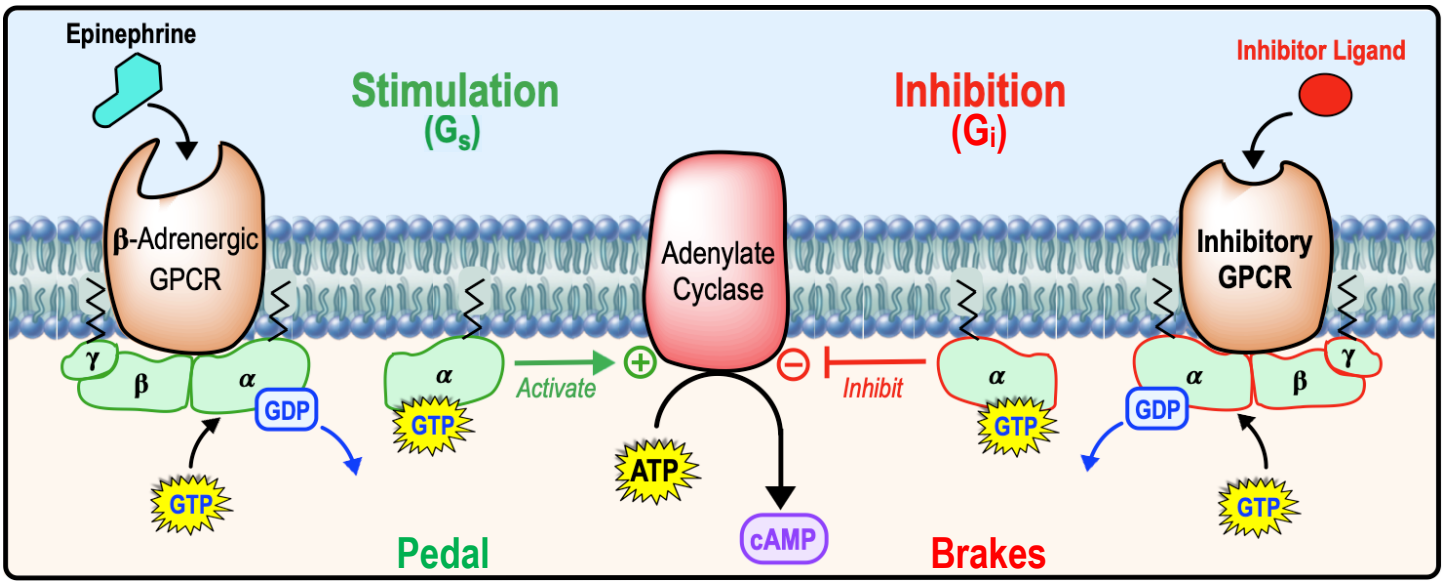
CONCEPT: INHIBITORY ADENYLATE CYCLASE GPCR SIGNALING

●Recall: some G proteins _____ the *effector enzyme*.

□ *Same* pathway as before, but α subunit of G-protein (G_i) ultimately _____ activity of the effector enzyme.

●*Integration* of stimulatory & inhibitory GPCR pathways can _____ the activity of the *effector enzyme*.

EXAMPLE: Adenylate Cyclase Activity Regulated by Inhibitory GPCR Pathway.



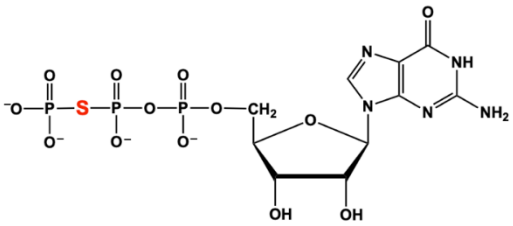
PRACTICE: The addition of the nonhydrolyzable GTP-analog, GTPγS (shown below), is a common cell culture technique. If only affecting the inhibitory pathway, what effect would GTPγS have on cellular cAMP levels?

a) cAMP levels increase because adenylate cyclase can no longer hydrolyze GTP.

b) cAMP levels decrease because G_s remains in its inactive state and can no longer bind GDP.

c) cAMP levels decrease because G_i remains in its active state since it can no longer hydrolyze GTP.

d) cAMP levels increase because G_i remains in its inactive state since it can no longer hydrolyze GTP.



PRACTICE: If a chemical is an inhibitor of the enzyme adenylyl cyclase, which of the following steps in the GPCR signaling pathway would be directly blocked?

a) Activation of gene transcription.

b) Exchange of GTP for GDP.

c) Ligand bound receptor activation.

d) Synthesis of the secondary messenger cAMP.

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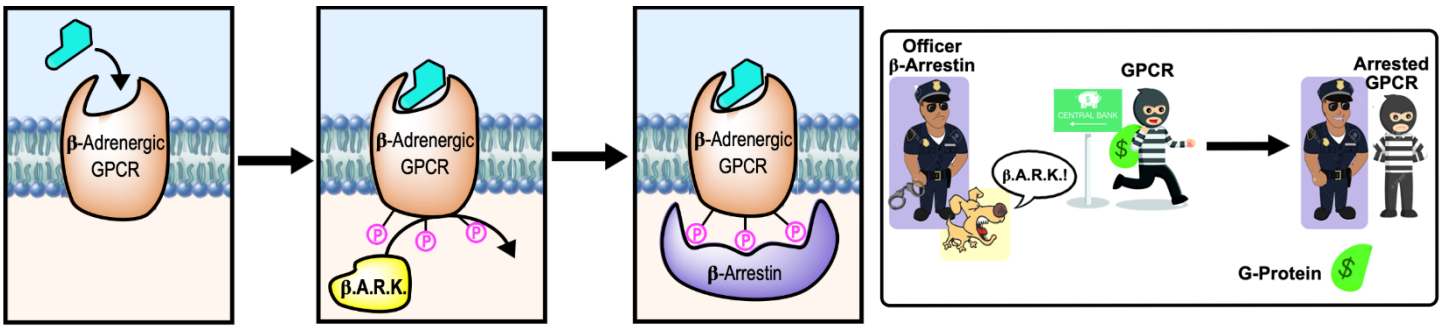
GPCR Desensitization

●When a signal is _____ present (repeated exposure), cells need the ability to *desensitize*.

●_____: *dampening* of the cellular response EVEN WHEN the primary messenger/ligand *persists*.

- □ **β-A**drenergic GPC**R K**inase (_____): _____ C-terminal Serines on *ligand-bound* GPCR.
- □ **β-Arr**estin (_____): protein that binds phosphorylated GPCR, _____ its interaction with G protein.
- □ β-Aarr can initiate temporary _____ of GPCR in vesicles (making GPCR inaccessible).



1) Epinepherine _____ **β**-Adrenergic GPCR.

2) **β**.A.R.K. _____ **β**-Adrenergic GPCR.

3) **β**-Arrestin _____ G-protein association with GPCR & triggers _____.

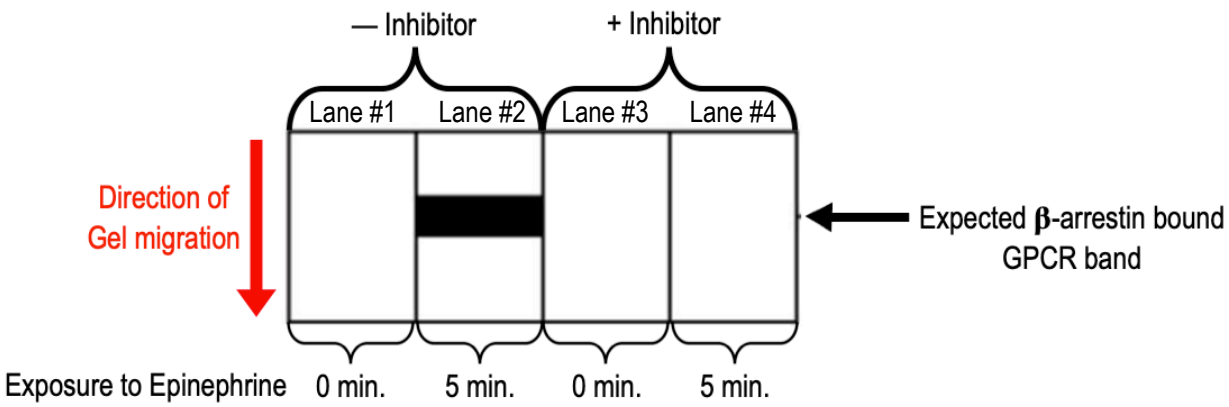
EXAMPLE: A cell line expressing the β-Adrenergic GPCR is incubated in epinephrine for 5 minutes. The cells are then lysed and the GPCR is purified. To determine if β-arrestin is bound to the GPCR, the purified GPCR solution is examined by Western Blot using an antibody against β-arrestin (Lanes #1 & 2). The experiment is repeated, but this time prior to and during epinephrine addition, the cells are incubated in an inhibitor that blocks β-Adrenergic GPCR phosphorylation by the GPCR kinase βARK (Lanes #3 & 4). Results are shown below. What conclusion can be made from the results/data?

a) In order for β-arrestin to bind, the receptor must be exposed/bound to its ligand.

b) In order for β-arrestin to bind, the receptor cannot be exposed/bound to its ligand.

c) In order for β-arrestin to bind, βARK must be bound to the receptor first.

d) In order for β-arrestin to bind, the inhibitor must first bind to the receptor.



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PRACTICE: How does GPCR termination differ from GPCR desensitization?

a) GPCR termination promotes GPCR signaling while GPCR desensitization overexpresses the GPCR.
b) GPCR desensitization involves degradation of the GPCR while GPCR termination promotes GTPase activity of G_α.
c) GPCR termination blocks the GTPase activity of G_α while GPCR desensitization promotes ligand binding.
d) GPCR termination resets the signaling pathway in the absence of ligand, whereas GPCR desensitization dampens the cell response even in the presence of ligand.

PRACTICE: Which of the following statements about beta-arrestin and GPCRs is TRUE?

a) Beta-arrestin phosphorylates activated GPCRs.
b) Beta-arrestin can cause desensitization of a signal by inducing GPCR exocytosis.
c) Beta-arrestin is pre-bound to inactive GPCRs (no ligand bound).
d) Beta-arrestin binds to phosphorylated residues on the carboxy-terminus region of GPCRs.
e) Beta-arrestin binds to phosphorylated residues on the amino-terminus region of GPCRs.

PRACTICE: Which of the following are involved in the desensitization of the β-adrenergic receptor?

a) β-adrenergic receptor kinase.
b) β-arrestin.
c) Protein Kinase A.
d) βARK.
e) GAPs.
f) A and B only.
g) A, B and D only.
h) A, B, and E only.