Slide 1	**Ligand Binding** **The Mechanistic Basis of the Emax Model** Nick Holford Dept Pharmacology & Clinical Pharmacology University of Auckland, New Zealand	Ligand binding describes the fundamental interaction between a drug molecule (a ligand) and its receptor (a binding site).
Slide 2	**Objectives** ➢ 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 	
Slide 3	**Binding Sites** ➢ Binding Site » Specific and Saturable ➢ Receptor » Binding Site + Effect 	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.

| Slide 4 | # Receptors

    ➢ Receptors (classical)
    ➢ Uptake carriers
    ➢ Ion channels
    ➢ Enzymes
    ➢ Plasma Proteins

 | Which one of these is only a binding site? |

| Slide 5 | # Plasma Proteins

    ➢ Albumin
      » acidic drugs
        – warfarin
    ➢ Alpha$_1$-acid-glycoprotein
      » basic drugs
        – lignocaine
    ➢ Transcortin
      » steroids
        – cortisol, prednisolone

 | 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. |

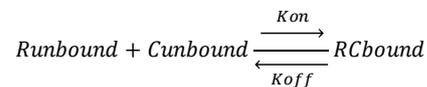| Slide 6 | # Affinity and Efficacy

    ➢ Affinity: the attraction of the drug for the binding site
      » high affinity: low concentrations bind
      » low affinity: high concentrations bind
      » no affinity: does not bind

    ➢ Efficacy: the intrinsic activity
      » Max. effect ⟹ intrinsic activity = 1
      » Min. effect ⟹ intrinsic activity = 0

 | Affinity is a physicochemical property of a binding site. Efficacy is a biological (functional) property of a receptor. |

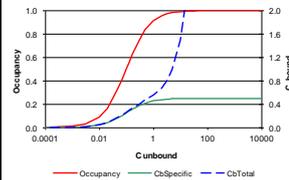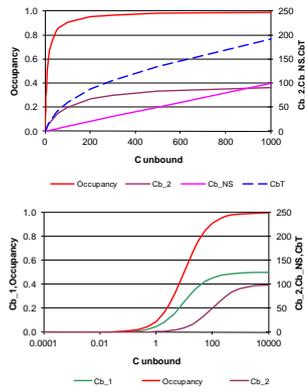| Slide 7	**Types of Binding** ➢ reversible 　» ionic attraction, hydrogen bonds ➢ slowly reversible /irreversible 　» high affinity non-covalent binding, 　　covalent binding 	Most drug binding is reversible and reaches binding equilibrium in a matter of a few seconds. If a ligand has a very high affinity 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** $$Runbound + Cunbound \underset{Koff}{\overset{Kon}{\rightleftharpoons}} RCbound$$ Mass Balance at Equilibrium $$Koff \cdot [RCbound] = Kon \cdot [Cunbound] \cdot [Runbound]$$ $$\frac{1}{T} \quad \cdot \quad C \quad = \quad \frac{1}{C \cdot T} \quad \cdot \quad C \quad \cdot \quad C$$ Equilibrium Dissociation Constant $$Kd = \frac{Koff}{Kon} = \frac{1}{T} \cdot \frac{C \cdot T}{1} = C$$ 	The Law of Mass Action is at the basis of all ligand binding and pharmacodynamic models. The rate constants describing association (Kon) and dissociation (Koff) determine the equilibrium dissociation constant known as Kd. Kd is a measure of affinity. The smaller the value of Kd then the higher the affinity.
Slide 9	**Binding and Occupancy** $$Occupancy = \frac{RCbound}{RCbound + Runbound}$$ $$Occupancy = \frac{Cunbound}{Kd + Cunbound}$$ $$CbTotal = RCbound + NS \cdot Cunbound$$ $$RCbound = \frac{B\max \cdot Cunbound}{Kd + Cunbound}$$ NS=Non-Saturable or Non-Specific 	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.
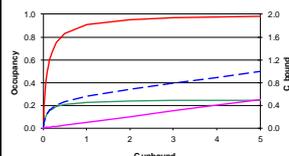
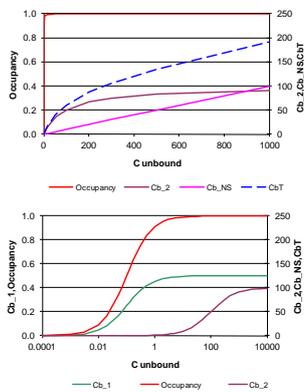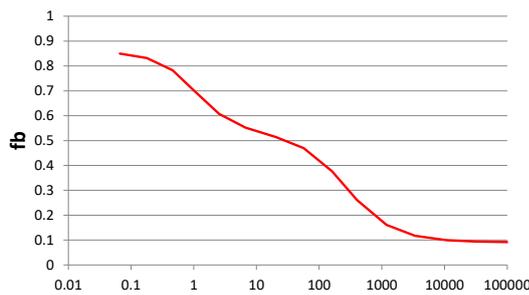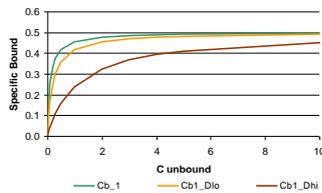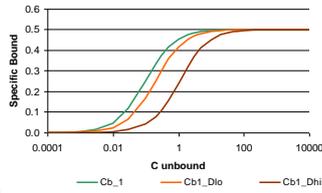| Slide 10	Two Sites – Different Affinity $$CbT = \frac{Bmax_1 \bullet Cu_L}{Kd_{1L} + Cu_L} + \frac{Bmax_2 \bullet Cu_L}{Kd_{2L} + Cu_L} + NS \bullet Cu_L$$ $Kd_{1L} = 10$ $Kd_{2L} = 100$ 	Ligands may bind specifically to more then one binding site. If the binding sites are associated with receptors 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 $$CbT = \frac{Bmax_1 \bullet Cu_L}{Kd_{1L} + Cu_L} + \frac{Bmax_2 \bullet Cu_L}{Kd_{2L} + Cu_L} + NS \bullet Cu_L$$ $Kd_{1L} = 0.1$ $Kd_{2L} = 100$ 	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 $$fb = \frac{Cb}{Ct}$$ 	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.

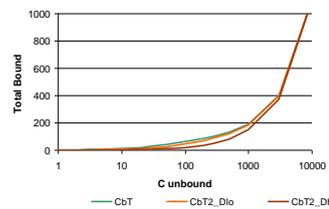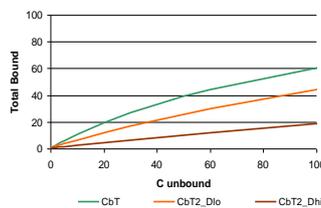| Slide 13	 **Competitive Binding** $$Cb_L = \frac{Bmax \bullet Cu_L}{Kd_L \bullet \left(1 + \dfrac{Cu_D}{Kd_D}\right) + Cu_L} + NS \bullet Cu_L$$ $$IC50 = Kd_L \cdot \left(1 + \frac{Cu_D}{Kd_D}\right)$$ Cheng & Prusoff Equation	The interaction between two ligands at the same binding site can usually be described by a competitive binding model. This is equivalent to a change in the apparent value of the Kd as the concentration of displacing ligand is increased. The concentration of displacer required to reduce binding of the measured ligan by 50% is often called the IC50. It is determined by the Kd of both ligands and the unbound concentration of the displacing ligand. The Cheng & Prusoff equation expresses this relationship.
Slide 14	 **Competitive Two Site** $$B_L = \frac{Bmax_1 \bullet Cu_L}{Kd_{L1} \bullet \left(1 + \dfrac{Cu_D}{Kd_{D1}}\right) + Cu_L} + \frac{Bmax_2 \bullet Cu_L}{Kd_{L2} \bullet \left(1 + \dfrac{Cu_D}{Kd_{D2}}\right) + Cu_L} + NS_L \bullet Cu_L$$	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 $$\mathrm{Cunbound} + \varepsilon = \mathrm{Ctotal} - (\mathrm{Cbound} + \varepsilon)$$ ▢ Predicted (1 site, 1 ligand) $a = 1 + NS$ $b = -NS \cdot (2 \cdot Ctotal + kd) - Bmax - Kd \cdot Ctotal$ $c = Ctotal \cdot (NS \cdot Ctotal + NS \cdot Kd + Bmax)$ $$\mathrm{Cbound} = \frac{-b - \sqrt{b^2 - 4 \cdot a \cdot c}}{2 \cdot a}$$	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 (Ct) and the binding model parameters. In the simplest case this involves a quadratic function of Ct.

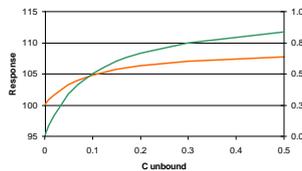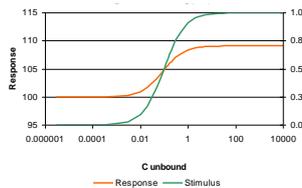|		
Slide 16	# Multiple Sites Multiple Ligands ➢ Given total ligand conc how can unbound ligand conc be obtained? ➢ Solve system of simultaneous equations 　» Feldman's Method in Munson & Rodbard (LIGAND) 　» http://www.curvefit.com (GraphPad Prism) 	A general method for predicting unbound ligand concentration 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 $$Occupancy = \frac{Cunbound}{Kd + Cunbound}$$ $$Stimulus = \text{intEfficacy} \cdot Occupancy$$ $$f = Stimulus$$ $$\mathrm{Re}\,sponse = R0 + E\max \cdot f$$ $$C50 = Kd$$ 	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 Kd for binding will be the same as the C50 for effect. The observable physiological response will be proportional to the effect (f) produced by the stimulus. The proportionality constant is Emax.
Slide 18	# Emax Model $$E = \frac{E\max \bullet Cunbound}{C_{50} + Cunbound}$$ ➢ E is the drug effect ➢ Cunbound is the conc at the receptor ➢ Emax is the maximum drug effect ➢ C_{50} is the conc at 50% of Emax 	The Emax 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 Emax model. When concentrations are low in relation to the C50 then the concentration effect relationship can be approximated by a straight line (the linear pharmacodynamic model): E=Slope*Conc

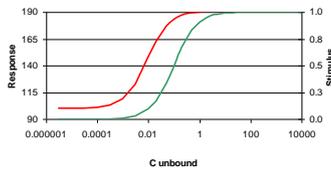| Slide 19 | | The hyperbolic stimulus-response relationship is more commonly observed than linear stimulus-response. |

Slide 19

Hyperbolic Stimulus and Response
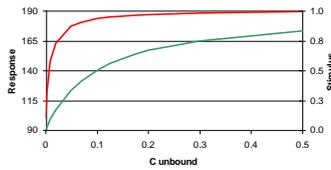
$$Occupancy = \frac{Cunbound}{Kd + Cunbound}$$

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

$$f = \frac{Stimulus}{S50 + Stimulus}$$

$$Response = R0 + E\max \cdot f$$

C50 < Kd "spare receptors"

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

It is commonly observed that the C50 is less than the Kd. This phenomenon has been attributed to 'spare receptors' because it seems that the effect is greater than the occupancy would predict. Clearly if C50 produces 50% of Emax and this concentration is less than the Kd 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 S50 then the apparent C50 of the drug will be determined by S50 and Bmax. If intEfficacy is 1 then C50=Kd*S50/(Bmax+S50).

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

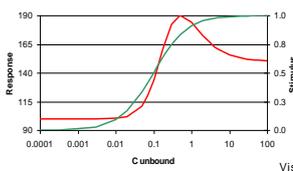## Slide 20

Parabolic Stimulus and Response
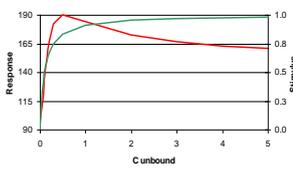
$$Occupancy = \frac{Cunbound}{Kd + Cunbound}$$

$$Stimulus = \text{int}Efficacy \cdot Occupancy$$

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

$$Response = R0 + a \cdot f$$

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.

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.

Caution - Fraction Unbound

➤ A common description of drug plasma protein binding is the fraction unbound:

» $fu = \dfrac{Cu}{Ctotal} = \dfrac{Cu}{Cbtotal+Cu}$

➤ This is a convenient descriptor especially if binding is linear and fu remains constant in the observed range of Cu

➤ A common mistake is to use fraction unbound as if it was unbound concentration.