

<p>Slide 1</p>	<p style="text-align: center;">Ligand Binding</p> <p style="text-align: center;">The Mechanistic Basis of the Emax Model</p> <p style="text-align: center;">Nick Holford Dept Pharmacology & Clinical Pharmacology University of Auckland, New Zealand</p>	<p>Ligand binding describes the fundamental interaction between a drug molecule (a ligand) and its receptor (a binding site).</p>
<p>Slide 2</p>	<p style="text-align: center;">Objectives</p> <ul style="list-style-type: none"> ● To appreciate ligand binding as the mechanistic basis for pharmacodynamics ● To learn the difference between binding sites and receptors ● To understand occupancy and the stimulus-response relationship <p><small>©Nick Holford, 2017. All rights reserved.</small></p>	
<p>Slide 3</p>	<p style="text-align: center;">Binding Sites</p> <ul style="list-style-type: none"> ● Binding Site <ul style="list-style-type: none"> » Specific and Saturable ● Receptor <ul style="list-style-type: none"> » Binding Site + Effect <p><small>©Nick Holford, 2017. All rights reserved.</small></p>	<p>Binding sites are defined by physicochemical properties. They are not a measure of biological function. Receptors are defined by a combination of a binding site and the ability to transform the binding interaction into a physiological effect.</p>

<p>Slide 4</p>	<p style="text-align: center;">Receptors</p> <ul style="list-style-type: none"> ● Receptors (classical) ● Uptake carriers ● Ion channels ● Enzymes ● Plasma Proteins <p><small>©NHG Hofstad, 2017, all rights reserved.</small></p>	<p>Which one of these is only a binding site?</p>
<p>Slide 5</p>	<p style="text-align: center;">Plasma Proteins</p> <ul style="list-style-type: none"> ● Albumin <ul style="list-style-type: none"> » acidic drugs <ul style="list-style-type: none"> – warfarin ● Alpha₁-acid-glycoprotein <ul style="list-style-type: none"> » basic drugs <ul style="list-style-type: none"> – lignocaine ● Transcortin <ul style="list-style-type: none"> » steroids <ul style="list-style-type: none"> – cortisol, prednisolone <p><small>©NHG Hofstad, 2017, all rights reserved.</small></p>	<p>From a pharmacological perspective all plasma proteins are simply binding sites and have no function. Although it is sometimes claimed that plasma proteins are required to help transport physiological substances around the body (e.g. transcortin, transferrin, etc) there is little convincing evidence that the function of the body is affected when these binding proteins are low.</p>
<p>Slide 6</p>	<p style="text-align: center;">Affinity and Efficacy</p> <ul style="list-style-type: none"> ● Affinity: the attraction of the drug for the binding site <ul style="list-style-type: none"> » high affinity: low concentrations bind » low affinity: high concentrations bind » no affinity: does not bind ● Efficacy: the intrinsic activity <ul style="list-style-type: none"> » Max. effect ⇔ intrinsic activity = 1 » Min. effect ⇔ intrinsic activity = 0 <p><small>©NHG Hofstad, 2017, all rights reserved.</small></p>	<p>Affinity is a physicochemical property of a binding site. Efficacy is a biological (functional) property of a receptor.</p>

Slide 7

Types of Binding

- reversible
 - » ionic attraction, hydrogen bonds

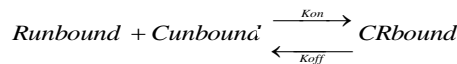
- slowly reversible /irreversible
 - » high affinity non-covalent binding, covalent binding

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Most drug binding is reversible and reaches binding equilibrium in a matter of a few seconds. If a ligand has a very high affinity for a binding site it can take longer to reach binding equilibrium and to dissociate. When this time is long enough it can seem like the ligand is irreversibly bound.

Slide 8

The Law of Mass Action



Mass Balance at Equilibrium

$$K_{off} \cdot [C_{Rbound}] = K_{on} \cdot [C_{unbound}] \cdot [R_{unbound}]$$

$$\frac{1}{T} \cdot c = \frac{1}{c_T} \cdot c \cdot c$$

Equilibrium Dissociation Constant

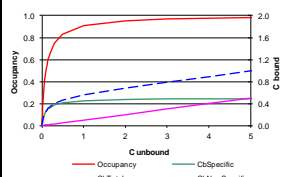
$$K_d = \frac{K_{off}}{K_{on}} = \frac{1}{T} \cdot \frac{c_T}{1} = C$$

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The Law of Mass Action is at the basis of all ligand binding and pharmacodynamic models. The rate constants describing association (K_{on}) and dissociation (K_{off}) determine the equilibrium dissociation constant known as K_d . K_d is a measure of affinity. The smaller the value of K_d then the higher the affinity.

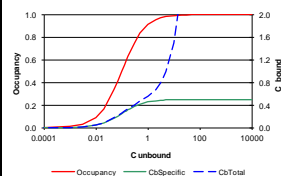
Slide 9

Binding and Occupancy



$$Occupancy = \frac{C_{unbound}}{K_d + C_{unbound}}$$

$$C_{bTotal} = C_{Rbound} + NS \cdot C_{unbound}$$



$$C_{Rbound} = \frac{B_{max} \cdot C_{unbound}}{K_d + C_{unbound}}$$

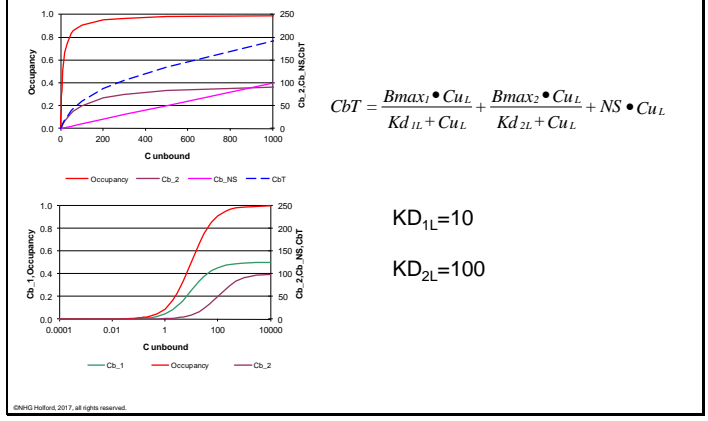
NS=Non-Saturable or Non-Specific

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Occupancy refers to the fraction of binding sites that are occupied by bound ligand. Non-saturable binding (at a different site) can increase with concentration even when the occupancy is essentially constant and close to 1.

Slide 10

Two Sites – Different Affinity

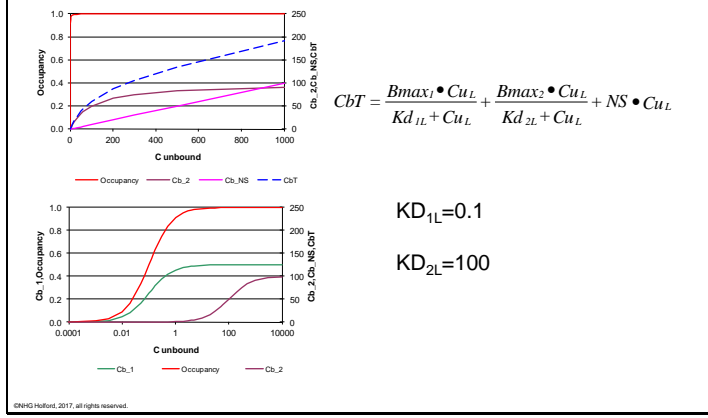


Ligands may bind specifically to more than one binding site. If the binding sites are associated with receptors that have opposing functional effects then this is another way to explain biphasic drug responses.

When the affinity for the binding sites differs by a factor of 10 or less then it is hard to distinguish the separate binding sites by measuring total binding.

Slide 11

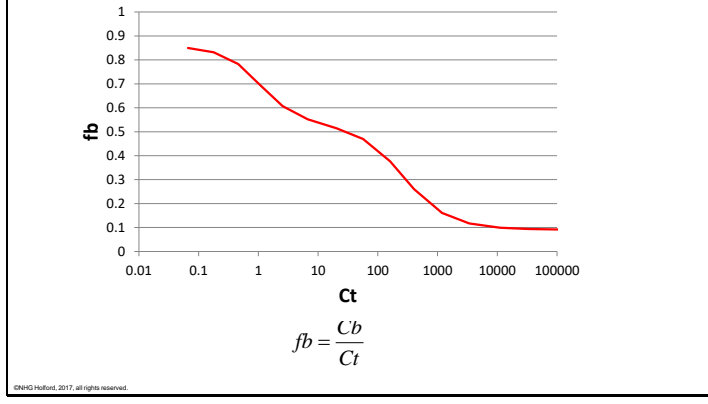
Two Sites – Really Different Affinity



If the binding affinity differs a lot then a simple saturation experiment design (shown here) does not clearly distinguish the binding sites by just observing total binding.

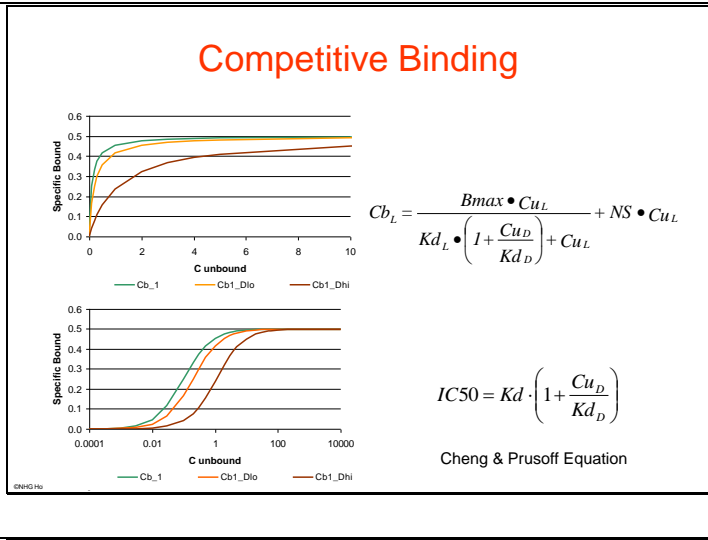
Slide 12

Two Site Binding Transformation



By transforming the data into the fraction bound then two binding sites can be identified. A more efficient way of distinguishing binding sites is to use selective displacing agents that affect one site more than another.

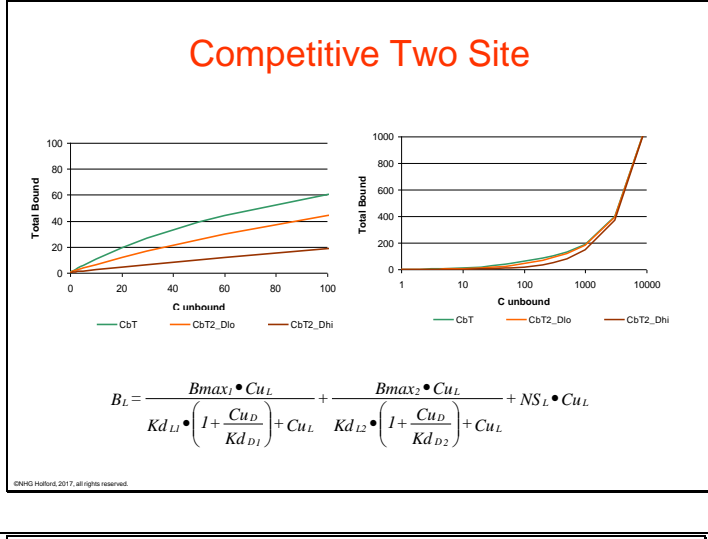
Slide 13



The interaction between two ligands at the same binding site can usually be described by a competitive binding model. This is equivalent to a change in the apparent value of the K_d as the concentration of displacing ligand is increased.

The concentration of displacer required to reduce binding of the measured ligand by 50% is often called the IC_{50} . It is determined by the K_d of both ligands and the unbound concentration of the displacing ligand. The Cheng & Prusoff equation expresses this relationship.

Slide 14



As the number of binding sites increases and the number of ligands increases then binding models get more complex. Note that the solution to the binding model is always expressed in terms of the unbound concentration of each ligand.

Slide 15

Obtaining Unbound Conc

- Naïve
 - $C_{unbound} + \epsilon = C_{total} - (C_{bound} + \epsilon)$
- Predicted (1 site, 1 ligand)
 - $a = 1 + NS$
 - $b = -NS \cdot (2 \cdot C_{total} + K_d) - B_{max} - K_d - C_{total}$
 - $c = C_{total} \cdot (NS \cdot C_{total} + NS \cdot K_d + B_{max})$
 - $C_{bound} = \frac{-b - \sqrt{b^2 - 4 \cdot a \cdot c}}{2 \cdot a}$

Practical ligand binding experiments involve the use of total ligand concentrations. Unbound concentrations are not directly measured but are often inferred from the measured bound concentration. This naïve method means that measurement error from the bound concentration will be incorporated in the unbound concentration used to predict binding. A better method uses the predicted unbound concentration based on the total ligand concentration (C_t) and the binding model parameters. In the simplest case this involves a quadratic function of C_t .

Slide 16

Multiple Sites Multiple Ligands

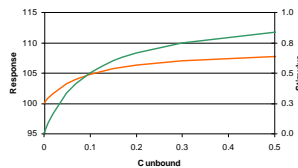
- Given total ligand conc how can unbound ligand conc be obtained?
- Solve system of simultaneous equations
 - Feldman's Method in Munson & Rodbard (LIGAND)
 - <http://www.curvefit.com> (GraphPad Prism)

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A general method for predicting unbound ligand concentration with an arbitrary number of binding sites and ligands was developed by Feldman, a mathematician, who worked with two endocrinologists (Munson & Rodbard).

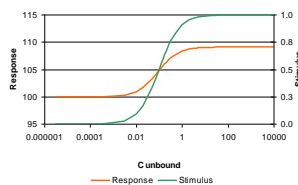
Slide 17

Linear Stimulus and Response



$$Occupancy = \frac{C_{unbound}}{K_d + C_{unbound}}$$

$$Stimulus = intEfficacy \cdot Occupancy$$



$$f = Stimulus$$

$$Response = R_0 + E_{max} \cdot f$$

$$C_{50} = K_d$$

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Occupancy is the essential link between binding and function. Occupancy is translated into a functional stimulus through intrinsic efficacy. If intrinsic efficacy is zero (e.g. a competitive antagonist) then occupancy by the ligand produces no direct effect. Partial agonists have an intrinsic efficacy that is less than that of a full agonist.

The simplest stimulus-response relationship is linear i.e. response is directly proportional to stimulus. The K_d for binding will be the same as the C_{50} for effect.

The observable physiological response will be proportional to the effect (f) produced by the stimulus. The proportionality constant is E_{max} .

Slide 18

E_{max} Model

$$E = \frac{E_{max} \cdot C_{unbound}}{C_{50} + C_{unbound}}$$

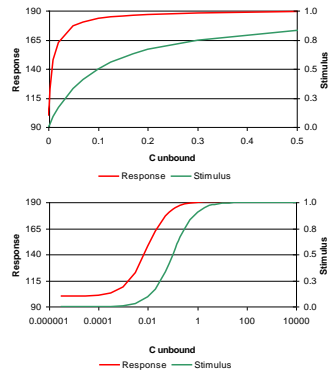
- E is the drug effect
- $C_{unbound}$ is the conc at the receptor
- E_{max} is the maximum drug effect
- C_{50} is the conc at 50% of E_{max}

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The E_{max} model is the most fundamental description of the concentration effect relationship. It has strong theoretical support from the physicochemical principles governing binding of drug to a receptor (the law of mass action). All biological responses must reach a maximum and this is an important prediction of the E_{max} model. When concentrations are low in relation to the C_{50} then the concentration effect relationship can be approximated by a straight line (the linear pharmacodynamic model): $E = Slope \cdot Conc$

Slide 19

Hyperbolic Stimulus and Response



$$Occupancy = \frac{C_{unbound}}{K_d + C_{unbound}}$$

$$Stimulus = \text{intEfficacy} \cdot Occupancy$$

$$f = \frac{Stimulus}{S_{50} + Stimulus}$$

$$Response = R_0 + E_{max} \cdot f$$

$C_{50} < K_d$ "spare receptors"

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The hyperbolic stimulus-response relationship is more commonly observed than linear stimulus-response.

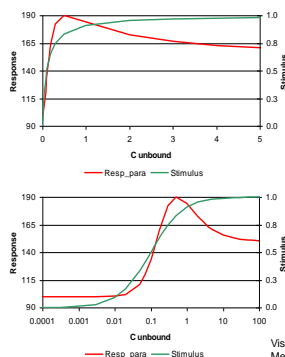
It is commonly observed that the C_{50} is less than the K_d . This phenomenon has been attributed to 'spare receptors' because it seems that the effect is greater than the occupancy would predict. Clearly if C_{50} produces 50% of E_{max} and this concentration is less than the K_d then less than 50% of binding sites must be occupied. However, the term 'spare receptors' is misleading because all receptors have an equal chance of being bound by ligand and participating in the drug effect.

A more physiological explanation recognizes that receptor binding leads to a chain of events involving 'second messengers' which eventually cause a functional effect (f). In the simplest case the formation of the second messenger is directly proportional to the stimulus but the effect is a non-linear function of second messenger concentration. If the stimulus-effect (or second messenger-effect) relationship is hyperbolic with half-maximum at a stimulus concentration S_{50} then the apparent C_{50} of the drug will be determined by S_{50} and B_{max} . If intEfficacy is 1 then $C_{50} = K_d \cdot S_{50} / (B_{max} + S_{50})$.

The observable physiological response will be proportional to the effect (f) produced by the stimulus. The proportionality constant is E_{max} .

Slide 20

Parabolic Stimulus and Response



$$Occupancy = \frac{C_{unbound}}{K_d + C_{unbound}}$$

$$Stimulus = \text{intEfficacy} \cdot Occupancy$$

$$f = b \cdot Stimulus^d - Stimulus^{2-d}$$

$$Response = R_0 + a \cdot f$$

Visser SA, Smulders CJ, Reijers BP, Van der Graaf PH, Peletier LA, Danhof M. Mechanism-based pharmacokinetic-pharmacodynamic modeling of concentration-dependent hysteresis and biphasic electroencephalogram effects of alphaxalone in rats. *J Pharmacol Exp Ther* 2002;302(3):1158-67.

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Sometimes the drug concentration-response relationship reaches a peak then the response decreases as concentration increases. This is called a bi-phasic response curve or an inverted U-shaped response curve.

Visser et al. (2002) were able to predict receptor occupancy *in vivo* and used a parabolic (quadratic) stimulus-effect function to describe a biphasic EEG response in rats treated with the neurosteroid alphaxalone. This stimulus-effect model is empirical and does not provide any mechanistic understanding of why the response is biphasic.