1. The binding of an extracellular ligand to a G protein-coupled receptor in the plasma membrane of a cell triggers intracellular signaling (Figure 1, A). After ligand binding, GTP replaces the GDP that is bound to $Gs\alpha$, a subunit of the G protein (Figure 1, B). This causes $Gs\alpha$ to activate other cellular proteins, including adenylyl cyclase that converts ATP to cyclic AMP (cAMP). The cAMP activates protein kinases (Figure 1, C). In cells that line the small intestine, a cAMP-activated protein kinase causes further signaling that ultimately results in the secretion of chloride ions (Cl^-) from the cells. Under normal conditions, $Gs\alpha$ hydrolyzes GTP to GDP, thus inactivating adenylyl cyclase and stopping the signal (Figure 1, A).
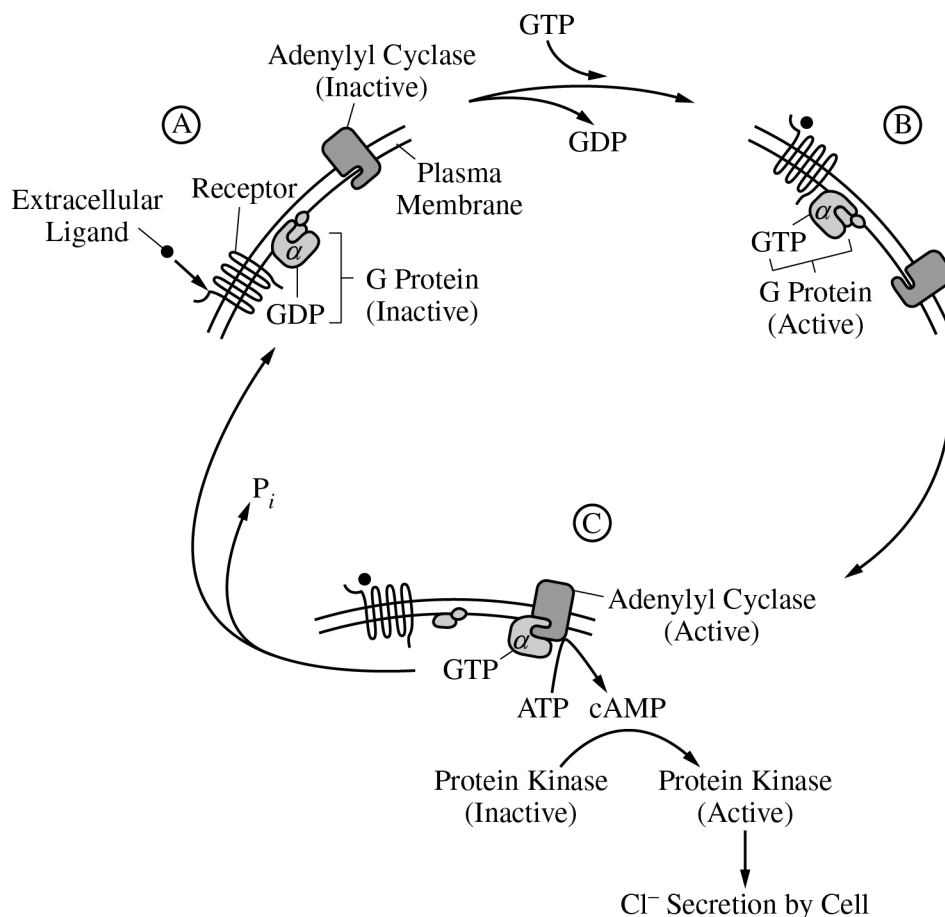

Figure 1. Under normal conditions, ligand binding to a G protein-coupled receptor results in chloride ion transport from an intestinal cell.

Individuals infected with the bacterium *Vibrio cholerae* experience severe loss of water from the body (dehydration). This is due to the effects of the bacterial cholera toxin that enters intestinal cells. Scientists studied the effects of cholera toxin on four samples of isolated intestinal cell membranes containing the G protein-related signal transduction components shown in Figure 1. GTP was added to samples II and IV only; cholera toxin was added to samples III and IV only. The scientists then measured the amount of cAMP produced by the adenylyl cyclase in each sample (Table 1).

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TABLE 1. AMOUNT OF cAMP PRODUCED FROM INTESTINAL CELL MEMBRANES IN THE ABSENCE OR PRESENCE OF CHOLERA TOXIN

Sample	GTP	Cholera Toxin	Rate of cAMP Production (pmol per mg adenylyl cyclase per min)
I	−	−	0.5
II	+	−	10.0
III	−	+	0.5
IV	+	+	127.0

present, +; absent, −

(a) **Describe** one characteristic of a membrane that requires a channel be present for chloride ions to passively cross the membrane. **Explain** why the movement of chloride ions out of intestinal cells leads to water loss.

(b) **Identify** an independent variable in the experiment. **Identify** a negative control in the experiment. **Justify** why the scientists included Sample III as a control treatment in the experiment.

(c) Based on the data, **describe** the effect of cholera toxin on the synthesis of cAMP. **Calculate** the percent change in the rate of cAMP production due to the presence of cholera toxin in sample IV compared with sample II.

(d) A drug is designed to bind to cholera toxin before it crosses the intestinal cell membrane. Scientists mix the drug with cholera toxin and then add this mixture and GTP to a sample of intestinal cell membranes. **Predict** the rate of cAMP production in pmol per mg adenylyl cyclase per min if the drug binds to all of the toxin. In a separate experiment, scientists engineer a mutant adenylyl cyclase that cannot be activated by $Gs\alpha$. The scientists claim that cholera toxin will not cause excessive water loss from whole intestinal cells that contain the mutant adenylyl cyclase. **Justify** this claim.

_____

Write your responses to this question only on the designated pages in the separate Free Response booklet.

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TABLE 1. AMOUNT OF cAMP PRODUCED FROM INTESTINAL CELL MEMBRANES IN THE ABSENCE OR PRESENCE OF CHOLERA TOXIN

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I	−	−	0.5
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IV	+	+	127.0

present,+; absent, −

(a) | **Describe** one characteristic of a membrane that requires a channel be present for chloride ions to passively cross the membrane. | **1 point**

Accept one of the following:

- The <u>interior of the membrane</u>/<u>phospholipid tail</u> is nonpolar.
- The <u>interior of the membrane</u>/<u>phospholipid tail</u> is not charged.
- The <u>interior of the membrane</u>/<u>phospholipid tail</u> is hydrophobic.

Explain why the movement of chloride ions out of intestinal cells leads to water loss. | **1 point**

Accept one of the following:

- The space outside of the cells becomes <u>hypertonic</u>/<u>hyperosmotic</u> compared with the cells, so water moves out of the cells.
- The space outside of the cells would have a lower water potential compared with the cells, so water will move out of the cells.

Total for part (a) | **2 points**

(b) | **Identify** an independent variable in the experiment. | **1 point**

Accept one of the following:

- The presence or absence of cholera toxin
- The presence or absence of GTP

Identify a negative control in the experiment. | **1 point**

Accept one of the following:

- <u>The sample lacking both cholera toxin and GTP /sample I</u>
- <u>The samples that lack cholera toxin /samples I and II</u>
- <u>The sample that lacks cholera toxin but contains GTP /sample II</u>
- <u>The samples that lack GTP /samples I and III</u>

Justify why the scientists included Sample III as a control treatment in the experiment. | **1 point**

Accept one of the following:

- (Sample III serves as a control) to compare cAMP production with that of <u>the sample having cholera toxin and GTP /sample IV</u> .
- Comparing sample III and sample IV enables the scientists to evaluate whether the activity of cholera toxin <u>requires GTP</u>/<u>acts via the G protein pathway</u>.

Total for part (b) | **3 points**

(c)	Based on the data, **describe** the effect of cholera toxin on the synthesis of $cAMP$.	**1 point**
	Accept one of the following:	

- Cholera toxin increases the production of $cAMP$ in the presence of GTP (IV vs II).
- Cholera toxin has no effect on the production of $cAMP$ in the absence of GTP (III vs I).

	Calculate the percent change in the rate of $cAMP$ production due to the presence of cholera toxin in sample IV compared with sample II.	**1 point**

- 1,170% [(127−10)/10 = 11.7 × 100]

		Total for part (c)	**2 points**

(d)	A drug is designed to bind to cholera toxin and prevent the toxin from crossing the intestinal cell membrane. Scientists mix the drug with cholera toxin and then add this mixture and GTP to a sample of intestinal cell membranes. **Predict** the rate of $cAMP$ production in $pmol$ per mg adenylyl cyclase per min if the drug binds to all of the toxin.	**1 point**

- The rate will be 10 ($pmol$ per mg adenyl cyclase per min).

	In a separate experiment, scientists engineer a mutant adenylyl cyclase that cannot be activated by $Gs\alpha$. The scientists claim that cholera toxin will not cause excessive water loss from whole intestinal cells that contain the mutant adenylyl cyclase. **Justify** this claim.	**1 point**

- (Even in the presence of the toxin) $cAMP$ will not be produced (by this pathway), the protein kinases will not be activated, and Cl^- ions will not be secreted (and less water will leave the intestinal cells).

		Total for part (d)	**2 points**

		Total for question 1	**9 points**