ESSAY 3

Answers must be written out in paragraph form. Outline form is not acceptable. Labeled diagrams may be used to supplement discussion, but a diagram without a written explanation will not receive credit. You must cite the source of all outside information you include. Include the page number of information from the course textbook or the web address of information found online.

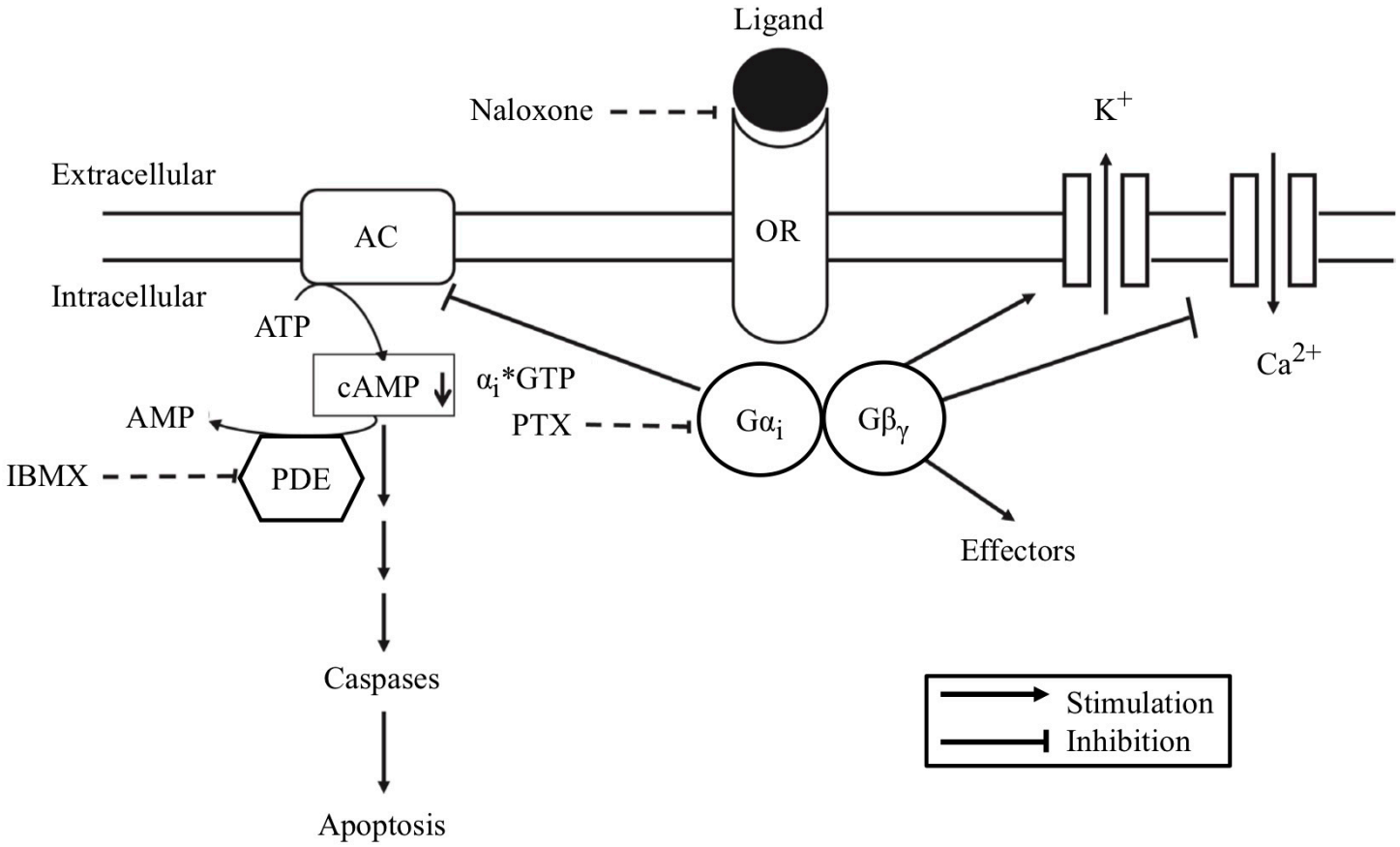
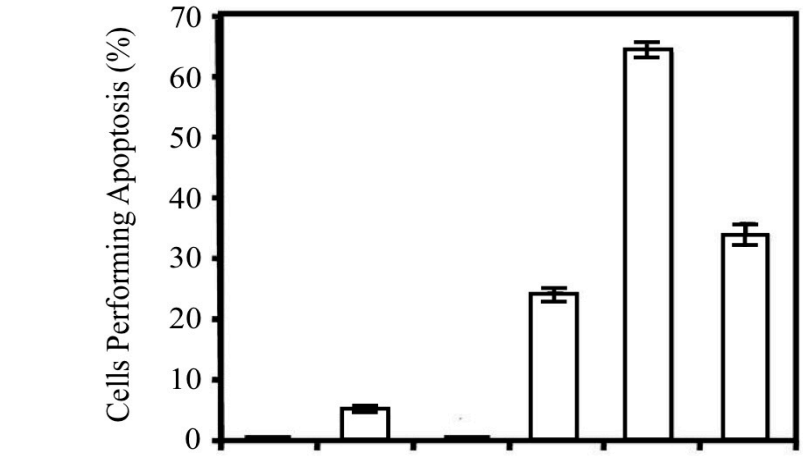


Figure 1. Opioid receptor signaling pathway. Stimulation of opioid receptors (OR) by ligands such as D,L-methadone leads to activation of the inhibitory G_i-protein. The α_i-subunit of the G_i-protein inactivates adenylyl cyclases (AC) resulting in a reduction of cAMP levels within the cell which in turn leads to apoptosis. The β_γ-subunit of the G_i-protein stimulates K^+ channel proteins and inhibits Ca^{2+} channel proteins. Naloxone competitively inhibits opioid receptors. Pertussis toxin (PTX) inactivates G_i-proteins and blocks downregulation of cAMP. 3-isobutyl-1-methylxanthine (IBMX) inhibits phosphodiesterase activity (PDE) and increases cAMP levels.

Cyclic AMP (cAMP) regulates a number of cellular processes and modulates cell death induction. cAMP levels are altered upon stimulation of specific G-protein-coupled receptors inhibiting or activating adenylyl cyclases. Opioid receptor stimulation can activate inhibitory G_i-proteins which in turn block adenylyl cyclase activity reducing cAMP. Opioids such as D,L-methadone induce cell death in leukemia cells, as shown in Figure 1. However, the mechanism by which opioids trigger apoptosis and activate caspases in leukemia cells is not understood.

Opioid receptor activation may be a promising strategy to improve anticancer therapies. In order to analyze the role of opioid-receptor triggering in apoptosis induction and consequently activation of apoptotic pathways, leukemia cells were treated with D,L-methadone, doxorubicin or with the opioid-receptor competitive inhibitor naloxone alone or in different combinations (Friesen et al., 2013). The percentage of cells undergoing apoptosis after 96 hours for each treatment group are shown in Figure 2.



Treatment	I	II	III	IV	V	VI
D,L-Methadone	–	+	+	–	+	+
Doxorubicin	–	–	–	+	+	+
Naloxone	+	–	+	–	–	+

Figure 2. Percent of leukemia cells performing apoptosis after incubation with 3 µg/mL D,L-methadone, 0.06 µg/mL doxorubicin, and 60 µg/mL naloxone, alone or in different combinations as indicated. After 96 hours, apoptotic cells were measured by FSC/SSC-analysis. Error bars represent the 95% confidence interval.

(a) **Describe** the physical property of naloxone that allows it to competitively inhibit opioid receptors. **Describe** ONE chemical property of D,L-methadone that prevents it from entering the cytoplasm.

(b) **Identify** the independent variable in the experiment. **Propose** a control group for the experiment. **Justify** the use of a bar graph to represent the data in Figure 2.

(c) Based on an analysis of the cell signaling model in Figure 1, **give ONE reason** why the stimulation of opioid receptors may be effective in improving anticancer therapies. Use the means and confidence intervals from the appropriate treatment groups in Figure 2 to **justify** the claim that the combination of D,L-methadone and doxorubicin is more effective in stimulating apoptosis in leukemia cells than D,L-methadone or doxorubicin alone. Use Figure 1 to **explain** why the percentage of cells performing apoptosis in treatment group VI of Figure 2 is lower than the percentage of cells performing apoptosis in treatment group V of Figure 2.

(d) Based on Figure 1, **predict** the effects of a decrease in PTX on the concentration of K^+ in a cell AND on the concentration of Ca^{2+} in a cell. **Provide reasoning** to support your predictions.

Reference

Friesen, C., Roscher, M., Hormann, I., Fichtner, I., Alt, A., Hilger, R. A., … Miltner, E. (2013). Cell death sensitization of leukemia cells by opioid receptor activation. *Oncotarget*, 4(5), 677–690.