International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on Terms and Symbols in Quantitative Pharmacology

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Abstract——The recommendations that follow have been updated from the proposals of a Technical Subcommittee set up by the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (Jenkinson DH, Barnard EA, Hoyer D, Humphrey PPA, Leff P, and Shankley NP (1995) International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. IX. Recommendations on terms and symbols in quantitative pharmacology. *Pharmacol Rev* 47:255–266).

I. Introduction

This update was undertaken to incorporate new information about multiple receptor conformational states

and the recognition that multiple distinct agonist responses may result that have different pharmacological properties (Kenakin, 1995). Nomenclature concerning the actions of allosteric (allotopic) ligands is presented based on recent literature (Christopoulos and Kenakin, 2002). The implications of high receptor numbers in heterologous expression systems for interpretation of agonist function are discussed. Additional changes address the fact that many receptors are not single mac-

romolecules but are made up of multiple subunits. Finally, there are new recommendations regarding nomenclature for equilibrium constants.

II. Working Definition of a Receptor

A cellular macromolecule, or an assembly of macromolecules, that is concerned directly and specifically in chemical signaling between and within cells. Combination of a hormone, neurotransmitter, drug, or intracellular messenger with its receptor(s) initiates a change in cell function. Thus NC-IUPHAR does not classify simple binding sites, without function (although truncated proteins without signaling function may be designated as such, to avoid confusion). Furthermore, a receptor may consist of several proteins, called subunits. In some cases the large number of combinatorial possibilities for assembly of multiple subunits may require NC-IUPHAR to use an interim nomenclature based on the individual subunits (Spedding et al., 2002). Nevertheless, the ultimate goal is to define the multi-subunit assemblies that occur in vivo.

The regions of the receptor macromolecule to which ligands bind are referred to collectively as the *recognition site(s)* of the receptor. Those at which the endogenous agonist binds are termed *primary* or *orthosteric* sites whereas other ligands may act through *allosteric* sites (see Table 1).

III. Use of Drugs in Definition of Receptors or of Signaling Pathways

When using drugs to define receptors or signaling pathways, it would be desirable to use a drug that acts only on the receptor or biological site of interest at all concentrations and doesn't interact with others at any achievable concentration. Unfortunately, there are very few or no drugs with this ideal property. Fortunately, there are numerous drugs with a detectable potency difference (in exceptional cases $>10^3$-fold but usually much less) between their primary target and other related receptors. Because these differences are not absolute, claims for the involvement of a particular receptor, or signaling protein, based on the use of such agents should be backed up by testing with multiple agents, and wherever possible, full concentration-response curves should be obtained for the definition of responses in in vitro experiments. Full dose-response curves should also be obtained in in vivo experiments, if ethical considerations allow.

A. The Expression of Amount of Drug: Concentration and Dose

1. Concentration. It is recommended that the molar concentration of substance X be denoted by either $[X]$ or c_x, with the former preferred. Decimal multipliers should be indicated by the use of either Le Système International d'Unités (International System of Units)

prefixes (e.g., μM, nM) or by powers of ten (e.g., 3×10^{-8} M), with the former preferred.

2. Dose. In some circumstances (e.g., in therapeutics and clinical pharmacology, in in vivo experiments, and when tissues are perfused in vitro and exposed to a bolus application of drug), absolute drug concentrations are uncertain, and it becomes more appropriate to specify the quantity of drug administered. This may be done in terms of either mass or molar quantity. Units and routes of administration should be specified. In the case of in vivo experiments with animals, the quantity of drug is to be expressed per unit of animal mass (e.g., mol/kg, mg/kg). In therapeutics, milligrams per kilogram will normally be appropriate. Negative indices should be used where confusion otherwise arises (e.g., mg min^{-1} kg^{-1}).

B. General Terms Used to Describe Drug Action

Table 1.

C. Experimental Measures of Drug Action

1. General Measures. Table 2.
2. Agonists. Table 3.
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D. Terms and Procedures Used in the Analysis of Drug Action

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2. Action of Agonists. Table 6.
3. Action of Antagonists. Table 7.

IV. Appendix

A. Microscopic and Macroscopic Equilibrium Constants

Microscopic and *macroscopic* equilibrium constants should be distinguished when describing complex equilibria, which occur with all agonists. The latter refers to a single constant describing the overall equilibrium (i.e., the value that would be obtained in a ligand binding experiment), whereas the former refers to each individual constant that describes each reaction step within the equilibrium. For the scheme

$$L + R \underset{}{\overset{K_1}{\rightleftharpoons}} LR \underset{}{\overset{K_2}{\rightleftharpoons}} LR^*$$
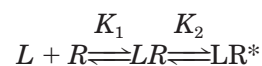
the *macroscopic equilibrium dissociation constant* (denoted here as K_{app} for "$K_{apparent}$") is given by

$$K_{app} = \frac{K_1 K_2}{1 + K_2}$$

Here K_1 and K_2 are the *microscopic equilibrium constants* for the first and second reactions, respectively. Note that in this scheme, saturation radioligand binding assays and Furchgott's (1966) irreversible antagonist method for determining the equilibrium dissociation

TABLE 1
General terms used to describe drug action

Term	Suggested Usage	Notes
Agonist	A ligand that binds to a receptor and alters the receptor state resulting in a biological response. Conventional agonists increase receptor activity, whereas *inverse agonists* (see Table 6) reduce it.	"Receptor activity" may be determined by: the proportion of receptor in an active conformation (e.g., R^* vs. R), post-translational modifications (e.g., phosphorylation), or some other mechanism such as subcellular targeting. Agonists may act by combining either with the same site(s) as the endogenous agonist (*primary* or *orthosteric site*) or, less commonly, with a different region of the receptor macromolecule (*allosteric* or *allotopic site*). Agonists in the second category are sometimes referred to as *allosteric* (*allotopic*) *activators* or *allosteric* (*allotopic*) *agonists*. Some agonists (e.g., glutamate) may only be effective in the presence of another ligand (e.g., glycine in the case of glutamate) that binds to a different site on the receptor macromolecule. Under these circumstances, glutamate is referred to as the *primary agonist* and glycine as a *co-agonist*.
Antagonist	A drug that reduces the action of another drug, generally an agonist. Many antagonists act at the same receptor macromolecule as the agonist. (see Table 7 for more details). Antagonism may also result from combination with the substance being antagonized (*chemical antagonism*). *Functional antagonism* occurs at cellular sites distinct from the receptor mediating the agonist response.	*Functional antagonism* may include mechanisms such as: *indirect antagonism,* which is competition by the inhibitor for the binding site of an intermediate macromolecule that links the binding of the administered agonist to the effect observed (e.g., adrenoceptor antagonist blockade of the actions of tyramine or protein kinase A inhibitors blocking β adrenoceptor agonist effects) or *physiological antagonism* in which the action of one agonist exerts an opposite effect to that of the original agonist—usually through a different receptor (e.g., muscarinic agonist inhibition of β adrenoceptor-stimulated adenylyl cyclase activity in the heart).
Allosteric (allotopic) modulator	A ligand that increases or decreases the action of an (primary or orthosteric) agonist or antagonist by combining with a distinct (allosteric or allotopic) site on the receptor macromolecule.	*Allosteric (allotopic) enhancers* are modulators that enhance orthosteric ligand affinity and/or agonist efficacy while having no effect on their own. *Allosteric (allotopic) antagonists* are modulators that reduce orthosteric ligand affinity and/or agonist efficacy. *Allosteric (allotopic) agonists* or *activators* are ligands that are able to mediate receptor activation in their own right by binding to a recognition domain on the receptor macromolecule that is distinct from the primary (orthosteric) site. *Neutral allosteric (allotopic) ligands* bind to an allosteric site without affecting the binding or function of orthosteric ligands but can still block the action of other allosteric modulators that act via the same allosteric site.
Syntopic interaction	An interaction between ligands that bind to the same recognition site, or to recognition sites that overlap, on the receptor macromolecule.	This term is most commonly associated with the description of competitive interactions between ligands that bind to the primary (orthosteric) site on a receptor, but need not be restricted to this specific situation. A syntopic interaction can also occur between different ligands that share a similar recognition domain (e.g., a common allosteric site) anywhere on the receptor macromolecule.
Allosteric (allotopic) interaction	An interaction between ligands that bind to distinct, non-overlapping, recognition sites on the receptor macromolecule.	The terms *syntopic* and *allotopic* are recommended to distinguish between interactions that occur at a *common (same) site* versus interactions that occur between *different sites,* respectively. Accordingly, the term *allotopic* can be used interchangeably with the term *allosteric* when describing cross-interactions between different sites on a receptor macromolecule. The term *syntopic* should be confined to defining interactions at a common site and should not be used interchangeably with the term *orthosteric;* the latter term specifically refers to the primary (endogenous agonist-binding) recognition *site* on the receptor.
Allosteric transition	The isomerization of a receptor macromolecule between multiple conformational states.	Different authors have used the term, *allosteric,* in different ways (see Colquhoun, 1998; Christopoulos and Kenakin, 2002). One common use of the term is to describe any mechanism that involves the isomerization of a receptor between two or more conformational states that can each display a different affinity for a given ligand. A second common use of the term is to explicitly describe an interaction between two topographically distinct recognition sites on a receptor macromolecule in a given conformational state. In order to accommodate both uses, it is recommended that the term *allosteric transition* be used when describing receptor isomerization mechanisms, and the term *allosteric* (or *allotopic*) *interaction,* be used when explicitly describing a cross-interaction between multiple ligands concomitantly bound to a receptor macromolecule.

constant for an agonist would each provide an estimate of K_{app} rather than K_1.

This distinction is also important when considering those receptors (e.g., ligand-gated ion channels) that have more than one binding site for the agonist.

B. Schild Equation and Plot—Further Detail

The Schild equation is based on the assumptions that (*a*) agonist and antagonist combine with the receptor macromolecule in a freely reversible but mutually exclusive manner, (*b*) equilibrium has been reached and that

TABLE 2
Experimental measures of drug action: general

Term	Suggested Usage	Notes
The relationship between concentration and effect: Hill equation	In the following, drug action is expressed in terms of the effect, E, produced when an agonist, A, is applied at a concentration $[A]$. The relationship between E and $[A]$ can often be described empirically by the Hill equation, which has the form: $$\frac{E}{E_{max}} = \frac{[A]^{n_H}}{[A]^{n_H} + [A]_{50}^{n_H}}$$ where E_{max} is the maximal action of A, n_H is the Hill coefficient and $[A]_{50}$ is the concentration that produces an effect that is 50% of E_{max}.	$[A]_{50}$ in the Hill equation is sometimes denoted by K, and E_{max} by α. The choice between $[A]_{50}$ and K will depend on the directness of the measurement. The former is appropriate if an indirect action, such as the contraction of an intact smooth muscle preparation, is observed. However, in a ligand binding experiment, K would be preferable, although whether the value of K corresponds to a single, microscopic, equilibrium dissociation constant (even if n_H is unity) will depend on the circumstances (see *Section IV. A.*). The *Hill equation* and the *logistic equation* are closely related but not identical (see *Section IV. C.*).
Potency	An expression of the activity of a drug, in terms of the concentration or amount needed to produce a defined effect; an imprecise term that should always be further defined (see EC_{50}, IC_{50}, etc.).	Drug potency depends on both receptor (affinity, efficacy) and tissue (receptor numbers, drug accessibility) parameters. The term is sometimes, incorrectly, used to refer to the maximum effect attainable.

TABLE 3
Experimental measures of drug action: agonists

Term	Suggested Usage	Notes
EC_{50} or $[A]_{50}$	The molar concentration of an agonist that produces 50% of the maximal possible effect of that agonist. Other percentage values (EC_{20}, EC_{40}, etc.) can be specified. The action of the agonist may be stimulatory or inhibitory.	The mass concentration (g/l) should be used if the molecular weight of the test substance is unknown.
		It may sometimes be preferable to express the activity of a drug in terms of the concentration that causes a specified change in a baseline measurement (e.g., a 20 mm Hg change in perfusion pressure; a 30% increase in a muscle twitch). If the EC_x (or $[A]_x$) terminology is to be used in this context, the appropriate units must be included (e.g., EC_{20mm} or $[A]_{30\%}$) to avoid confusion with EC_{20} or $[A]_{30}$ as here defined.
		Because the relation between receptor occupancy and response is usually nonlinear, the EC_{50} generally does not directly measure the equilibrium dissociation constant of the agonist and therefore is only a descriptive term.
		The term ED_{50} is sometimes used interchangeably with EC_{50}, but the former term is best reserved for in vivo use where actual doses, as opposed to concentrations, are used.
ED_{50}	Either the dose of a drug that produces, on average, a specified all-or-none response in 50% of a test population or, if the response is graded, the dose that produces 50% of the maximal response to that drug.	Units (e.g., mg/kg, mmol/kg or mg/l, mmol/l when a tissue is perfused) to be given.
		Applicable to in vivo measurements and to those in vitro experiments (e.g., with a perfused tissue) in which absolute concentration is uncertain. Otherwise use EC_{50}.
		In some circumstances, the maximum response will be unknown. This will often be so in clinical pharmacology, for considerations of safety. The effectiveness of a drug may then be best expressed in terms of the dose that causes, for example, a certain change in blood pressure or heart rate. If the ED terminology is to be used for such measurements, the appropriate units must be included (e.g., ED_{20mm}) to avoid confusion with the usage recommended in the left column.
pEC_{50} or $p[A]_{50}$	The negative logarithm to base 10 of the EC_{50} of an agonist.	The term pD_2 has also been used, particularly in the earlier literature.
Maximal agonist effect	The maximal effect that an agonist, whether conventional or inverse, can elicit in a given tissue under particular experimental conditions. It is best expressed as a fraction of the effect produced by a full agonist of the same type acting through the same receptors under the same conditions.	Also referred to historically as *intrinsic activity* and designated as α. The generic term *maximal agonist effect* is preferred because maximal effects are highly dependent on the experimental conditions such as tissue used, level of receptor expression, the type of measurement used (e.g., IP_3 vs. Ca^{2+}, vs. contraction or secretion), and changes in signal transduction efficiency. Thus intrinsic activity should not be used as a primary pharmacologic characteristic of an agonist as it is not a constant. A simple description of "maximal effect in (specified) assay" is preferred.
EMR	Equi-effective molar concentration ratio; the ratio of the molar concentrations of test and reference substances that produce the same biological effect (whether activation or inhibition).	Should be specified only if the log concentration-effect curves for the substances being compared are parallel.
EDR	Equi-effective dose ratio, as above, but used when doses rather than concentrations are compared, as in in vivo work.	

EMR, equi-effective molar concentration ratio; EDR, equi-effective dose ratio.

TABLE 4
Experimental measures of drug action: antagonists

Term	Suggested Usage	Notes
Concentration ratio, r	The ratio of the concentration of an agonist that produces a specified response (often but not necessarily 50% of the maximal response to that agonist in an assay) in the presence of an antagonist, to the agonist concentration that produces the same response in the absence of antagonist.	The traditional term *dose ratio* is considered less appropriate.
IC_{50}	This term is used in a number of ways: (*i*) the molar concentration of an antagonist that reduces the response to an agonist by 50%; the concentration of agonist should be given; (*ii*) the molar concentration of an unlabeled agonist or antagonist that inhibits the binding of a radioligand by 50%; the concentration of radioligand should be given; (*iii*) the molar concentration of an inhibitory agonist that reduces a response by 50% of the maximal inhibition that can be attained; this latter usage is not recommended—instead the term, EC_{50}, should be used since this is an agonist effect.	In functional studies, the antagonist IC_{50} is most useful if the concentration of the stimulatory agonist is submaximal. Higher concentrations of the agonist will increase the IC_{50} of the competitive antagonist well above its equilibrium dissociation constant. Even with low agonist concentrations, the IC_{50} from functional studies, like an agonist EC_{50} or maximal response, is dependent on the conditions of the experiment (tissue, receptor expression, type of measurement, etc.). Thus, IC_{50} should only be used for comparison of drugs under the specific conditions of the experiment and may have limited relevance to absolute affinity. However, when determined in radioligand binding studies (point *ii*) under equilibrium conditions for competitive ligands, the IC_{50} may be converted to a true dissociation constant using the Cheng and Prusoff equation (Cheng and Prusoff, 1973), which takes into consideration the concentration of the radioligand.
pA_2	The negative logarithm to base 10 of the molar concentration of an antagonist that makes it necessary to double the concentration of the agonist needed to elicit the original submaximal response obtained in the absence of antagonist (Schild, 1947, 1949).	An empirical measure of the activity (in concentration terms) of an antagonist that is not dependent on how the antagonist acts. The pA_2 is determined by measuring the value of the concentration ratio r at several antagonist concentrations, allowing an estimate of the antagonist concentration at which r would be 2. This is commonly done by graphical extrapolation or interpolation (Arunlakshana and Schild, 1959). If certain experimental conditions are fulfilled, the value of pA_2 can provide an estimate of the equilibrium dissociation constant for the combination of a competitive antagonist with its binding site (see *Section IV. B.* for further details).

the law of mass action can be applied, (*c*) a particular level of response is associated with a unique degree of occupancy or activation of the receptors by the agonist, (*d*) the response observed is mediated by a uniform population of receptors, and (*e*) the antagonist has no other relevant actions, e.g., on the relationship between receptor and response. Under these circumstances, the slope of the Schild plot should be 1 and the resulting estimate of the pA_2 should be equal to the pK (negative logarithm of the antagonist equilibrium dissociation constant).

For an antagonist to be classified as *reversible and competitive* on the basis of experiments in which a biological response is measured, the following criteria must hold:

1. In the presence of the antagonist, the log agonist concentration-effect curve should be shifted to the right in a parallel fashion.
2. The relationship between the extent of the shift (as measured by the *concentration ratio*) and the concentration of the antagonist should follow the Schild equation over as wide a range of antagonist concentrations as practicable. Usually, the data are presented in the form of the Schild plot, and adherence to the Schild equation is judged by the finding of a linear plot with unit slope (see *Note 2* below). Nonlinearity and slopes other than unity can result from many causes. For example, a slope greater than 1 may reflect in-

complete equilibration with the antagonist or depletion of a potent antagonist from the medium, as a consequence either of binding to receptors or to other structures. A slope that is significantly less than 1 may indicate removal of agonist by a saturable uptake process, or it may arise because the agonist is acting at more than one receptor (the Schild plot may then be nonlinear). See Kenakin (1997) for a detailed account.

Note 1: The finding that the Schild equation is obeyed over a wide range of concentrations does not prove that the agonist and antagonist act at the same site. All that may be concluded is that the results are in keeping with the hypothesis of mutually exclusive binding, which may of course result from competition for the same site but can also arise in other ways (see Allosteric Modulators in Table 1 and Competitive Antagonism in Table 7).

Note 2: Traditional Schild analysis is based on the use of linear regression. Nowadays, with the almost ubiquitous availability of computers in most research environments, a more accurate approach to performing Schild analysis is to use computerized nonlinear regression to directly fit agonist/antagonist concentration-response data to the Gaddum/Schild equations. The advantages of this approach over traditional Schild analysis are described elsewhere (Waud, 1975; Black et al., 1985; Lew and Angus, 1995). One simple

TABLE 5
Terms and procedures used in the analysis of drug action: the quantification of ligand-receptor interactions

Term	Suggested Usage	Notes
"Concentration" of receptors	$[R]$ for notional concentration of ligand-free receptors; $[R]_T$ or $[R]_{tot}$ for total receptors.	
Number of receptors, N	The total number of receptors, expressed in terms of unit area of membrane, or per cell, or per unit mass of protein.	Proportional to the quantity B_{max} (the maximal specific binding of a ligand, often expressed in units of mol ligand/mg protein, or ligands bound/cell) measured in radioligand binding studies, in the absence of complications. The relationship between B_{max} and N is influenced by the number of ligand binding sites possessed by each receptor. For ligand-gated ion channels, this is generally greater than one. Also referred to as *receptor density*.
Proportion of receptors in specified states	p_R for proportion (fraction) of receptors or binding sites free of ligand. p_{LR} for the proportion of receptors or binding sites occupied by the ligand L. If a distinction is made between inactive and active states of the receptor, then p_{LR} refers to the inactive state. p_{LR}^* for the proportion of receptors in which L occupies its binding site(s) and which are in an active state. $p_{LR'}$ for the proportion of receptors in which L occupies its binding site(s) and which are in a distinct (R') state that differs from both the inactive and the fully active states. This may exhibit some classical signaling activity or it may differ from R or R^* in another property such as activation of different effectors, rates of internalization, or cellular trafficking (Berg et al, 1998; Kenakin and Onaran, 2002).	
Rate constants for the binding of a ligand	k_{+1} for the association (forward) rate constant, and k_{-1} for the dissociation (backward) rate constant, in the reaction $$L + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} LR$$ Here, L represents a ligand and R the unoccupied binding site.	Units to be specified ($M^{-1} s^{-1}$ or $M^{-1} min^{-1}$ for k_{+1}, s^{-1} or min^{-1} for k_{-1} in the scheme illustrated). Lowercase symbols to be used to denote rate constants (cf., uppercase for equilibrium constants). Where there are several ligands, alphabetical subscripts can be added: e.g., k_{+1A}, k_{-1B}. For more complicated schemes involving several reactions, subscripts 2, 3, etc., can be used: e.g., $$L + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} LR \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} LR^*$$
Equilibrium dissociation constant for ligand-receptor interactions, K	In the simple scheme below, K is numerically equal to the ratio of dissociation to association rate constants (k_{-1}/k_{+1}), and has the dimension M (mol/l). $$L + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} LR$$	K can be used in combination with subscripts for clarity. Lowercase letter subscripts are used to designate the type of experimental approach used to determine the constant (e.g., K_d, K_i, K_b—see below) and uppercase letter subscripts designate the compound to which the constant refers (e.g., K_A, K_B, or K_{dA}, K_{dB}, for compounds A and B, respectively). The choice of lowercase subscript that is used in combination with K is based on the following conventions: (i) K_d refers to the equilibrium dissociation constant of a ligand determined directly in a binding assay using a labeled form of the ligand. (ii) K_i refers to the equilibrium dissociation constant of a ligand determined in inhibition studies. The K_i for a given ligand is typically (but not necessarily) determined in a competitive radioligand binding study by measuring the inhibition of the binding of a reference radioligand by the competing ligand of interest under equilibrium conditions. (iii) K_b refers to the equilibrium dissociation constant of a ligand (traditionally, a competitive antagonist) determined by means of a functional assay. When a subscript indicates the type of method used, K_d, K_i and K_b should be used in preference to K_D, K_I, and K_B, respectively.[a]. Uppercase subscripts (either alphabetical, e.g., K_L, numerical, e.g., K_2 or a combination of the two, e.g., K_{2L}) are recommended only to identify the particular ligands and equilibria under consideration, especially when dealing with more complicated schemes involving several steps such as binding followed by isomerization. Two alternative examples of such a scheme are shown below: $$L + R \overset{k_1}{\rightleftharpoons} LR \overset{k_2}{\rightleftharpoons} LR^*$$ $$L + R \overset{k_{1L}}{\rightleftharpoons} LR \overset{k_{2L}}{\rightleftharpoons} LR^*$$ Note: The reciprocal of the equilibrium dissociation constant (the *equilibrium association constant* or *affinity constant,* in units of M^{-1} can also be used, although this is not preferred.

Continued.

TABLE 5—*Continued*
Terms and procedures used in the analysis of drug action: the quantification of ligand-receptor interactions

Term	Suggested Usage	Notes
pK	The negative logarithm to base 10 of the equilibrium dissociation constant, K in molar concentration units. The term can be used in combination with subscripts as described above for equilibrium dissociation constants (pK_d, pK_i, pK_b, etc.).	There are two major benefits to using the pK measures of pharmacological potency rather than the equilibrium constant (K) itself. Since pharmacological potency often ranges over many orders of magnitude (K values from 10^{-10} M to $>10^{-3}$ M), it is easier to present and discuss these differences in a pK form (i.e., values generally range from about 10 to 3). More importantly from a statistical point of view, concentration parameters are generally distributed in a log normal manner (Christopoulos, 1998) so standard deviations are symmetrical for pK values but not for K values.
Hill-Langmuir equation	$p_{LR} = \dfrac{[L]}{[L]+K}$ in which p_{LR} is the fraction (proportion) of binding sites occupied by a ligand L at equilibrium. It is assumed that the interaction between L and the sites obeys the law of mass action and can be described by the simple scheme $$L \;+\; R \underset{}{\overset{K}{\rightleftharpoons}} LR$$ in which K is the equilibrium dissociation constant.	Described as the Langmuir absorption isotherm in physical chemistry. More complicated expressions may hold, especially if L is an agonist (see *Section IV. A.*).

[a] The original usage of K_B by Gaddum represented the binding constant of ligand B to distinguish it from that of ligand A. More recent usage of K_B or pK_B usually refers to values derived from pharmacological blocking experiments. Thus, to maintain consistency with the use of lower case subscripts for inhibition and direct binding experiments (i.e., K_i and K_d) we recommend using K_b or pK_b for estimates of the dissociation constant that are derived from pharmacological blocking experiments (e.g., Schild plots.).

TABLE 6
Terms and procedures used in the analysis of drug action: agonists

Term	Suggested Usage	Notes
Desensitization, fade, tachyphylaxis	Overlapping terms that refer to a spontaneous decline in the response to a continuous application of agonist, or to repeated applications or doses. The following usages are suggested: *fade,* the waning of a response in the continued presence of agonist; *tachyphylaxis,* a decline in the response to repeated applications or doses of agonist. No mechanism is implied by either term. It is recommended that *desensitization* be used when the fade or tachyphylaxis is considered to be a direct consequence of receptor activation.	
Efficacy, e	The concept and numerical term introduced by Stephenson (1956) to express the degree to which different agonists produce varying responses, even when occupying the same proportion of receptors. (See also *Maximal agonist effect,* Table 3).	In Stephenson's formulation (1956), combination of an agonist with its receptors is considered to result in a signal or "stimulus" S, which is equated to the product of the efficacy of the agonist A and the proportion of receptors occupied: $S_A = e_A p_{AR}$ When the response of a tissue is half-maximal, S is assigned the value unity. Hence, a partial agonist that when occupying all the receptors produces a maximal response that is half that of a full agonist (under the same experimental conditions), has an efficacy of unity. Efficacy is both agonist- and tissue-dependent. The expression *intrinsic efficacy,* ε, was introduced by Furchgott (1966) to denote the notional efficacy associated with a single receptor: $e = \varepsilon[R]_T$ in which $[R]_T$ indicates the total concentration of receptors. This term is now also used in a wider sense (see below). Black and Leff (1983) provided another description of differences in the ability of agonists to produce a maximal effect. They defined the term τ (tau) as $[R]_T/K_E$ in which K_E is the midpoint parameter of an explicit function relating receptor occupancy to the response of a tissue. Recent advances in the understanding of receptor function have identified the importance of distinguishing between the *occupation* of a receptor by an agonist and the *activation* of that receptor. This distinction was not considered in the earlier work. More detailed models of receptor action are therefore required, and these provide a better framework for expressing, and explaining, differences in the ability of agonists to activate receptors. The term *intrinsic efficacy* is now often used when discussing the agonist, rather than the tissue-dependent component of efficacy in such schemes [e.g., the isomerization model of del Castillo and Katz (1957), also Colquhoun (1987); the ternary model of DeLean et al. (1980), also Samama et al. (1993)]. However, Stephenson's *efficacy,* and Black and Leff's τ, can still serve as useful comparative measures of the activity of agonists on intact tissues.

TABLE 6—*Continued*
Terms and procedures used in the analysis of drug action: agonists

Term	Suggested Usage	Notes
Full agonist	When the receptor stimulus induced by an agonist reaches the maximal response capability of the system (tissue), then it will produce the system maximal response and be a full agonist in that system. If the maximum tissue response is reached at less than full receptor occupancy it results in a so-called a *spare receptor* situation (see below). Several agonists may thus elicit the same maximal response, albeit at different receptor occupancies. They are all full agonists in that experimental system but have different efficacies. This designation of full vs. partial agonist is system-dependent, and a full agonist for one tissue or measurement may be a partial agonist in another.	
Inverse agonist	A ligand that by binding to receptors reduces the fraction of them in an active conformation (see also agonist, Table 1). This can occur if some of the receptors are in the active form (R^*), in the absence of a conventional agonist: $R \rightleftharpoons R^*$ (inactive)　　(active) If the ligand L, combines preferentially with inactive receptors, it will reduce the fraction in the active state: $LR \rightleftharpoons L + R \rightleftharpoons R^*$ (inactive)　(inactive)　(active)	An inverse agonist may combine either with the same site as a conventional agonist, or with a different site on the receptor macromolecule (see Table 1).
Intrinsic efficacy	See *Efficacy* (above in this table).	
Partial agonist	An agonist that in a given tissue, under specified conditions, cannot elicit as large an effect (even when applied at high concentration, so that all the receptors should be occupied) as can another agonist acting through the same receptors in the same tissue (see also *Full agonist* and *Efficacy,* above in this table, and *Maximal agonist effect,* Table 3).	As noted for *Full agonist* above, the designation partial agonist is system-dependent and a partial agonist in one experimental system may be a full agonist in another (e.g., one in which there were more receptors expressed). Recent advances make it clear that the inability of a particular agonist to produce a maximal response can have several explanations. Perhaps the most important is that not enough of the receptors occupied by the agonist convert to an active form, and the term *partial agonist* is now sometimes applied to this situation alone. The distinction between such usages can be illustrated by the action of decamethonium at the neuromuscular junction. Decamethonium cannot match the conductance increase caused by acetylcholine. However, this is not because decamethonium is less able to cause the receptors to isomerize to an active form: rather, the smaller maximal response is largely a consequence of the greater tendency of decamethonium to block the ion channel that is intrinsic to the nicotinic receptor. Hence, decamethonium would not be regarded as a partial agonist with respect to receptor conformational equilibria defined above but would be in the broader sense of the term.
Spare receptors	A pharmacological system has *spare receptors* if a full agonist can cause a maximum response when occupying only a fraction of the total receptor population. Thus not all of the receptors in the tissue are required to achieve a maximal response with some high efficacy agonists. This has been amply demonstrated experimentally by Furchgott (1966) and others in that irreversible chemical inactivation of some receptors results in a decrease in agonist potency without a decreased maximal response. At sufficiently high degrees of receptor inactivation, the maximum response even to full agonists is finally reduced.	The term *spare receptors* is widely misunderstood with some readers thinking that the "spare" receptors are nonfunctional. The phrase *receptor reserve* means essentially the same thing and may help avoid this confusion though it is less frequently used in the literature. Although all receptors may not be needed for a maximal response, all receptors contribute to the measured responses, thus the potency of full agonists (and often the physiological agonists) is enhanced by the presence of the *spare receptors* In analyzing pharmacological properties of ligands or interpreting results with receptor mutants in heterologous expression systems, which often have very high levels of receptor expression, it is essential to understand and account for the *spare receptor* phenomenon. Many compounds that are partial agonists in normal tissues are full agonists in expression systems due to the high receptor number (see for example, Brink et al., 2000).

method is to fit agonist EC_{50} data, determined in the absence and presence of antagonist, to the following equation:

$$pEC_{50} = -\log([B]^S + 10^{-pA_2 \cdot S}) - \log c$$

where pEC_{50} and pA_2 are as defined previously in Tables 3 and 4, respectively, [B] denotes the antago-

nist concentration, S is a logistic slope factor analogous to the Schild slope and $\log c$ is a fitting constant (Motulsky and Christopoulos, 2003). This equation is based on a modification of the original Gaddum/Schild equations that results in more statistically reliable parameter estimates than those obtained using the original equations for nonlinear regression (Waud

TABLE 7
Terms and procedures used in the analysis of drug action: antagonists

Term	Suggested Usage	Notes
Competitive antagonism	In competitive antagonism, the binding of agonist and antagonist is mutually exclusive. This may be because the agonist and antagonist compete for the same binding site or combine with adjacent sites that overlap (syntopic interaction). A third possibility is that different sites are involved but that they influence the receptor macromolecule in such a way that agonist and antagonist molecules cannot be bound at the same time. If the agonist and antagonist form only short-lasting combinations with the receptor, so that equilibrium between agonist, antagonist, and receptors is reached during the presence of the agonist, the antagonism will be surmountable over a wide range of concentrations (*reversible competitive antagonism*). In contrast, some antagonists, when in close enough proximity to their binding site, may form a stable covalent bond with it (*irreversible competitive antagonism*), and the antagonism becomes *insurmountable* when no spare receptors remain. More generally, the extent to which the action of a competitive antagonist can be overcome by increasing the concentration of agonist is determined by the relative concentrations of the two agents, by the association and dissociation rate constants for their binding, and by the duration of the exposure to each.	In practice, it is difficult to distinguish syntopic orthosteric antagonism from very strong allosteric antagonism (i.e., allosteric antagonism that is characterized by very high negative cooperativity between the orthosteric site and the allosteric site).
Noncompetitive antagonism	Agonist and antagonist can be bound to the receptor simultaneously; antagonist binding reduces or prevents the action of the agonist with or without any effect on the binding of the agonist.	Current usage should be limited to the action of blockers on the same receptor as the agonist (such as channel block of the nicotinic receptor). Prior use to describe the inhibition by adrenoceptor antagonists of the response to tyramine would be better termed *indirect antagonism* (Table 1).
Insurmountable antagonism	A descriptive term indicating that the maximum effect of the agonist is reduced by either pretreatment or simultaneous treatment with the antagonist. This can encompass several distinct molecular mechanisms such as: (*a*) irreversible competitive antagonism; (*b*) noncompetitive antagonism; and (*c*) functional antagonism (see Table 1). The converse phenomenon *surmountable antagonism* is generally observed with reversible competitive antagonism though it may also occur with chemical antagonism, with irreversible antagonists in the case of spare receptors, or with certain forms of allosteric antagonism.	In dissecting mechanisms of insurmountable antagonism, it is often helpful to distinguish between the locus of the action (competitive, noncompetitive, or indirect) and the kinetics of the action (reversible and irreversible). This can usually be done with appropriately designed time course or preincubation/blocking experiments.
Gaddum equation	$$p_{AR} = \frac{[A]}{[A] + K_A \left(1 + \frac{[B]}{K_B}\right)}$$ The relationship (Gaddum, 1937, 1943) that replaces the Hill-Langmuir equation (see Table 5) when two ligands, *A* and *B*, are in equilibrium with a common binding site. p_{AR} is the proportion of the binding sites occupied by *A*; K_A and K_B are the equilibrium dissociation constants of *A* and *B*, respectively.	Equating occupancies by an agonist first in the absence and then in the presence of a reversible competitive antagonist leads to the *Schild equation* (see below), and the terms *Schild equation* and *Gaddum equation* have sometimes been regarded as interchangeable.
The Schild equation	$$r - 1 = \frac{[B]}{K_B}$$ The relationship (Schild, 1949) that would be expected to hold between the concentration ratio, *r*, and the concentration of a reversible competitive antagonist, *B*. K_B is the equilibrium dissociation constant for the combination of *B* with the receptor.	See also *Gaddum equation* (item above), *Schild plot* (item below), and (*Section IV. B.*).
The Schild plot	A graph of log ($r - 1$) against log antagonist concentration, where *r* is the concentration ratio (see Table 4). This should yield a straight line of unit slope if the Schild equation is obeyed (Arunlakshana and Schild, 1959).	The linearity and slope provide information about the nature of the antagonism. In practice, it is preferable to analyze agonist/antagonist interaction data by direct curve fitting to the Gaddum or Schild equations using computer-assisted nonlinear regression, but the Schild plot remains a useful graphical aid (see *Section IV. B.*).

et al., 1978; Lazareno and Birdsall, 1993). If S is not significantly different from 1, then it should be constrained as such and the equation re-fitted to the data.

C. The Relationship between the Hill and Logistic Equation

The logistic function is defined by the equation

$$y = \frac{1}{1 + e^{-(a+bx)}}$$

where a and b are constants. If a is redefined as $-\log_e(K^b)$, and x as $\log_e z$, then

$$y = \frac{z^b}{K^b + z^b}$$

which has the same form as the Hill equation.

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