Acetylcholine Synthesis and Release
Choline acetyltransferase catalyzes the formation of ACh from choline and acetyl coenzyme A. Acetate (which is used to make acetyl coenzyme A) and choline are transported into the nerve cell by specific transporters. Once it is made, ACh is stored in vesicles until its release into the synapse. One way of decreasing the amount of ACh in the cell is to block the choline transporter. If the cell does not have enough choline, ACh synthesis does not proceed.

The structure of hemicholinium (Figure 4) is similar to that of choline. Because of this structural resemblance, hemicholinium is able to bind to the choline transporter. This action blocks choline from entering the cell, interrupting the synthesis of ACh and resulting in less neurotransmitters available. (Quaternary amines are positively charged and are formulated with 1 or more anions to form salts.)

Blocking its release from the presynaptic neuron can also block ACh from entering the synapse. Botulism toxin, for example, inhibits the release of ACh into the synapse. Decreased levels of ACh in the synapse will lead to a diminished ACh response.

Acetylcholine Metabolism (Acetylcholinesterase Inhibition)
Once ACh is in the synapse, it is quickly metabolized by acetylcholinesterase (AChE) to choline and acetate (Figure 5). These products can be transported back into the presynaptic cell to be used again for ACh synthesis. If AChE is inhibited, an increased amount of ACh will accumulate in the synapse and be available to bind to the cholinergic receptors on the postsynaptic cell. In this case, the postsynaptic cell will stay stimulated longer, and the nerve signal will continue.

![Figure 4. Structure of hemicholinium.](image-url)
The first way to inhibit AChE is by blocking the binding site of ACh on the enzyme with another molecule, which is an example of competitive inhibition. Edrophonium and ambenonium (Figure 6) are 2 molecules that compete with ACh for binding to AChE. The second method of inhibiting AChE is to use a molecule that will form a covalent bond with the enzyme (Figure 7). In this way, the molecule will stay bound to the binding site (also called the active site), which prevents binding and metabolism of ACh. Several molecules inhibit AChE in this way. Molecules that make a covalent bond with the active site, so that the natural action of the enzyme is disrupted, are called irreversible inhibitors.

Figure 5. Action of acetylcholinesterase (AChE) to metabolize acetylcholine. The enzyme cleaves the ester of ACh to produce choline and acetate.

Figure 6. Structures of molecules that inhibit AChE.