Cell Biology

Unit 5 · Lesson 2 · Messengers, kinases and drugs

The notes for Lesson 2 of Unit 5: chapters 5.4 to 5.6 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 2 of 3 | 3 chapters | Chapters 5.4 to 5.6

About these notes

These are the notes for **Lesson 2 · Messengers, kinases and drugs** of the University free n easy video lessons on Cell Biology Unit 5, Cell Signaling and Cell Communication. They are chapters 5.4 to 5.6 of the unit book, unchanged, so a page here is the same page in the full book.

5.4 Second messengers and signal amplification

The hormone that reaches a cell is the first messenger. It stays outside. Inside, the receptor and G protein make or release small molecules that spread the message through the cytosol: these are **second messengers**. The main ones are cyclic AMP (cAMP), cyclic GMP (cGMP), inositol 1,4,5-trisphosphate (IP_3), diacylglycerol (DAG) and calcium ions (Ca^{2+}). Because each enzyme in the chain makes many molecules of the next, a few hormone molecules can change millions of molecules inside the cell. That multiplication is **signal amplification**.

You need: the G-protein cycle and the table of Gs, Gi and Gq from Chapter 5.3.

The cAMP pathway

cAMP was the first second messenger discovered; Earl Sutherland received the 1971 Nobel Prize in Physiology or Medicine for the work, done on how adrenaline makes liver cells release glucose.

1. Active Gsα switches on **adenylyl cyclase**, a membrane enzyme that turns ATP into cAMP (and pyrophosphate).

2. cAMP activates **protein kinase A** (PKA). Inactive PKA is a tetramer of two regulatory (R) and two catalytic (C) subunits, R_2C_2. Each R binds two cAMP, so four cAMP release two free, active C subunits.
3. C subunits phosphorylate target proteins on serine and threonine. In the liver they start the glycogen breakdown cascade (worked example below). In the nucleus they phosphorylate the transcription factor **CREB** (cAMP response element binding protein), which turns on genes.
4. The signal ends when **cAMP phosphodiesterase** breaks cAMP to AMP, and protein phosphatases remove the phosphates PKA added. Methylxanthines such as theophylline, used in asthma, inhibit phosphodiesterase and so prolong cAMP.

The IP_3–DAG–Ca^{2+} pathway

1. Active Gqα switches on **phospholipase C-β** (PLCβ).
2. PLCβ cuts a minor membrane lipid, phosphatidylinositol 4,5-bisphosphate (PIP_2), into two messengers: water-soluble **IP_3**, which diffuses into the cytosol, and lipid **DAG**, which stays in the membrane.
3. IP_3 binds the **IP_3 receptor**, a Ca^{2+} channel in the endoplasmic reticulum (ER) membrane. Ca^{2+} rushes out of the ER into the cytosol.
4. DAG, together with Ca^{2+} and the membrane lipid phosphatidylserine, activates **protein kinase C** (PKC), which phosphorylates its own set of targets.
5. Ca^{2+} pumps return Ca^{2+} to the ER and out of the cell; DAG and IP_3 are converted back to lipids. The signal ends.

Calcium and calmodulin

Resting cytosolic Ca^{2+} is about 10^{-7} M (0.1 μM), while outside the cell and inside the ER it is about 10^{-3} M (1 mM). This 10,000-fold gradient, kept by ATP-driven pumps (Unit 2, *Active transport*), means that opening a few channels raises cytosolic Ca^{2+} fast. Ca^{2+} acts mainly through **calmodulin**, a small protein with four Ca^{2+}-binding sites. Ca^{2+}-calmodulin changes shape and activates enzymes such as the Ca^{2+}/calmodulin-dependent kinases (CaM-kinases). In muscle, Ca^{2+} triggers contraction (Unit 6, *Muscle cells*).

cGMP

cGMP is made by guanylyl cyclase. Nitric oxide activates the soluble form inside smooth muscle, cGMP activates protein kinase G, and the muscle relaxes, widening the blood vessel. Sildenafil works by inhibiting phosphodiesterase 5 (PDE5), which breaks cGMP. In rod cells of the eye the logic runs the other way: light lowers cGMP through transducin (Chapter 5.3).

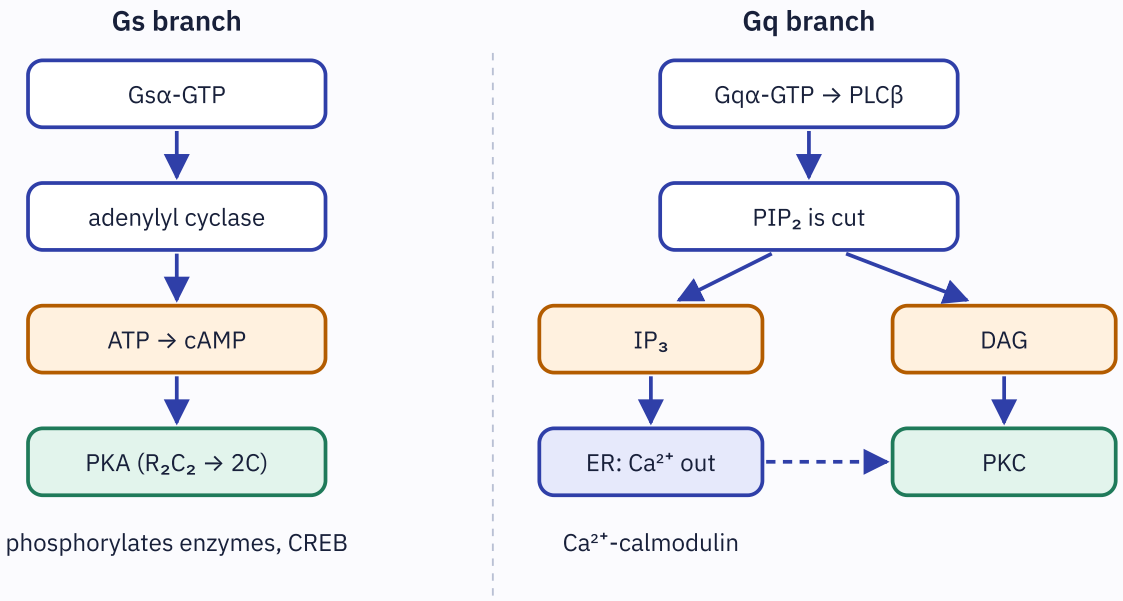


Figure 5.4.1 The two main GPCR second-messenger branches. Orange boxes are the second messengers. Notice that the Gq branch makes two messengers from one lipid, and that PKC needs both of them (DAG directly, Ca^{2+} via the dashed arrow).

Why a cascade amplifies

Each enzymatic step is a multiplier. One active receptor activates many G proteins in turn; each adenylyl cyclase makes many cAMP; each kinase phosphorylates many substrate molecules. Multiplying the gains of several steps gives a huge total gain. A cascade also gives control points: the cell can damp any step (phosphodiesterase, phosphatases, RGS proteins), and different branches can be combined.

WORKED EXAMPLE

Question (6 marks). In a liver cell, adrenaline acts through a β-adrenergic receptor, Gs, adenylyl cyclase, cAMP, PKA, phosphorylase kinase and glycogen phosphorylase, which releases glucose 1-phosphate from glycogen. Use these illustrative gains: each receptor activates 10 Gs; each Gs–cyclase pair makes 100 cAMP; PKA needs 4 cAMP to release 2 C subunits; each C activates 10 phosphorylase kinase; each phosphorylase kinase activates 10 phosphorylase; each phosphorylase releases 1,000 glucose 1-phosphate. How many glucose 1-phosphate molecules come from one adrenaline-bound receptor?

1. Gs: 1 × 10 = 10 active Gs.
2. cAMP: 10 × 100 = 1,000 cAMP.
3. PKA: 1,000 cAMP ÷ 4 per holoenzyme = 250 holoenzymes, giving 250 × 2 = 500 C subunits.
4. Phosphorylase kinase: 500 × 10 = 5,000.
5. Glycogen phosphorylase: 5,000 × 10 = 50,000.
6. Glucose 1-phosphate: 50,000 × 1,000 = 5×10^7 molecules.

Answer: about 50 million glucose 1-phosphate molecules from one receptor, an overall gain of 5×10^7. Notice that the PKA step divides by 2 (four cAMP give two C), the only step that is not a gain. The real gains differ from cell to cell; the method is what the examiner checks.

EXAM TRAPS

- IP_3 releases Ca^{2+} from the ER, not from mitochondria and not from outside the cell.
- DAG stays in the membrane; IP_3 diffuses. Students often swap them.
- PKA's regulatory subunits bind cAMP; the catalytic subunits do the phosphorylating. cAMP does not bind the catalytic subunits.
- Phosphodiesterase ends the cAMP signal; it is not part of the activation path.
- Favourite question: "What are second messengers? Explain the cAMP and IP_3/DAG pathways." Name all five messengers and draw both branches.

CHECK YOURSELF

– REMEMBER **List five second messengers.** 2 marks

cAMP, cGMP, IP_3, DAG, Ca^{2+}.

– REMEMBER **Which enzyme makes cAMP and which destroys it?** 2 marks

Adenylyl cyclase makes cAMP from ATP; cAMP phosphodiesterase breaks it to AMP.

– UNDERSTAND **How does cAMP activate PKA?** 3 marks

Inactive PKA is R_2C_2. Two cAMP bind each R subunit; the R subunits change shape and release the two C subunits, which are then active kinases.

– UNDERSTAND **Why can a small rise in Ca^{2+} influx make a large signal?** 3 marks

Resting cytosolic Ca^{2+} is very low (about 10^{-7} M) against about 10^{-3} M outside and in the ER, so opening channels raises cytosolic Ca^{2+} many-fold quickly, and calmodulin then switches on many enzymes.

– APPLY **A drug blocks phosphodiesterase in heart cells given adrenaline. Predict the effect on cAMP and PKA.** 3 marks

cAMP is not broken down, so it stays high for longer; more PKA stays active and its effects (for example stronger heart contraction) last longer.

– APPLY **With gains of 20, 50 and 10 at three steps, what is the total gain?** 2 marks

20 × 50 × 10 = 10,000. Gains multiply, they do not add.

– ANALYZE **Why does a cell use a multi-step cascade instead of letting the receptor act directly on glycogen phosphorylase?** 4 marks

Each step multiplies the signal, giving large amplification from very few hormone molecules. Each step is also a control point that can be damped or combined with other signals, and different branches can spread one signal to several responses (metabolism, gene expression).

BEYOND THE SYLLABUS

Second messengers do not flood the cell evenly. Anchoring proteins called AKAPs (A-kinase anchoring proteins) hold PKA near particular targets, so cAMP signals form local "nanodomains". Researchers watch cAMP and Ca^{2+} in living cells with fluorescent sensors such as the GCaMP calcium indicators. This is not for the exam; search "cAMP compartmentalisation" and "genetically encoded calcium indicators" to read further.

5.5 Kinase pathways: PI3K/Akt, JAK/STAT and AMPK

Many signals act not through second messengers alone but through chains of protein kinases, enzymes that add phosphate groups to other proteins. Three chains appear again and again in physiology and disease: PI3K/Akt, which tells a cell to survive, grow and take up glucose; JAK/STAT, which carries cytokine signals straight to the nucleus; and AMPK, which senses low energy and switches the cell to saving mode. Elsewhere this chapter is extra reading.

You need: receptor tyrosine kinases and SH2 domains from Chapter 5.2.

PI3K/Akt: the survival and growth pathway

1. Insulin or a growth factor activates its RTK. The phosphorylated receptor (or, for insulin, the docking protein IRS-1) binds **phosphoinositide 3-kinase** (PI3K) through SH2 domains.
2. PI3K adds a phosphate at position 3 of the inositol ring of the membrane lipid PIP_2, making **PIP_3**.
3. PIP_3 is a docking site for proteins with a **PH domain** (pleckstrin homology domain). Two of them arrive: the kinase PDK1 and the kinase **Akt** (also called protein kinase B).
4. PDK1 phosphorylates Akt on threonine 308, and the complex mTORC2 phosphorylates it on serine 473. Akt is now fully active and leaves the membrane.
5. Akt phosphorylates many targets: it inactivates the pro-death protein BAD (so the cell survives), keeps FOXO transcription factors out of the nucleus, activates mTORC1 (more protein synthesis and growth), and in muscle and fat cells moves GLUT4 glucose transporters to the surface.
6. The phosphatase **PTEN** removes the 3-phosphate from PIP_3 and ends the signal. PTEN is a tumour suppressor; losing it leaves Akt on, which is common in cancer (Unit 4, *Cancer and oncogenes*).

JAK/STAT: a short road to the nucleus

Receptors for cytokines such as interferons, erythropoietin and many interleukins have no kinase domain of their own. Instead, a **Janus kinase** (JAK) is bound to each receptor chain inside the cell. Ligand binding brings the chains together, and the two JAKs phosphorylate each other and then the receptor tails. **STAT** proteins (signal transducers and activators of transcription) dock on the phosphotyrosines through their SH2 domains and are phosphorylated by JAK. Phosphorylated STATs let go, pair up through their SH2 domains, enter the nucleus and

switch on genes. The pathway is turned off by phosphatases and by **SOCS** proteins (suppressors of cytokine signalling), which STAT itself induces: a negative feedback loop. Erythropoietin, for example, signals through JAK2 and STAT5 to make red blood cells.

AMPK: the energy sensor

AMP-activated protein kinase (AMPK) has three subunits: α (the kinase), β and γ. The γ subunit binds AMP, ADP or ATP. When ATP falls and AMP rises, AMP binding helps the upstream kinase LKB1 phosphorylate α on threonine 172 and protects that phosphate from removal. Active AMPK switches off ATP-using processes (fatty acid synthesis by inhibiting acetyl-CoA carboxylase; protein synthesis by inhibiting mTORC1) and switches on ATP-making ones (glucose uptake, fatty acid oxidation). Exercise activates AMPK in muscle, and the diabetes drug metformin activates it indirectly.

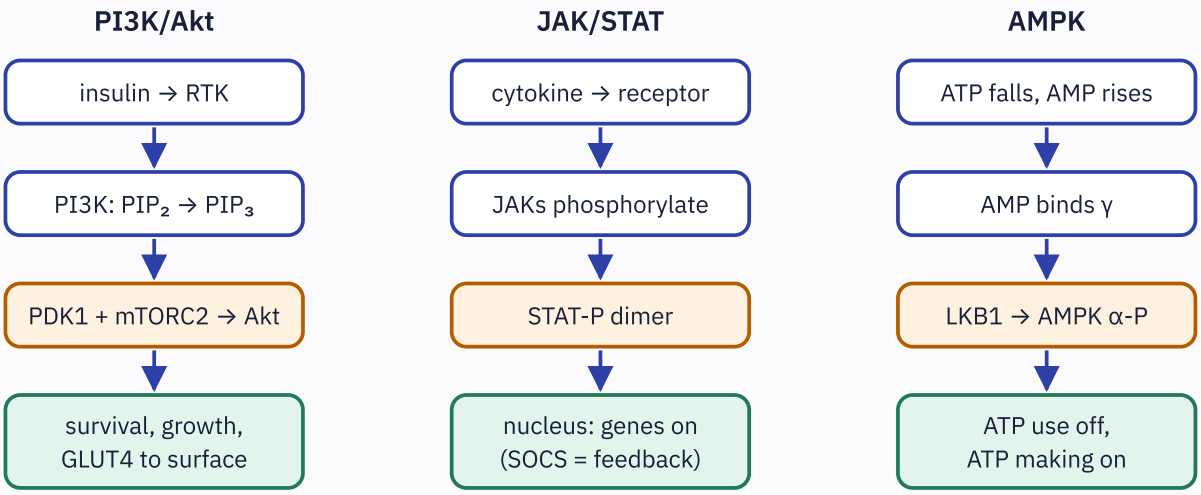


Figure 5.5.1 The three kinase pathways. The first two start at a receptor; AMPK starts from the cell's own energy state. Notice that Akt drives growth through mTORC1 while AMPK brakes it, so the two pathways pull mTORC1 in opposite directions.

WORKED EXAMPLE

Question (5 marks). The enzyme adenylate kinase keeps 2 ADP ⇌ ATP + AMP near equilibrium, with $K = [ATP][AMP]/[ADP]^2 \approx 1$. A resting muscle cell has ATP = 5.0 mM, ADP = 0.50 mM. (a) Find AMP. (b) During exercise ATP falls to 4.4 mM and ADP rises to 0.95 mM. Find AMP now and compare the percentage changes in ATP and AMP. Why is AMP, not ATP, the signal AMPK reads?

1. (a) $[AMP] = K[ADP]^2/[ATP] = 1 \times (0.50)^2 / 5.0 = 0.25 / 5.0 = 0.050$ mM.
2. (b) $[AMP] = (0.95)^2 / 4.4 = 0.9025 / 4.4 = 0.205$ mM. Check the total: 4.4 + 0.95 + 0.205 = 5.555 mM, the same as 5.0 + 0.50 + 0.05 = 5.55 mM, so adenine nucleotides are conserved.
3. ATP change: (5.0 – 4.4)/5.0 = 12% fall. AMP change: 0.205/0.050 = 4.1 times, a 310% rise.
4. Because AMP depends on the square of ADP, a small fall in ATP gives a large rise in AMP. AMP is therefore a far more sensitive signal of energy stress than ATP.

EXAM TRAPS

- PI3K phosphorylates a lipid ($PIP_2 \rightarrow PIP_3$), not a protein. Akt is the protein kinase.
- Cytokine receptors are not RTKs; the kinase (JAK) is a separate protein bound to them.
- AMPK is not activated by cAMP. The name refers to AMP; do not confuse it with PKA.
- PTEN is a phosphatase that opposes PI3K; losing PTEN activates, not blocks, Akt signalling.

CHECK YOURSELF

– REMEMBER **What does PI3K make, and which domain binds it?** 2 marks

PIP_3 from PIP_2; PIP_3 is bound by PH domains (on Akt and PDK1).

– REMEMBER **Expand JAK and STAT.** 2 marks

Janus kinase; signal transducer and activator of transcription.

– UNDERSTAND **How do STATs reach the nucleus?** 3 marks

They dock by SH2 domains on phosphorylated receptor tyrosines, are phosphorylated by JAK, then dimerise through SH2–phosphotyrosine contacts and move into the nucleus to bind DNA.

– APPLY **A tumour has lost PTEN. Predict PIP_3, Akt and cell survival.** 3 marks

PIP_3 stays high, Akt stays active, BAD is kept inactive, so the cells resist apoptosis and keep growing.

– APPLY **Why does exercise raise glucose uptake in muscle even without insulin?** 3 marks

Exercise uses ATP, AMP rises, AMPK is activated and promotes glucose uptake (GLUT4 to the surface) through a route independent of insulin and Akt. (Ca^{2+} signals from contraction help too.)

– ANALYZE **Compare how JAK/STAT and cAMP/PKA reach gene expression.** 4 marks

JAK/STAT is short: the transcription factor itself is phosphorylated at the receptor and goes straight to the nucleus. cAMP/PKA goes through G protein, cyclase, second messenger and kinase before PKA phosphorylates CREB; it amplifies more but has more steps.

BEYOND THE SYLLABUS

Each pathway is a drug target. JAK inhibitors such as ruxolitinib treat myelofibrosis and some autoimmune diseases, and a single JAK2 mutation (V617F) drives the blood cancer polycythaemia vera. PI3K inhibitors are used in some breast cancers. This is not for the exam; search "mTOR signalling network" to see how Akt and AMPK meet.

5.6 Agonists and antagonists

A receptor does not know whether the molecule in its pocket is the body's own hormone or a tablet. Any molecule that binds a receptor and switches it on is an **agonist**; any that binds and blocks it without switching it on is an **antagonist**. Most drugs work this way: salbutamol inhalers are agonists at airway β_2 receptors, and propranolol, a "beta blocker", is an antagonist at heart β receptors. Other colleges meet it in pharmacology.

You need: K_d and occupancy from Chapter 5.2.

Two separate properties: affinity and efficacy

Affinity is how tightly a molecule binds, measured by K_d. **Efficacy** is how well the bound molecule activates the receptor. An antagonist can have high affinity and zero efficacy. This split

gives four kinds of ligand:

1. **Full agonist**: gives the maximum response the system can make. Adrenaline at β receptors; morphine at μ-opioid receptors.
2. **Partial agonist**: even when every receptor is occupied, gives less than the maximum. Buprenorphine at μ-opioid receptors. Because it occupies receptors, it also blocks a full agonist from acting fully.
3. **Antagonist** (neutral): binds, gives no response, and blocks agonists. Naloxone reverses opioid overdose this way; atropine blocks muscarinic acetylcholine receptors.
4. **Inverse agonist**: some receptors are partly active even when empty (constitutive activity). An inverse agonist lowers that activity below the resting level.

Competitive and non-competitive antagonists

A **competitive** antagonist binds reversibly to the same site as the agonist. Enough agonist outcompetes it, so the maximum response is still reached, but more agonist is needed: the dose-response curve shifts to the right without lowering the top. A **non-competitive** antagonist either binds elsewhere on the receptor (an allosteric site) or binds the agonist site irreversibly. Adding more agonist cannot overcome it, so the maximum response falls.

The **EC_{50}** is the agonist concentration giving half the maximum response; a lower EC_{50} means higher **potency**. For a competitive antagonist B with dissociation constant K_B, the factor by which the EC_{50} shifts, the **dose ratio** r, is given by the Gaddum–Schild equation:

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r = 1 + [B] / KB
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where [B] is the antagonist concentration. A plot of log(r − 1) against log[B], the Schild plot, is a straight line of slope 1 for a truly competitive antagonist.

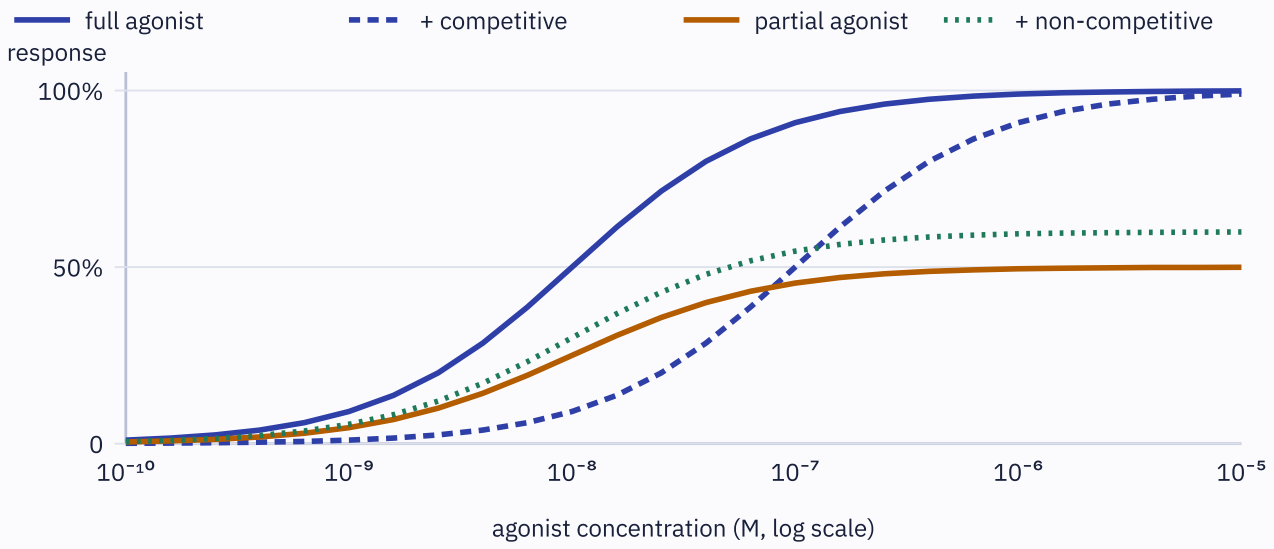


Figure 5.6.1 Dose-response curves computed for an agonist with EC_{50} = 10 nM. The competitive antagonist (dashed blue, dose ratio 10) shifts the curve right but still reaches 100%; the partial agonist tops out at 50%; the non-competitive antagonist lowers the maximum to 60%. Notice that only the competitive case can be overcome by more agonist.

WORKED EXAMPLE

Question (5 marks). An agonist has EC_{50} = 10 nM. A competitive antagonist with K_B = 2 nM is added at 18 nM. (a) Find the new EC_{50}. (b) What antagonist concentration would shift the EC_{50} to 1 µM? (c) Will the maximum response change?

1. (a) r = 1 + 18/2 = 1 + 9 = 10. New EC_{50} = 10 × 10 nM = 100 nM.
2. (b) Needed r = 1 µM / 10 nM = 1000 nM / 10 nM = 100. So $[B]/K_B$ = 99 and [B] = 99 × 2 nM = 198 nM.
3. (c) No. A competitive antagonist is surmountable; at high enough agonist concentration the full response returns.

EXAM TRAPS

- High affinity does not mean high efficacy. Antagonists often bind more tightly than the natural agonist.
- A partial agonist acts as an antagonist when a full agonist is present.
- Potency (EC_{50}) and efficacy (maximum) are different; a curve can shift without changing its top.
- A competitive antagonist shifts the curve right; a non-competitive one lowers the maximum.

CHECK YOURSELF

– REMEMBER **Define agonist and antagonist.** 2 marks

An agonist binds a receptor and activates it; an antagonist binds and does not activate it, blocking agonist action.

– REMEMBER **Give one clinical antagonist and its receptor.** 1 mark

Propranolol (β-adrenergic), atropine (muscarinic), or naloxone (μ-opioid).

– UNDERSTAND **What is an inverse agonist?** 2 marks

A ligand that lowers the activity of a receptor that is partly active on its own, below the resting level.

– APPLY **K_B = 5 nM and [B] = 45 nM. By what factor does the EC_{50} rise?** 2 marks

r = 1 + 45/5 = 10; the EC_{50} rises ten-fold.

– APPLY **Why can naloxone reverse a morphine overdose?** 3 marks

Naloxone is a competitive antagonist at μ-opioid receptors with high affinity; it displaces morphine from the receptors and gives no activation itself, so breathing recovers.

– ANALYZE **From a set of dose-response curves, how would you tell a competitive from a non-competitive antagonist?** 4 marks

Competitive: parallel right shift, same maximum, Schild slope 1. Non-competitive: maximum falls and is not restored by more agonist; the curve may also shift.

BEYOND THE SYLLABUS

Modern pharmacology adds allosteric modulators, which bind away from the agonist site and turn the receptor's response up or down, and biased agonists that favour one downstream pathway. This is not for the exam; search "allosteric modulator GPCR" and "Schild analysis" to read further.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Second messengers	cAMP, cGMP, IP_3, DAG, Ca^{2+}	5.4
PKA activation	R_2C_2 + 4 cAMP → $R_2(cAMP)_4$ + 2 C (active)	5.4
Cascade gain	Total gain = product of the gains of each step	5.4
PI3K/Akt	PIP_2 → PIP_3 (PI3K); PTEN reverses; Akt drives survival and growth	5.5
Adenylate kinase	2 ADP ⇌ ATP + AMP; [AMP] ≈ $[ADP]^2/[ATP]$, so AMP signals energy stress to AMPK	5.5
Dose ratio (Gaddum–Schild)	r = 1 + $[B]/K_B$ for a competitive antagonist	5.6

Glossary (terms used in this lesson)

Agonist A ligand that binds a receptor and activates it.

Akt Protein kinase B; activated by PIP_3, PDK1 and mTORC2; drives survival and growth.

AMPK AMP-activated protein kinase; switches the cell to energy-saving mode when AMP rises.

Antagonist A ligand that binds a receptor without activating it and blocks agonists.

Calmodulin Small Ca^{2+}-binding protein (four sites) that activates enzymes when Ca^{2+} rises.

cAMP Cyclic AMP; second messenger made by adenylyl cyclase, activates PKA.

EC_{50}	Agonist concentration giving half the maximum response; a measure of potency.
IP_3	Inositol 1,4,5-trisphosphate; releases Ca^{2+} from the ER.
JAK/STAT	Cytokine pathway in which Janus kinases phosphorylate STAT transcription factors.
K_d	Dissociation constant; ligand concentration at which half the receptors are bound.
PI3K	Phosphoinositide 3-kinase; makes PIP_3 from PIP_2.
PKA	Protein kinase A; R_2C_2 tetramer activated by cAMP.
Second messenger	Small intracellular molecule that relays a signal from a receptor (cAMP, cGMP, IP_3, DAG, Ca^{2+}).

The full unit book

Every chapter of Unit 5, the unit summary, the exam question bank with model answers and the full glossary: Cell Biology · Unit 5 · Cell Signaling and Cell Communication (one file, works offline).

Sources and further reading

- Alberts B. et al., *Molecular Biology of the Cell*, Garland Science / W. W. Norton: the chapters on cell signalling and on cell junctions and the extracellular matrix (from memory; check the edition you are prescribed).
- Lodish H. et al., *Molecular Cell Biology*, W. H. Freeman: the chapters on signal transduction and on integrating cells into tissues (from memory; check).
- Karp G., *Cell and Molecular Biology: Concepts and Experiments*, Wiley: the chapters on cell signalling and on interactions between cells and their environment (from memory; check).
- Cooper G. M., *The Cell: A Molecular Approach*, Sinauer / Oxford University Press: the chapter on cell signalling (from memory; check).
- Alberts B. et al., *Molecular Biology of the Cell*, 4th edition (2002), "General Principles of Cell Communication", free on NCBI Bookshelf: ncbi.nlm.nih.gov/books/NBK26813 (source of the hormone and acetylcholine concentrations and the nitric oxide half-life used in Chapter 5.1).
- Wikipedia, "Gap junction": en.wikipedia.org/wiki/Gap_junction (consulted for the connexon structure and the range of size limits quoted in Chapter 5.7).

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