measure of the affinity of the non radioactive drug. This will be discussed later when drug antagonism is described.

5.1. Micro- and Macro-Affinity

The affinity for a molecule binding to a given protein state, can be defined as, a micro-affinity for that particular state. Proteins often exist in multiple states with the proportions of the various states changing with changes in the free energy of the complete system. What can be measured experimentally usually is the macro-affinity which is the total affinity of the ligand for the complete collection of states. This becomes an issue when two experimentally relevant states of the protein are prevalent in the system and a measurement of affinity is made. For example, consider the situation where a receptor R can convert into a conformation R^* upon binding of a ligand A. The equilibrium system can be depicted as:

$$A+R \underset{k_2}{\overset{k_1}{\rightleftarrows}} AR \underset{\sigma}{\overset{\theta}{\rightleftarrows}} AR^*$$

where the forward rate constant for production of the R^* species after binding of A is θ and for the backward rate constant for reversion of R^* back to R is σ The equilibrium equations for this system are:

$$k_2/k_1 = [A][R]/[AR] \tag{5}$$

$$\theta/\sigma = [AR]/[AR^*] \tag{6}$$

The receptor conservation equation is:

$$[R_{tot}] = [R] + [AR] + [AR^*] \tag{7}$$

Therefore, the quantity of end product $[AR^*]$ formed for various concentrations of $[A]$ is given as:

$$\frac{[AR^*]}{[R_{tot}]} = \frac{[A]/K_A}{[A]/K_A(1+\sigma/\theta)+\sigma/\theta} \tag{8}$$

where $K_A = k_2/k_1$. The macro affinity of A for R is the observed equilibrium dissociation constant (K_{obs}) of the complete two-stage process is given as:

$$K_{obs} = \frac{K_A \cdot \theta/\sigma}{1+\theta/\sigma} \tag{9}$$

It can be seen that for non-zero positive values of θ/σ (binding promotes formation of R^*), $K_{obs} < K_A$. Therefore the system will not yield measured values of K_A unless the process of isomerization of receptor from R to R^* can either be quantified and accounted for (Eq. (9)) or negated experimentally. This inability to measure micro-affinity is important in binding experiments where isomerization of protein species leads to aberration of measured affinity and misleading estimates.

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Biographical Sketch

Terry Kenakin obtained his B.S. in chemistry and Ph.D. in Pharmacology at the University of Alberta, Canada. After 3 years at University College, London, UK he joined Burroughs-Wellcome as a Senior Research Pharmacologist. He then went on to join Glaxo which became Glaxo-Wellcome and subsequently GlaxoSmithKline where he heads a research team interested in the use of quantitative receptor pharmacology and theory to advance new drug discovery programs within GlaxoSmithKline Molecular Discovery Drug Discovery. He has written 7 books on Pharmacology and is co-Editor-in-Chief of the International Journal of Receptors and Signal Transduction.