Neuroscience of Human Movement
Department of Multidisciplinary
Indian Institute of Technology, Madras

Lecture - 13
Disorders of Neuromuscular Junction

Welcome to this class on Neuroscience of Human Movement. In today's class we will be discussing about Disorders of the Neuromuscular Junction. So, in the previous class we discussed neuromuscular junction let us briefly review that concept and then discuss the various disorders.

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So, in today's class we will be talking about disorders of the neuromuscular junction, specifically we will discuss two autoimmune disorders; Lambert-Eaton syndrome, myasthenia gravis. And then we will be discussing the role of toxins in affecting the function of neuromuscular junction.

There are several such toxins, there is Botulinum toxin, there is Hemicholinium, Vesamicol, Tetanus, Tubocurarine and other toxins whose mechanisms and actions we do not discuss in this class right. So, let us briefly review what happens with the neuromuscular junction.

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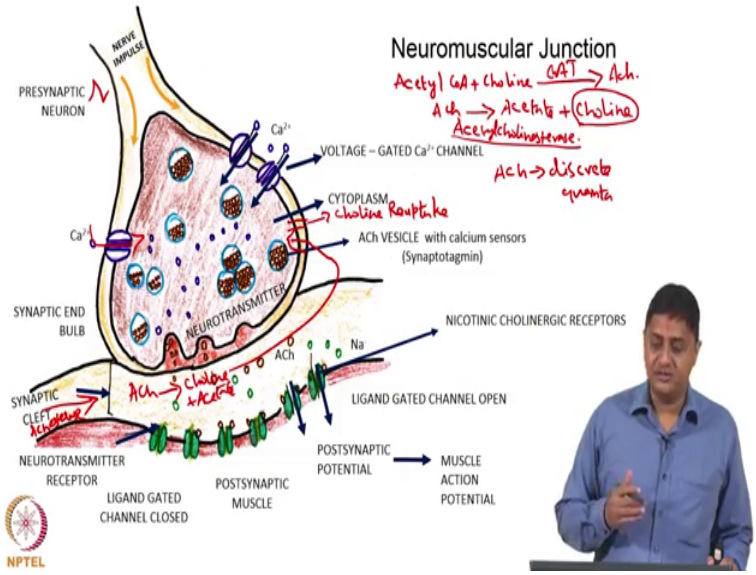

Before we discuss neuromuscular junction we will have to remind ourselves of how acetylcholine is synthesized or the life cycle of acetylcholine. Let us remember that acetylcholine is synthesized by choline acetyltransferase by the reaction of acetyl coenzyme A and choline niptons of choline acetyltransferase it is sometimes called CAT or sometimes called chat. Choline acetyltransferase is the protein that takes acetyl coenzyme A and choline and makes acetylcholine and packs them into bags called vesicles we said that meshed. And now acetylcholine itself is broken down into acetate plus choline by water called as esterases; in these particular clays, that is acetylcholinesterase ok.

So, acetylcholinesterase breaks acetylcholine into acetate and choline and choline acetyltransferase brings acetyl coenzyme A and choline together to make acetylcholine. So, this is the lifecycle of acetylcholine. So, also remember acetylcholinesterase when it breaks acetylcholine into two parts: basically into acetate and choline, part of this choline is reuptaken back into the presynaptic terminals.

So, where is this acetylcholinesterase acting at the synaptic cleft Ach esterase? Acetylcholine esterase is acting at the synaptic cleft where it breaks acetylcholine into choline plus acetate. The acetate group escapes the system whereas, choline is reuptaken back to the presynaptic terminal by choline by water called as. So, choline is reuptaken to the presynaptic terminal by water called as choline reuptake mechanisms ok. So, there are mechanisms to bring choline back into the presynaptic terminal and this choline will be recycled by choline acetyltransferase to make more acetylcholine.

So, this is the life cycle of acetylcholine, and so this acetylcholine is packed into bags called vesicles such that whenever one vesicle is opened, all the molecules of acetylcholine in that vesicle are released. So, the idea that acetylcholine release happens in discrete quanta, where each quanta describes one vesicle was first described by Bernard Kats and further he received a Nobel Prize we saw that in a previous class. Ach is released in discrete quanta not continuously and the amount of this release is related to the depolarization or the amount of calcium that is entering the cell due to depolarization or due to action potential.

So, when an action potential arise here, the calcium channels open letting in the lad of calcium and synaptotagmin sensors the presence of this calcium synaptotagmin is the calcium sensor on the vesicle. So, synaptotagmin sensors the presence of this calcium and moves the vesicle closure to the cell body by the action of synaptobrevin and syntaxin has shown in the next this.

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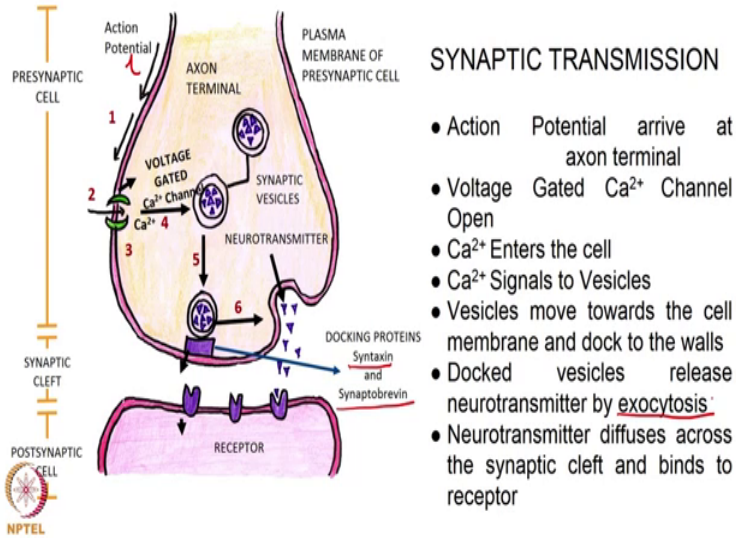

So, the action potential arise here and the calcium is detected by synaptotagmin, and it moves the vesicles closure to the cell membrane by the action of docking proteins syntaxin and synaptobrevin, the vesicles fusses to the membrane. And then opens up on the outside releasing the neurotransmitter by a process called as exocytosis we saw this in the previous class.

Now the question is what are the various things that could go wrong and what could be the effect of these problems that is the question.

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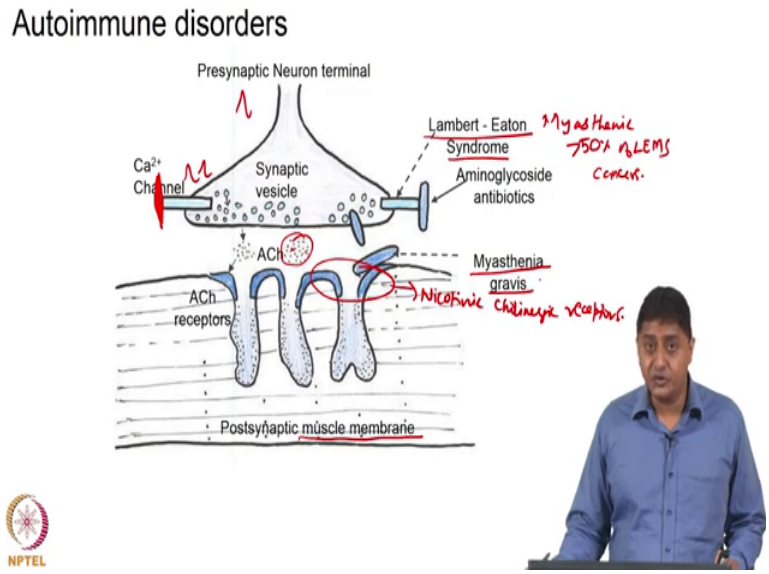

First we start out with autoimmune disorders. There are two specific cases that we will discuss in the context of neuromuscular junction. One is for whatever reason the immune system wrongly believes or wrongly labels the voltage gated calcium channels at the presynaptic terminal as an invader as an outsider. Then what happens the immune system attacks the voltage gated calcium channel destroying them, preventing their function it is what happens.

So, when this calcium channel is blocked say for example, when this calcium channel is blocked or its function is reduced, action potential will happen in the presynaptic neuron and it will reach the voltage gated calcium channel. But since the voltage gated calcium channel is not functioning, it will not open since calcium is not entering the cell, synaptotagmin will not sense the presence of a calcium so, there will be no exocytosis.

So, essentially acetylcholine will not be released what is the effect? The effect is basically weakness and inability to perform voluntary movements' etcetera. So, this situation is called as Lambert-Eaton Myasthenic Syndrome. Lambert-Eaton Myasthenic Syndrome is due to blockage of calcium channels by the immune system in an autoimmune disorder.

Usually this happens as side effect as one of the followed as one of the problems associated with cancers; are in many cases and about greater than 50 percent of Lambert-Eaton Myasthenic Syndrome cases or due to cancers ok, but there are other variance of this disease that also happens without the cancer ok. In many cases this is due to side effect or one of the other affects of cancer. What else could go wrong? Other thing is when the nicotinic cholinergic receptors and the postsynaptic muscle side.

So, this is the postsynaptic muscle membrane, here there is the nicotinic cholinergic receptors right that open whenever acetylcholine comes and binds to these receptors, these channels open causing an inflects of sodium in the muscle membrane. It turns out that for whatever reason if the immune system wrongly labels or wrongly believes that the nicotinic cholinergic receptors or if for whatever reason, the immune system wrongly believes that the nicotinic cholinergic receptors or the outside agent. Then it attacks the nicotinic cholinergic receptors, causing basically no more function. So, if the nicotinic cholinergic receptors are attacked by the immune system then it they when they are destroyed.

So, if the nicotinic cholinergic receptors are labeled as outside agents by the immune system for whatever reason, then what happens is that the nicotinic cholinergic receptors and their functions are blocked. So, acetylcholine that is released from the presynaptic terminal cannot attach to the receptor.

So, then it is not able to attach to the receptor what happens? The sodium will not enter inside the postsynaptic muscle cell, thus causing weakness and paralysis etcetera. So, in the case of myasthenia gravis, what happens is the action potential enters in the case of myasthenia gravis action potential reaches the synaptic end bulb. And calcium is released into the presynaptic terminal and this calcium is detected. And the vesicles start moving and vesicles actually fuse and exocytosis of acetylcholine actually happens, but this acetylcholine cannot attach to any point on the postsynaptic terminal.

So, because of that reason acytylcholine remains here, but it cannot attach to the receptors. So, sodium cannot enter inside causing weakness and other problem that come with this disease myasthenia gravis ok.

Now, let us discuss the role of neurotoxins and how they affect the function at the neuromuscular junction right.

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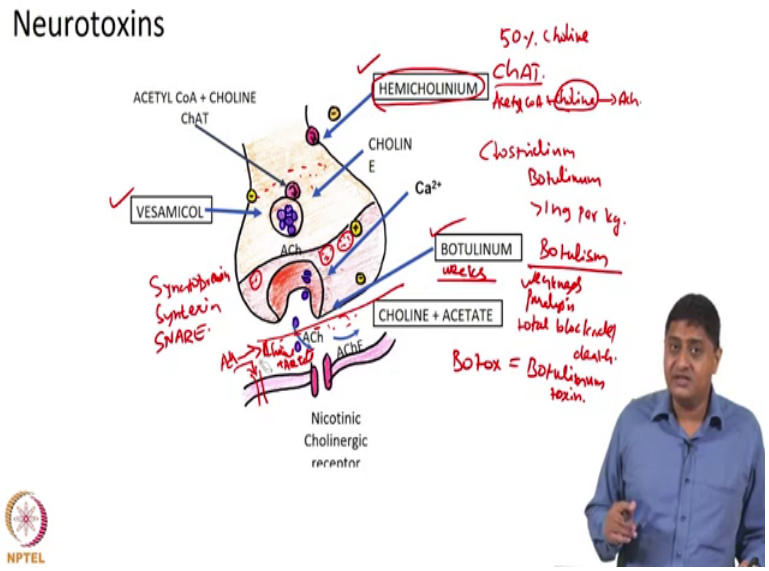

So, there are several things several places; let us remember choline is entering inside the system from the choline reuptake system right entering from the extra cellular fluid, to the presynaptic terminal via the choline reuptake system right. If the choline reuptake system is somehow compromised, then you will have no more choline entering the system. Let us remember only about 50 percent of the choline is reuptaken the other 50 percent is not reuptaken; the for choline acetyltransferase to produce.

So, choline acetyltransferase basically combines acetyl coenzyme A and choline to produce acetylcholine this is what we have seen. If this ingredient in acetylcholine is lesser or if choline is reduced by 50 percent, essentially what will happen is acetylcholine cannot be produced right after sometime acetylcholine cannot be produced.

So, that action is caused by the toxin hemicholinium. Hemicholinium basically blocks the choline reuptake system from reuptaking choline this will lead to a situation where choline cannot enter the system and whatever choline is already present in the presynaptic terminal only can be used, but essentially what will happen is after sometime all the choline will be taken by choline acetyltransferase to make acetylcholine and all the acetylcholine would be released into a synaptic cleft right.

After sometime there will be no more choline or there will be a very small amount of choline that is remaining that can be used by choline acetyltransferase to make acetylcholine. Essentially this will reduce the amount of the acetylcholine in the

presynaptic terminal right. Here, no more acetylcholine can be manufactured by choline acetyltransferase there because there is no more raw material which is choline. Now another thing that could go wrong is the case of what happens with the toxin vesamicol.

Now vesamicol what it does is it causes vesicles to go empty by the following process. Essentially it does not allow the choline acetyl transferase to pack acytylcholine into bags now that is essential right. Acetylcholine will be remaining distributed, but they cannot go outside by themselves, because there is no acetylcholine channel right. They cannot go out like that, they have to be packed in bags, or they have to be packed in vesicles by choline acetyltransfarase and these vesicles must be in a position to detect the presence of calcium and then move and fuse with the membrane.

So, as to exocytose acetylcholine; this is the mechanism through which acetylcholine can actually leave the presynaptic membrane is it not. When acetylcholine is not packed into the synaptic vesicles, then it is not possible for acetylcholine to be released from the presynaptic terminal to the post synaptic terminal.

So, this chemical vesamicol actually prevents packing of acetylcholine into the vesicles right. So, this is the other situation. Botulinum toxin is one of the most potent neurotoxin found, if not the most potent neurotoxin.

So, how is it produced? Botulinum toxin is actually produced by the action of clostridium botulinum, which is a bacteria and aerobic bacteria that produces this very powerful toxin. There are several variations of this toxin there are several forms of this toxin, but all of them have almost similar actions on the neuromuscular junction. What it does? How is it synthesized? How is it produced right? Clostridium botulinum produces is another big bacteria, and it produces botulinum toxin turns out that this is found in a large quantities in honey for example, which is why it is not a good idea to give honey to infants, right.

Infants do not have the good bacteria or the healthy bacteria in their digestive tract; that can fight the invaders like botulinum or clostridium botulinum and other such dangerous bacteria right. So, infants when they get honey right it is possible for clostridium botulinum to produce botulinum toxin in their system and since infants can do not have the healthy bacteria to fight clostridium botulinum, clostridium botulinum will colonize

the digestive tract of these infants and basically it start producing botulinum toxin in relatively large quantities.

And actually the tolerable limits is slightly above 1 nano gram per kilo gram of body mass. So, we are talking about relatively a small quantity that is why I said this is one of the most potent neurotoxins right. So, since children infants are about a few kilograms like 3 4 kilograms we are talking about. If they are given honey they can only tolerate upto 3 nano grams which are which can be easily produced by this bacteria right. So, what this causes in infants is a condition called botulism.

So, the infants eyelids will droop and the face will drop, and will become the body will become lymph the eye and hand and leg movements will become very minimized right. So, that is the symptom. Fortunately it is possible to save these children from botulism if immediate action is taken ok. How does botulinum toxin itself work that is the question.

So, the release of acetylcholine is caused by the action of synaptobrevin and syntaxin, which basically form a part of group of proteins called as snare proteins ok. Botulinum toxin what it does is it prevents the ability of these snare proteins to interact basically it cleaves the snare proteins, then what will happen? Then the vesicles can no longer fuse with the membrane and exocytose the acetylcholine. So, vesicles will be present they will have acetylcholine, but they will not be able to fuse and release acetylcholine into the synaptic cleft. Essentially leading to a situation, where acetylcholine all the acetylcholine is present in the presynaptic terminal no acetylcholine is released in the synaptic cleft.

So, the receptor is not able to sense the presence of acetylcholine and sending sodium so, that force can be produced essentially this leads to a situation where there is weakness or paralysis. In many cases this leads to total blockade and in some cases it also leads to death right. It turns out that there is a very crucial very critical clinical use for botulinum toxin.

If used in relatively controlled and small quantities by surgeons it is possible to release; tight muscles or those that are spastic those that are having rigidity. Those that cause rigidity to loosen up a little bit. Fortunately this is a very very crucial step in the process of rehabilitation of these patients, unfortunately it turns out that this is only temporary,

after sometime the rigidity will return as in this last for some weeks few weeks after sometime then they will have to take one more dose of botox right.

The details of how; this is used is beyond the scope of this class, those who are interested can check the clinical and cosmetic use of botox or botulinum toxin. So, botox is the short form for this toxin. It is possible to synthesize and isolate this toxin in the lab, which is then used for rehabilitation and cosmetic purposes. Those who are interested can check; what are the cosmetic and clinical applications of botox can check.

Now so, far what we have seen we have seen hemicholinium we have seen vesamicol we have seen botulinum toxin and there are a few more.

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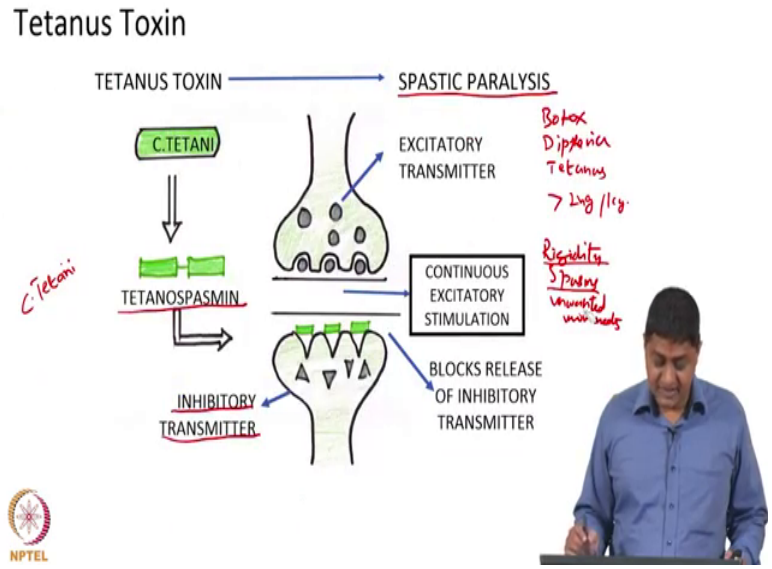

The other one is tetanus toxin. Tetanus toxin is tetanospasmin that is produced by the bacteria C Tetani or Clostridium or Clostridium Tetani. This is one of the most potent toxins known actually it is the third most potent after botox basically there is botox, there is diphtheria toxin, then there is tetanus toxin these are the order of this. You need a little bit more than two grams sorry two nano grams per kilogram, for this to be very potent like. So, what is its action?

What this does is, it blocks the release of inhibitory neurotransmitters. So, what happens in the neuromuscular junction right? Once acetylcholine is released, some acetylcholine may remain in the system for it to stop acting anymore acetylcholine esterase breaks

acetylcholine into choline plus we saw this, it acetylcholine esterase breaks acetylcholine into choline plus acetate, but its action is also stopped by other receptors that take Gaba. Whenever Gaba enters into the picture it hyperpolarizes the postsynaptic membrane right, this way the effect of acetylcholine is neutralized. Tis needed in real life everyday movement this happens in the healthy case.

So, this inhibitory neurotransmitter if it is blocked, then what happens is acetylcholine is present for a relatively long period causing rigidity, spasms and unwanted movements. So, tetanospasmin essentially blocks the release of the inhibitory neurotransmitters such as Gaba, and prevents them from neutralizing the effect of acetylcholine when it is not wanted. The action of acetylcholine is balanced by the inhibitory neurotransmitter such as Gaba, and the action of esterases such as acetylcholine esterase and other such things.

So, when this fine balance is skipped or disturbed in favor of acetylcholine, what happens is excess acetylcholine remains at the postsynaptic terminal, causing unwanted movements causing spasms causing rigidity etcetera ok. So, this leads to a situation where there is spasm or spastic paralysis. Let us remember what happens with the case of botox right it causes flaccid paralysis. This causes flaccid paralysis, whereas tetanus toxin or tetanospasmin actually causes spastic paralysis.

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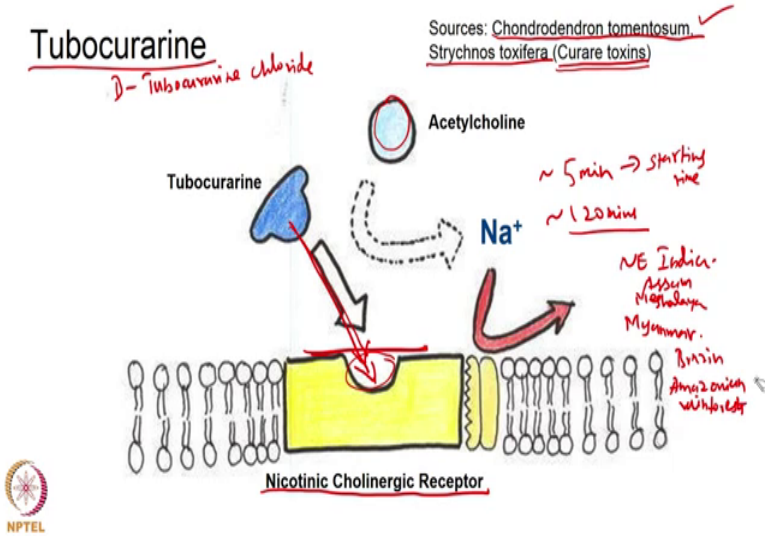

There is a group of aero poisons, there is a group of drugs called curare toxins right that are source from this can be source from plant and animal sources. From plants such as

strychnos toxifera, curare toxins are produced, but specifically from a plant called chondrodendron tomentosum, tubocurarine or more specifically D-Tubocurarine chloride is produced. What this does is this competes with acetylcholine for space in the nicotinic cholinergic receptor, basically it blocks the nicotinic cholinergic receptor from accepting anymore acetylcholine it blocks this right.

So, when acetylcholine molecule reaches this point, it cannot attach to that point because already tubocurarine has attached because of this reason acetylcholine will remain in the synaptic cleft. Until and unless acetylcholine attaches to the nicotinic cholinergic receptor, the channel will not open and sodium will not enter inside leading to a situation where there is flaccidity weakness paralysis.

Actually when it when it reaches the lung muscles breathing muscles, it could stop breathing and the essentially cause death this is how aero poisons are used. So, basically tubocurarine chloride is quoted on the aero and it when it is shot on the animal or the game of interest or the particular target of interest, this animal is paralyzed by the action of tubocurarine. Essentially what happens is that this blocking of the nicotinic cholinergic receptor by tubocurarine causes paralyses of the animal, but it is a relatively slow process, it takes about 5 minutes, its affect last for about for about 120 minutes. For the affect to start for the affect to take role it takes about 5 minutes. In comparison with botulinum toxin in comparison with the other toxins, this is relatively slow poison right and its affect last for only about 120 minutes approximately.

So, these are all these are all approximate values; this is used in South American rain forest by tribes who hunt for animals using this aero poisons. This is still used in north eastern India state such as Assam, Meghalaya and in Myanmar for hunting it is still used and of course, it is used in Brazil and Amazonian rain forest, where the tribes of the Amazonian rainforest still used these aero poisons to hunt animal's right.

So, this is the action of that.

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Disorder	Action	Effect on the Neuromuscular Transmission
Myasthenia Gravis (Autoimmune Disease)	Blocks ACh Receptors in postsynaptic terminals	Drooping of eyelids, difficulty swallowing, muscle weakness.
Lambert-Eaton Syndrome (Autoimmune Disease)	Blocks Ca^{2+} Channels in presynaptic terminals	Difficulties in climbing stairs and rising from a sitting position because of muscle weakness in lower limbs
Botulinum toxin	Blocks ACh release from presynaptic terminals Cleaving SNARE protein	Total blockade, paralysis of respiratory muscles, and death
Hemicholinium	Blocks reuptake of choline into presynaptic terminal	Depletes ACh stores from presynaptic terminal
Vesamicol	Inhibits ACh uptake into synaptic vesicles and reducing its release	Failure of transmission, paralysis and respiratory blockade.
Tetanus Toxin	Blocks the release of inhibitory neurotransmitters	Difficulty in controlling or stopping the movements, Rigidity, Spasms.
tubocurarine	Competes with ACh for binding to postsynaptic receptor.	Failure of sodium influx and the action potential transmission does not occur.

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So, in summary what are the various things that we have seen? We have seen Myastenia Gravis which is an autoimmune disorder what this does it is it blocks the nicotinic cholinergic receptors in the postsynaptic terminal.

So, essentially causing drooping of eyelids, difficulty in swallowing, weakness of the muscle right. Then that is a Lambert-Eaton Myastenic syndrome this is again an autoimmune disorder, this blocks calcium channels in the presynaptic terminals right causing difficulty in climbing stairs, raising from sitting position and because muscles are weakened right because of muscle weakness. Then there is botulinum toxin; botulinum toxin essentially prevents the release of acetycholine from the presynaptic terminal, how? By cleaving the snare proteins actually prevents the action of the docking protein synaptobrevien syntaxin from fusing to the membrane and releasing acetylcholine into the cleft right.

It leads to total blockade paralysis as in flaccid paralysis and death right. Hemicholinium basically prevents the reuptake of choline into the presynaptic terminal, essentially depleting acetylcholine stores from the presynaptic terminal under some point; Acetylcholine will become empty or basically at some point presynaptic terminal will not have any more acetylcholine to release and causing weakness right.

Vesamicol what it does is it inhibits acetylcholine uptake into synaptic vesicles, basically it blocks packaging of acetylcholine into the vesicles thus reducing the possibility of its release right. Essentially this leads to a failure of neurotransmission paralysis and when it

reaches the respiratory system, respiratory blockade right. Tetanus toxin what it does is it blocks the release of inhibitory neurotransmitters not the excitatory neurotransmitters. It blocks the release of inhibitory neurotransmitters thus reducing the possibilities of neutralizing the effect of acetylcholine.

The affect of acetylcholine is excitatory on the postsynaptic terminal, on the muscle, until and unless and hyper polarization arise or in inhibitory neuro transmitter arise basically Gaba or other inhibitory neurotransmitters arrive, there will be some activity in the muscle right. Tetanospasmin basically blocks the release of these inhibitory neurotransmitters leading to spasm, unwanted movements, rigidity and difficulty in stopping the movements.

Then you have curare drugs or aero poison such as tubocurarine chloride, which completes with the acetylcholine for basically occupying the space allotted for acetylcholine, which is basically the nicotinic cholinergic receptor is blocked by d tubocurarine chloride, which is in aero poison thus preventing acetylcholine from binding.

Note: only when acetylcholine is bound to the receptor the channel will open and let in sodium not when anything is binding. So, because of this reason sodium will not enter inside when tubocurarine is attached to the postsynaptic receptor, basically to the nicotinic cholinergic receptor. This leads to failure of sodium influx and action potential transmission to the muscle, basically leads to weakness, paralysis, total blockade depending on the amount it could also lead to total blockade or death due to failure of respiratory muscles, right.

So, with this we come to the end of this lecture.

Thank you very much for your attention.