

A Botched Botox Party in the Hamptons

by

Adam J. Kleinschmit

The Department of Biology and Earth Sciences

Adams State University, Alamosa, CO



Part I – Synaptic Vesicle Exocytosis

Jackie: Thank goodness you were available to stop by my villa right away this morning after my call Dr. Peterson, I am freaking out! Who is this young lady you have with you? Can I get both of you to join me in having a mimosa?

Dr. Peterson: As the Hamptons most sought-after concierge doctor, I am always on call to assist. This is Carmen, a pre-med student that is shadowing me and we will have to pass on the mimosas since we are visiting other patients today. Let's start by having you take a deep relaxing breath. Now, please tell us what is bothering you.

Jackie: It's my right eyelid! It has been drooping all morning to the point where I am having impaired vision! I attended my first ever Botox party three days ago in an attempt to hide the 11's between my eyebrows and to my dismay I have a lifeless eyelid that has progressively become worse over the last 24 hours! I don't know what to do. I can't fulfill my summer social obligations looking like this!

Dr. Peterson: This is a common side effect of Botox injections known as ptosis, which is observed in approximately five percent of Botox forehead region injections. Your 11's or glabellar skin lines, which are observed when frowning and squinting, are formed by the contraction of muscles in the glabellar complex. Poor injection technique can lead to Botox inadvertently diffusing into the region of the levator palpebrae superioris muscle, a small muscle that serves to elevate the upper eyelid, which can result in ptosis (Figure 1).

Jackie: Botox targets muscles? I thought Botox only acted directly on wrinkled skin. How could Botox affect the muscles controlling my eyelid?

Dr. Peterson: Botulinum neurotoxin (BoNT), which is marketed under the brand name Botox, temporarily removes dynamic skin wrinkles induced by muscles by paralyzing somatic motor neurons, which stimulate skeletal muscle. BoNT does this by entering a cell by receptor-mediated endocytosis and functions as a neuroparalytic toxin within the cytosol of neurons (Figure 2).

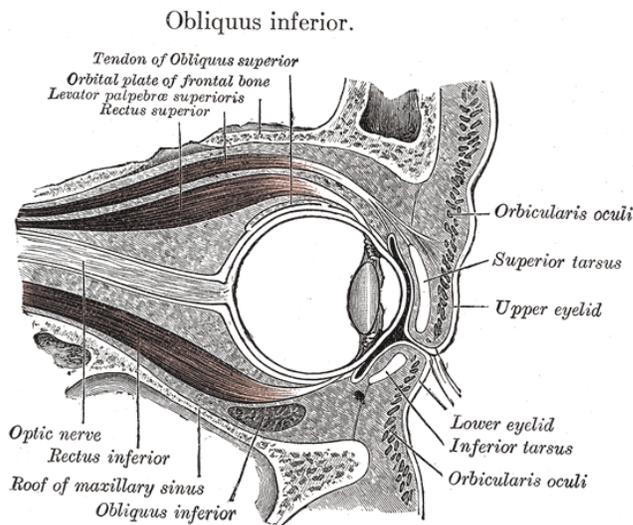


Figure 1. Sagittal section of the orbital cavity. Note the levator palpebral superioris, the skeletal muscle that is flaccidly paralyzed (relaxed state) in Jackie's condition. Additionally, note the superior tarsus, the smooth muscle that is stimulated by lidopidine eye drops to counter ptosis. Credit: *Gray's Anatomy*, Plate 888, PD.

Carmen: BoNT's molecular mechanism of cell entry sounds very similar to how cells take up cholesterol via low-density lipoproteins (LDL) from the bloodstream. Our general biology class learned that receptor-mediated endocytosis of LDL is clathrin-dependent; is the same true for BoNT?

Dr. Peterson: That's correct Carmen. BoNT also uses a clathrin-dependent process. Once BoNT gets into the cytosol it targets a protein essential in mediating the exocytosis of the excitatory neurotransmitter acetylcholine (ACh) that is temporarily stored in secretory vesicles, which inhibits communication to muscle tissue (Figure 2).

Carmen: That makes sense! So, the neuron cannot release ACh at the axon terminus, thus the post-synaptic muscle cell does not receive the chemical signal to contract.

Dr. Peterson: Exactly! BoNT inhibits the active transport of ACh via exocytosis into the synapse. The muscle on the post-synaptic side relies on the ACh to bind to ACh receptors on the muscles to stimulate contraction. Dynamic wrinkles appear when muscles in your face contract while making animated facial expressions. When these muscle groups are paralyzed, the muscles do not contract, which hides dynamic wrinkles (Figure 2).

Carmen: Oh yeah, that's right! Both endocytosis and exocytosis are energy-requiring active transport processes even though they do not necessarily move molecules against a concentration gradient.

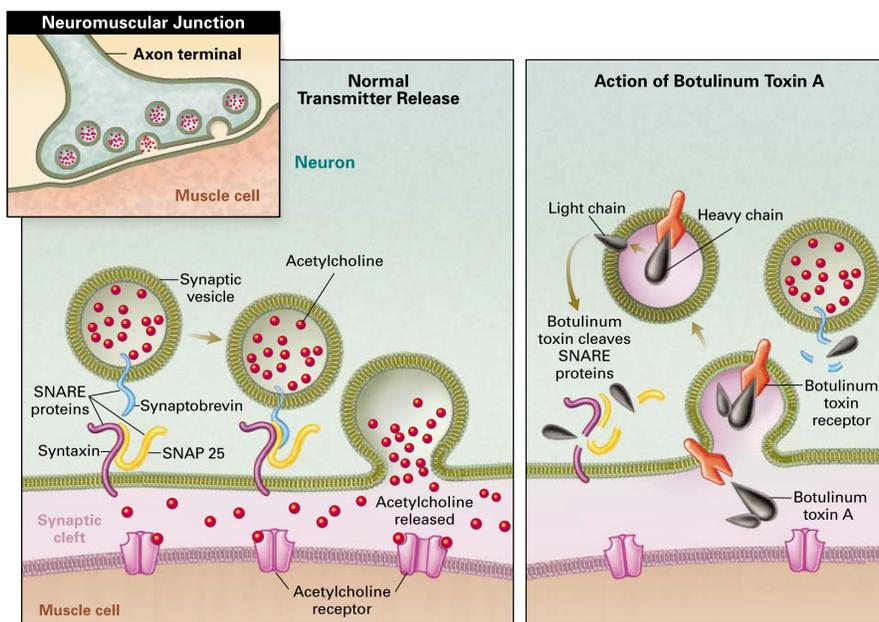


Figure 2. Molecular mechanism of BoNT in somatic motor neurons. *Credit:* Rowland, L.P. Stroke, spasticity, and botulinum toxin. *N Engl J Med.* 347(6):382–383. Copyright ©2002 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

Questions

1. Why are endocytosis and exocytosis considered active transport mechanisms if they do not necessarily move molecules against a concentration gradient?
2. What is clathrin and what is its role in receptor-mediated endocytosis?
3. Compare and contrast receptor-mediated endocytosis with phagocytosis and pinocytosis.
4. What are some other examples of proteins secreted by exocytosis that are essential for cell and tissue function?
5. From memory, sketch a section of the phospholipid bilayer of an axon terminal and diagram how neurotransmitter-containing vesicles secrete protein in the absence of BoNT via exocytosis. On the drawing, add transmembrane receptor proteins that BoNT binds to and diagram receptor-mediated endocytosis of the receptor bound to BoNT and how it can influence exocytosis.

Part II – Postsynaptic Signaling

Jackie: So I take it that acetylcholine (ACh) is important for my eyelid to go back to normal. What does ACh do to my eyelid when it is present?

Dr. Peterson: ACh binds to nicotinic acetylcholine receptors (nAChRs), which are ligand-gated ion channels, on skeletal muscle. Binding of the ligand acetylcholine to the nAChRs induces these ion channels to open, allowing Na^+ ions to rush into the cell (Figure 3).

Carmen: Oh yeah, in General Biology we learned that ion channels can function as receptors that do not require secondary messengers for signal transduction. The movement of Na^+ ions in this case would be an example of facilitated diffusion, right?

Dr. Peterson: Yes, that's correct—ion channels can mediate facilitated diffusion, as they are an example of a channel protein! Remember though that active transport mechanisms are essential for establishing an electrochemical gradient needed for facilitated diffusion of Na^+ and other molecules into the muscle cell.

Carmen: That's right, Na^+/K^+ pumps are essential for helping set up a membrane potential or membrane voltage across the membrane of all cells, especially electrically excitable cells.

Jackie: Okay...so it sounds like my neurons and muscle cells associated with my eyelid are functional, but my neurons are just having trouble talking to the muscles because no ACh is being released. Can I get some eye drops or medication with ACh to bypass the neurons to get my eyelid muscle contracting again?

Dr. Peterson: Unfortunately, there are numerous muscles in the area in question, which would make targeting the correct muscle very difficult and probably not in a sustainable fashion. The best prescription here is time.

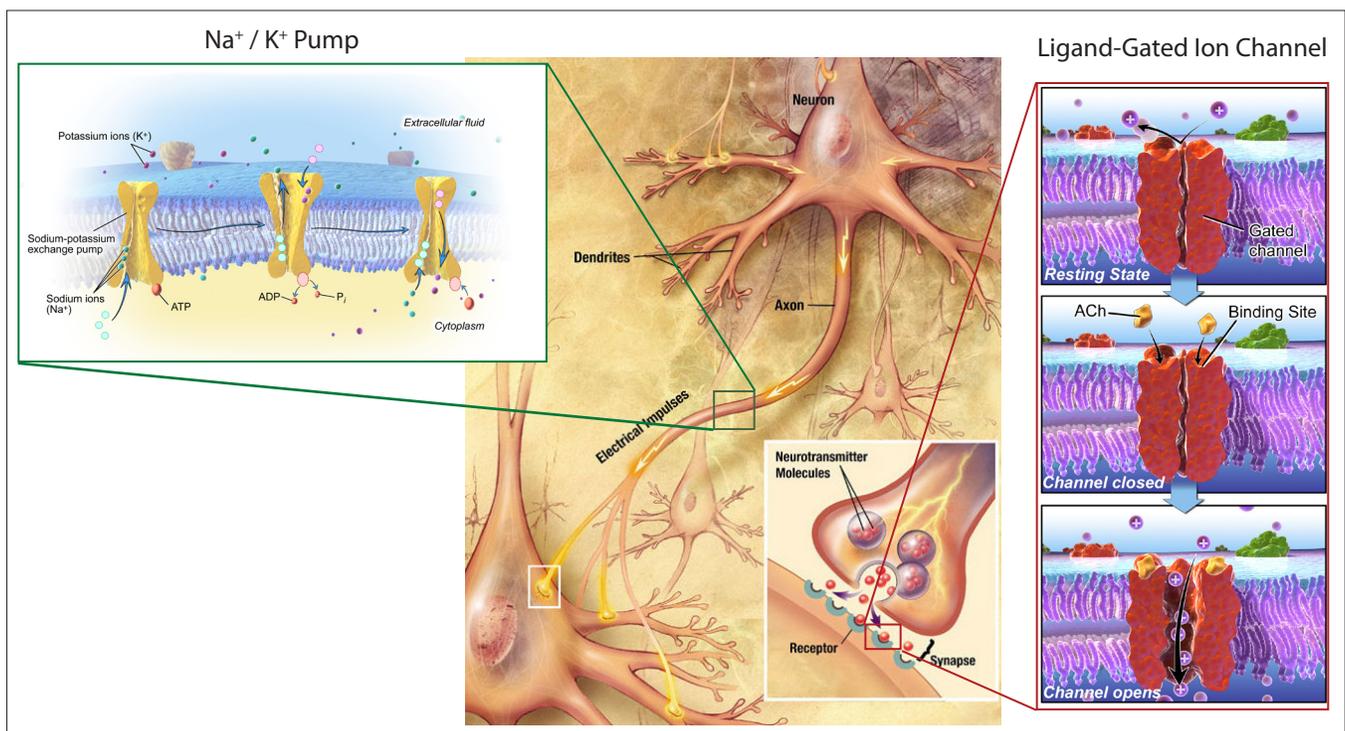


Figure 3. Neuronal signaling at a synapse. Acetylcholine (ACh) target ligand-gated ion channel conformational change and Na^+/K^+ mechanistic diagram inlayed schematics. *Credit:* Composite image; center image of synapse by US NIH, PD; image of Na^+/K^+ pump (left) and of ligand-gated ion channel (right) are courtesy (CC BY3.0) of: Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014." *WikiJournal of Medicine* 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436.

Questions

1. Compare and contrast uniporter, symporter, and antiporter proteins including the relative direction of molecule movement and any associated energetic needs. Which protein class would nicotinic acetylcholine receptors (nAChRs) be associated with?
2. In cardiac muscle, acetylcholine binds to muscarinic acetylcholine receptors (mAChRs), which are G-protein coupled receptors, which elicit a signal transduction pathway that induces cardiac muscle to relax. Outline how G-protein coupled receptors induce signal transduction pathways including relevant secondary messengers.
3. Interpret Dr. Peterson's statement that active transport is essential for facilitated diffusion of Na^+ ions to occur in muscle cells. How are Na^+/K^+ pumps involved in this process? Sketch a Na^+/K^+ pump indicating conformational changes and energy usage steps and identify ions that are moved during the cyclic process.
4. Generally, after neurotransmitters are released in the neuromuscular junction, many of the neurotransmitters or some of their components are recycled by a reuptake mechanism inherent in the pre-synaptic neuron. Considering that the reuptake mechanism is often a sodium-dependent process that utilizes membrane potential, what type of membrane transport might you expect to be in play here?

Part III – Molecular Mechanisms for the Inhibition of Neuronal Chatter

Jackie: In the past year, I have been given local and general anesthetics while having some work done at my plastic surgeon's office. I feel like the anesthetics had similar effects in that I could not move some of my muscles after my face lift and had a similar experience with my breast augmentation, but it wore off after a couple hours to a half a day. Can I expect a similar time frame for the Botox effect to wear off?

Dr. Peterson: The muscle paralysis you experienced was likely due to a neuromuscular blocking agent added to the anesthetic. That being said, anesthetics and Botox work using different molecular mechanisms, yet both can result in interrupted communication between nerves and target cells. Unfortunately, for your ptosis, Botox's effects are long lived, hence why it is used for wrinkles and not for surgery.

Carmen: If I remember correctly, in my physiology class we learned how many local anesthetics block ion channels.

Dr. Peterson: That's correct; for example, lidocaine, a commonly used local anesthetic, sterically blocks Na^+ channels from the cytoplasmic side, thus even though acetylcholine (ACh) may bind to ligand-gated ion channels, Na^+ is unable to pass through the channels due to steric interference by lidocaine. Lidocaine in the unprotonated form enters neurons by diffusion. Once inside the cell, lidocaine becomes protonated and interacts with the cytoplasmic side of Na^+ ion channels (Figure 4).

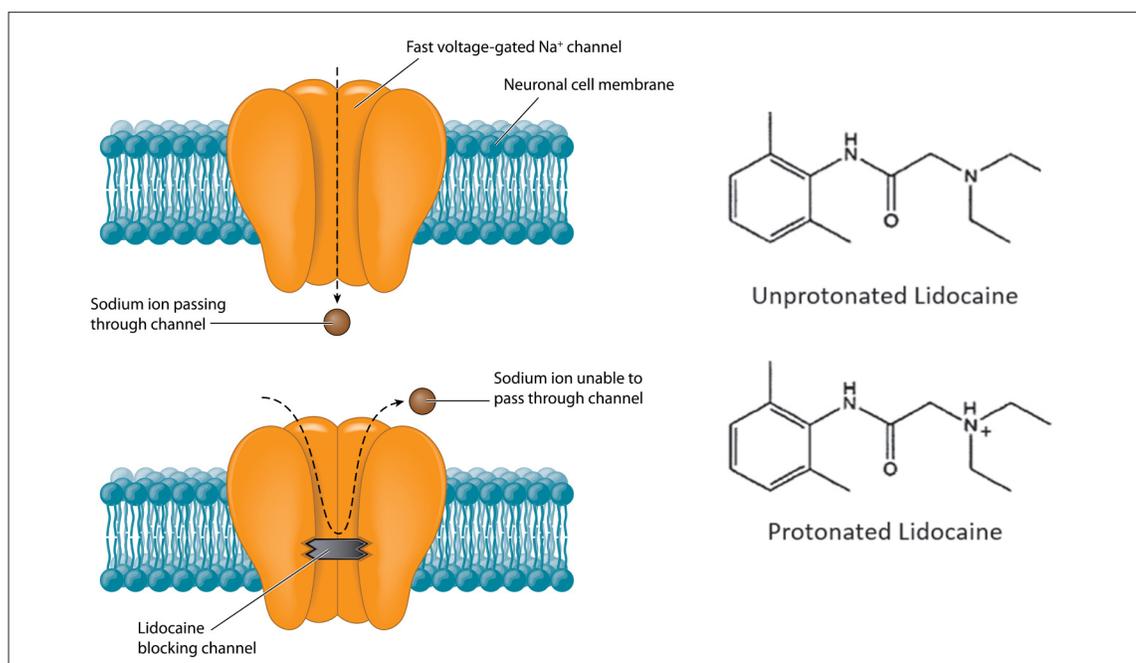


Figure 4. Molecular mechanism of Na^+ ion channel inhibition by lidocaine and chemical structure of protonated and unprotonated lidocaine. Credit: © Legger | Dreamstime, ID54908281, licensed.

Carmen: Okay, but I don't remember learning about general anesthetics. Do they work through the same mechanism as local anesthetics, but on a broader scale?

Dr. Peterson: General anesthetics are still not well understood, but some are thought to influence the fluidity and compactness of biological membranes by inserting themselves directly into the lipid bilayer of the plasma membrane. Through this putative mechanism, it is possible that some general anesthetics influence the ability of transmembrane proteins, like ion channels, from undergoing conformational changes needed to create a pore for ions to flow through (Figure 5).

Jackie: Your student sure knows a lot about human biology! Now is there something you can do to alleviate my ptosis so I can look presentable for my appearance at the yacht club this evening?

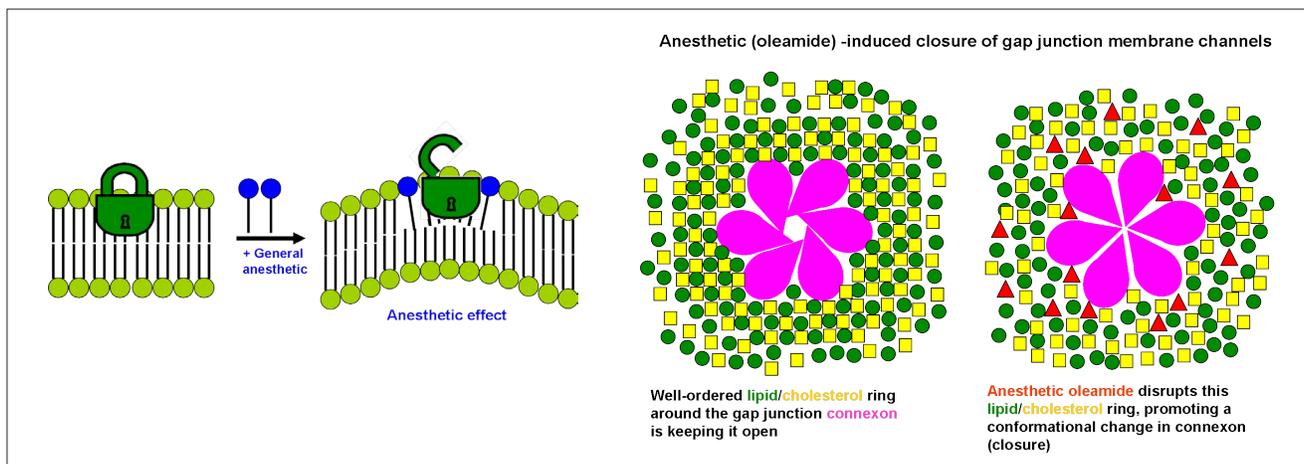


Figure 5. Molecular mechanism of general anesthetic asymmetric integration into lipid bilayer creating lateral pressure on membrane proteins. Credit: Akuznetsova, PD, Wikimedia Commons.

Dr. Peterson: Unfortunately, I cannot reverse the relaxed paralysis of your levator muscle, but I can provide you with a refillable prescription for 0.5% lopidine eye drops to help partially counter your eyelid situation so that you can see better.

Jackie: Refillable?! How long will it take for my eyelid to look normal?

Dr. Peterson: You will need to use lopidine until the Botox paralytic effect wears off, which may take two to four months. Lopidine is an α -adrenergic agonist which causes the tarus superior, a smooth muscle in the eyelid, to contract, elevating the upper lid. Specifically, the tarus superior has both α -1 and α -2 adrenoceptors, a type of G protein-linked receptor, on the surface that are used as part of the flight-or-fight response (Figure 1, in Part I). By stimulating contraction of the tarus superior muscle we can elevate your eyelid approximately 2mm to help you with your eyesight!

Jackie: I suppose the eye drops are better than nothing, but this is going to require some careful party planning as there are only so many masquerade balls a girl can host before somebody notices my ptosis! I am going to make myself a mimosa as I wait for my personal assistant to pick up my eye drops!

Questions

1. You have isolated a long hydrophobic molecule that inserts itself within biological membranes. Specifically, it spans both layers of the lipid bilayer after insertion. Would you expect this molecule to mimic the effect that some general anesthetics have? Why or why not? Refer to the general anesthetic mechanism outlined in Figure 5 as a resource.
2. Compare and contrast local anesthetics, some general anesthetics, and botulinum neurotoxin in terms of the effect they have on neuron excitability and function.
3. Predict possible clinical outcomes of injecting botulinum neurotoxin intervenously, like a general anesthetic would be administered to a patient.
4. In the protonated form, lidocaine does not freely leave cells until it is metabolized. Since lidocaine can freely diffuse into the cell, why does it not diffuse back out when lidocaine levels drop over time in the region of administration (refer to structural differences in Figure 4)?
5. α -latrotoxin, a neurotoxin in black widow spider venom, also effects acetylcholine (ACh) stimulation of muscle. Interestingly, organisms injected with α -latrotoxin exhibit uncontrollable muscle cramps and spasms, which after some time is then followed by complete flaccid (relaxed) muscle paralysis. Considering what you know about the botulinum neurotoxin, hypothesize how α -latrotoxin may modulate exocytosis of ACh.

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