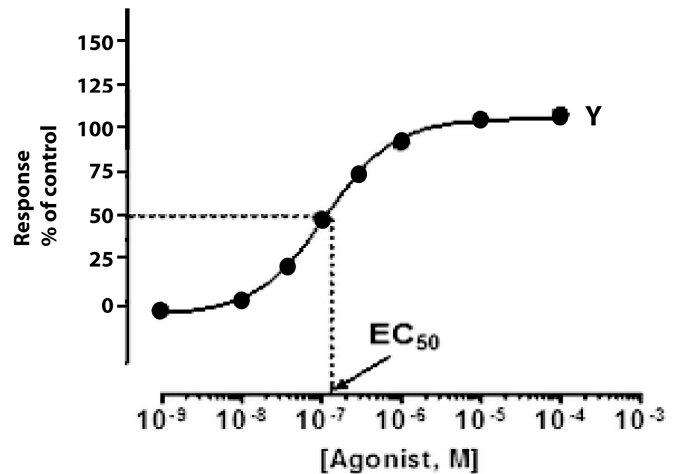
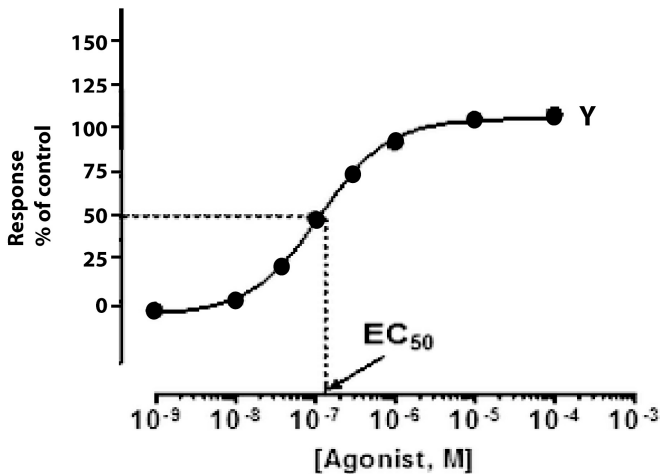


1. You have two agonist drugs A and B at the same receptor, X. Agonist A has a 50 fold higher affinity for X compared to the endogenous ligand Y whose dose response is shown on the graph below (Emax 100%, EC50 100nM). However, agonist A is a partial agonist (Emax 50% of the endogenous ligand). Agonist B has a 10x lower affinity for X compared to the endogenous ligand Y, but is a super agonist (Emax 150% of the endogenous ligand).

a. Draw the dose response curves for A and B on the graphs below, which shows the endogenous ligand Y's dose response curve.

Draw Drug A on this graph

Draw Drug B on this graph

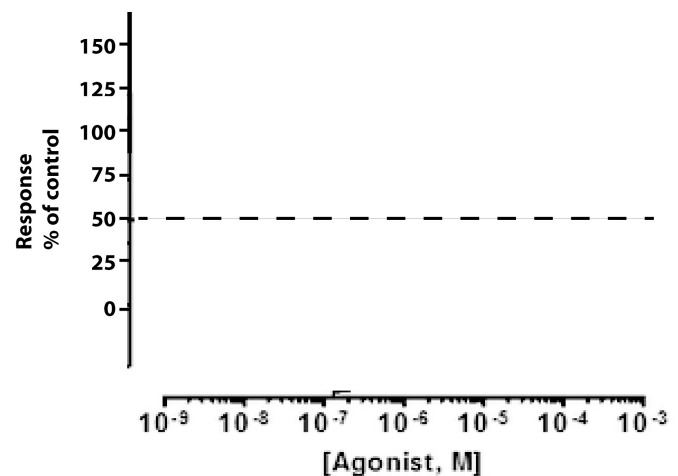
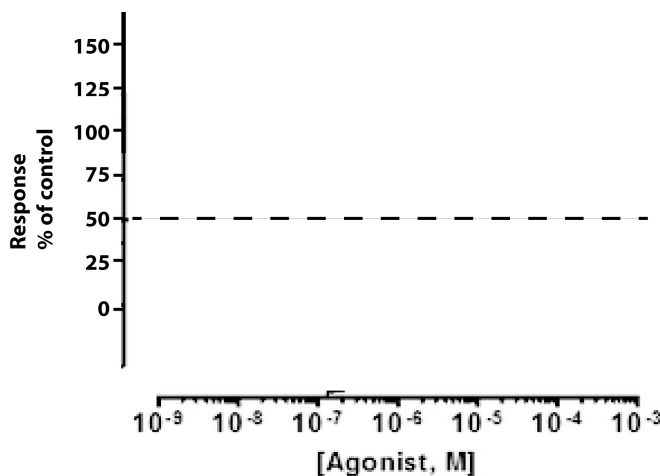


b. Draw the dose response curves when you hold the concentration of the endogenous ligand Y at 100nM (the EC₅₀ dashed line in figure below) and add increasing concentrations of agonist A.

c. Draw the dose response curves when you hold the concentration of the endogenous ligand constant at 100nM (dashed line in figure below) and add increasing concentrations of B.

b. Draw agonist A here

c. Draw agonist B here



d. Which of these agonist drugs when administered to give 1μM effective concentration at the target receptor in the target tissue will have different functional effects depending on whether the “tone” of endogenous ligand Y is high at that target (10μM) or low at that target (1nM)? Why?

2. All antihistamines are inverse agonists at H1 histamine receptors. After taking Loratidine (Claritin) for several weeks, Joe notices that it is no longer effective.

a. Propose at least two possible explanations (other than PK or PD) for why Loratidine has become less effective at treating Joe's symptoms (other than the amount of allergens in the environment have increased). Extra credit for coming up with more than 2 (I can think of 4).

Joe switches to Cetirizine (Zyrtec). It is much more effective.

b. propose at least two mechanisms by which Zyrtec can be more effective than Claritin under these conditions. Extra credit for coming up with more than 2.

At work the next week, Joe realizes he forgot to take his Zyrtec that morning and he has a big presentation. His colleague gives him a Claritin. Amazingly, Joe finds it works just fine. Does this empirical finding of Joe's inform which of the two mechanisms you proposed in (b) are responsible for why switching to Zyrtec improved Joe's symptoms?

3. Administration of neuropeptide S (NPS), the endogenous peptide agonist of the neuropeptide S receptor (NPSR) decreases anxiety--like behavior in animal models. A point mutation in the NPSR found in ~15% of people has been linked to increased susceptibility to anxiety disorders in humans, suggesting there is decreased function of the NPSR in humans with this mutation. Mice with this mutation also show increased anxiety like behavior. This mutation changes a single amino acid in the receptor. However, this mutation does not alter the affinity of the endogenous ligand, neuropeptide S.

a. Give at least two possible explanations for how a point mutation that does not change ligand affinity could result in decreased activity of this receptor in vivo.

b. What experiments would you do in mice to distinguish between these possibilities?

4. You are interested in identifying new small molecule agonists for your favorite receptor (YFR), a GPCR that is highly expressed in the amygdala. Microinjection of the endogenous peptide ligand, Chill, for YFR, into the amygdala, reduces anxiety in rodent models without causing the sedation that is produced by existing anxiolytics.

You perform a screen for new agonist ligands at YFR using a cell line that overexpresses YFR. You identify two new agonists, Hello and Goodbye. You determine, using a radiolabeled antagonist for YFR, that both agonists have the same affinity for YFR, and that they both compete for binding at the orthosteric site. Furthermore, both Hello and Goodbye reach the same E_{max} for G protein signaling, in your cell line, as the endogenous ligand, Chill.

- a) True or false: both hello and goodbye will have the same potency for G protein signaling in your cell line. Why?
- b) You next decide to examine the pharmacological properties of Hello and Goodbye on endogenously expressed receptors. You know YFR is expressed in the amygdala, so you prepare membranes from this brain region for use in determining ligand affinity and activity. You find that, just as in the cell line, both Hello and Goodbye have equal affinity for YFR in these brain membranes. However, while Goodbye is a full agonist, Hello is only a partial agonist for G protein signaling in brain membranes, with an E_{max} 50% of Chill. Propose at least two distinct mechanisms whereby the efficacy of Hello can be different in the brain membranes versus the heterologous system.
- c) You decide to go ahead and determine which agonist will be better at reducing anxiety when directly injected into the amygdala. You begin by administering each compound at the same dose. Which compound do you expect to show a greater affect on anxiety? Why?

- d) To be thorough, you test 4 doses of each drug and run Chill as your control. You find that Goodbye is completely ineffective at reducing anxiety even at the highest dose, while both Hello and Chill reduce anxiety in a dose dependent manner. Give at least one possible explanation, other than unfavorable pharmacokinetics/dynamics, for why Goodbye is ineffective.
- e) What experiment would you do to test your hypothesis?
- f) One goal of your work is to identify potential new GPCR targets for reducing anxiety in treatment-seeking alcoholics. Thus, you decide to compare the effects of your new compound, Hello, in water drinking and ethanol drinking rodents. As expected, Hello is not as effective as Chill in water drinking rodents consistent with your observation in (b) that it is a partial agonist. However, it is as effective as Chill in ethanol drinking rodents. What are two distinct mechanisms that can explain the increased efficacy of Hello in ethanol drinking rodents? What experiments would you do to differentiate between these two possibilities (4 points)?

5. The population of PFC neurons that express the Gi coupled D2R are restricted to pyramidal neurons in layer V. A recent study found that applying the D2 receptor agonist quinpirole to mouse PFC neurons elicited an afterdepolarization that results in voltage fluctuations and spiking for hundreds of milliseconds after initial stimulation.

- a) Would this be the expected effect of a D2 receptor agonist on a neuron? Why or why not? (2 points)
- b) The afterdepolarization does not occur immediately upon application of quinpirole, but rather it emerges 15 minutes after application of quinpirole. What might be occurring during this 15 minutes? Does this information help you reconcile (a) (2 points)?
- c) This afterdepolarization is only apparent if the neuron also receives physiological levels of synaptic input. Why might physiological levels of synaptic input be necessary to observe the afterdepolarization at the synapse (2 points)?

6. The CCR5 receptor is a co-receptor for HIV entry. Humans that carry a mutation in the CCR5 receptor that prevent its proper folding and secretion are resistant to HIV infection. Based on this finding, you do a screen for new antagonist ligands at this receptor in hopes that they will be novel anti-HIV drugs. You identify two new ligands Jack and Jill.

Neither Jack nor Jill have any effect on basal receptor activity in a GTPγS assay.

Both Jack and Jill have the same affinity at the CCR5 receptor.

Both have equivalent potency at blocking G protein activation by the natural ligands of CCR5, CCL3, 4, and 5.

However, only Jill effectively blocks HIV entry in your cell-based infection model.

What might explain why Jill but not Jack shows efficacy for blocking HIV entry?