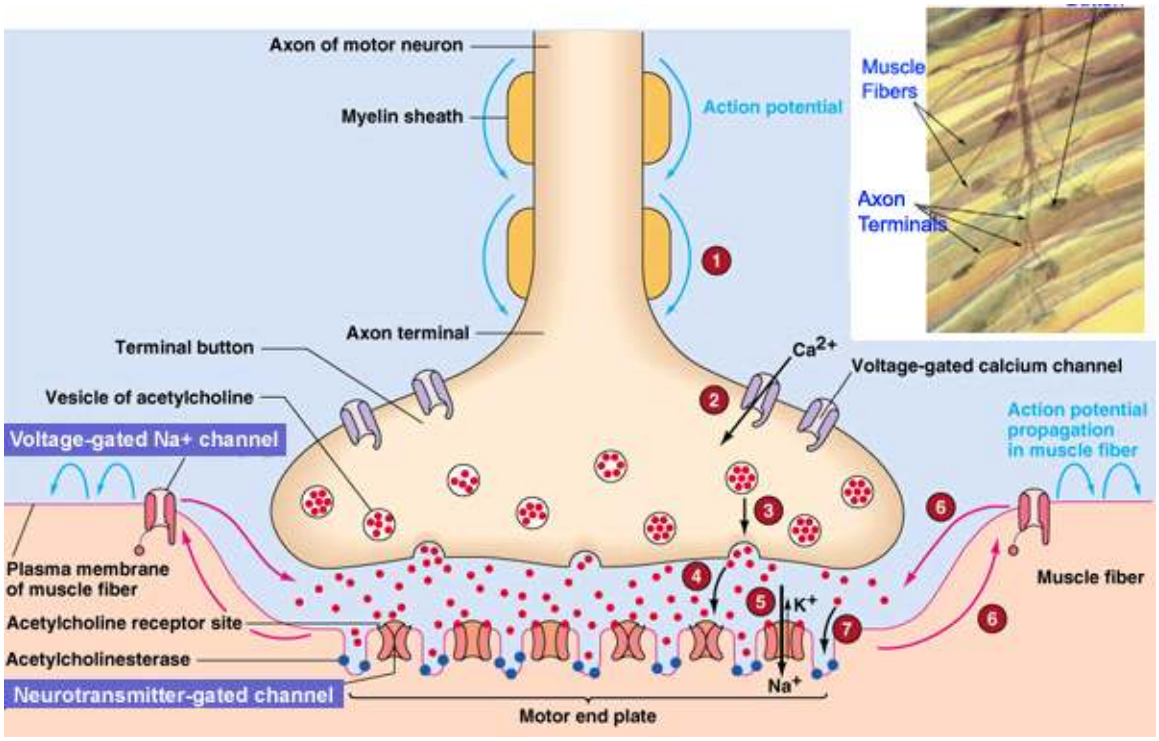
# Chapter 4
# Neuromuscular and Synaptic Transmission

## 1. Neuromuscular junctions

The neuromuscular endplate is the *contact zone* between the axons of motor neurons and striated muscle fibres. Axon terminals have vesicles containing *acetylcholine*. The vesicles dock on the *active zones* or *release sites* of the presynaptic membrane with high affinity. The muscle cell membrane at the endplate is folded in junctional folds or *crypts*. *Nicotinic acetylcholine receptors* are concentrated at the openings of these junctional crypts. The release sites are located directly over the acetylcholine receptors. The postsynaptic membrane has *acetylcholinesterase* all over its surface.

The *nicotinic acetylcholine receptor* is related to a ligand (acetylcholine)-gated ion channel found not only in the neuromuscular junction, but also at all autonomic ganglia and in the central nervous system (CNS). The receptor is fixed into the postjunctional membrane, whereas *acetylcholinesterase* is loosely attached to its surface. The receptor has five integral protein subunits surrounding a *central ion channel pore* that is opened by the binding of 2 acetylcholine molecules to the proteins. Opening of the ion channel increases the conductance for small cations ($Na^+$ and $K^+$) across the postjunctional membrane, depolarising the membrane potential of the cell. These ion channels are not voltage-gated (not dependent on changes in membrane potential), like most cation channels in neurons, cardiac and skeletal muscle cell membranes.




The acetylcholine-vesicles are probably already stored close to the *release zones*, awaiting the release signal. When the action potential (AP) reaches the axon terminals, the axon membrane is depolarised, and voltage-gated $Ca^{2+}$-channels are transiently activated. This causes $Ca^{2+}$ to flow down its concentration gradient from the outside into the axon terminal. The influx of $Ca^{2+}$ at the release zones causes the vesicles to fuse with the axon membrane, and empty acetylcholine into the 50 nm wide cleft by *exocytosis* .

After crossing the synaptic cleft by diffusion, acetylcholine binds to its *receptor protein* on the muscle cell membrane. This binding complex opens the ion channel and increases the conductance for small cations across the muscle cell membrane. The influxes of $Na^+$ depolarise the *endplate* temporarily, the transient depolarization is termed the *endplate potential* (EPP). The EPP dies away when acetylcholine is hydrolysed to acetate and choline by the enzyme, *acetylcholinesterase*. The EPP has a large safety margin, as a *single action potential* in the motor axon will produce an EPP that always reaches the threshold potential in the muscle fibre.

Rapid contraction of the muscle fibre is achieved by propagation of the muscle action potential along the whole length of the muscle fibre membrane and into the small, transverse tubules, which penetrate all the way through the muscle fibre (T-tubules).

The acetylcholine binding at the motor endplate increases endplate conductance and generates an action potential (AP) in all directions from the end plate. The electrical excitation of the sarcolemma and the transverse tubules (T-tubules) during the AP triggers – by an unknown mechanism - the *sarcoplasmic reticulum* to release a pulse of $Ca^{2+}$ ). The $Ca^{2+}$-channels opens transiently in the vicinity of each sarcomere The sarcoplasmic [$Ca^{2+}$] increases from $10^{-7}$ to $10^{-6}$ M (which is the threshold). This $Ca^{2+}$ diffuses to the adjacent myofilaments, where they bind strongly to troponin C on the active filament, and end the troponin-tropomyosin blockade. This enables cyclic crossbridges to work as long as the high [$Ca^{2+}$] is maintained, whereby contraction occurs. A continually active $Ca^{2+}$-pump returns $Ca^{2+}$ to the sarcoplasmic reticulum, and another $Ca^{2+}$-pump in the cell membrane also reduces sarcoplasmic  $Ca^{2+}$ . Then the thin filament is *off duty*, because $Ca^{2+}$ is withdrawn from its troponin C, the troponin-tropomyosin-blockade is re-established and *relaxation* ensues. The terminal cisternae of the sarcoplasmic reticulum contain granules of calsequestrin, a protein that can bind $Ca^{2+}$ and reduce the concentration gradient

*Neurons* with *motor* function have the ability to synthesise acetylcholine, because they contain *choline-acetyltransferase*. This enzyme catalyses the production of acetylcholine from acetyl-CoA and choline. Almost all cells produce *acetyl-CoA* and *choline*. Choline is also actively taken up from the extracellular fluid via a mechanism indirectly powered by the $Na^+$-$K^+$-pump. There is a 50% reuptake of choline from the synaptic cleft; hence some choline must be synthesized in the motor nerve.

The postjunctional membrane depolarizes spontaneously - resulting in so-called *miniature endplate potentials* (MEP-potentials). A miniature endplate potential is probably caused by the spontaneous release of a single vesicle into the cleft. This is called *quantal release*.

An endplate potential is prolonged when *cholinesterase-inhibitors* are present in the synaptic cleft. This is because these substances (eserine, edrophonium, malathion, parathion etc.) inhibits the enzyme and thereby protects acetylcholine from being hydrolysed by the enzyme. The life dangerous parathion poisoning is described in chapter 6. Under normal conditions, the endplate potential is terminated by the rapid hydrolysis of acetylcholine by acetyl-cholinesterase.

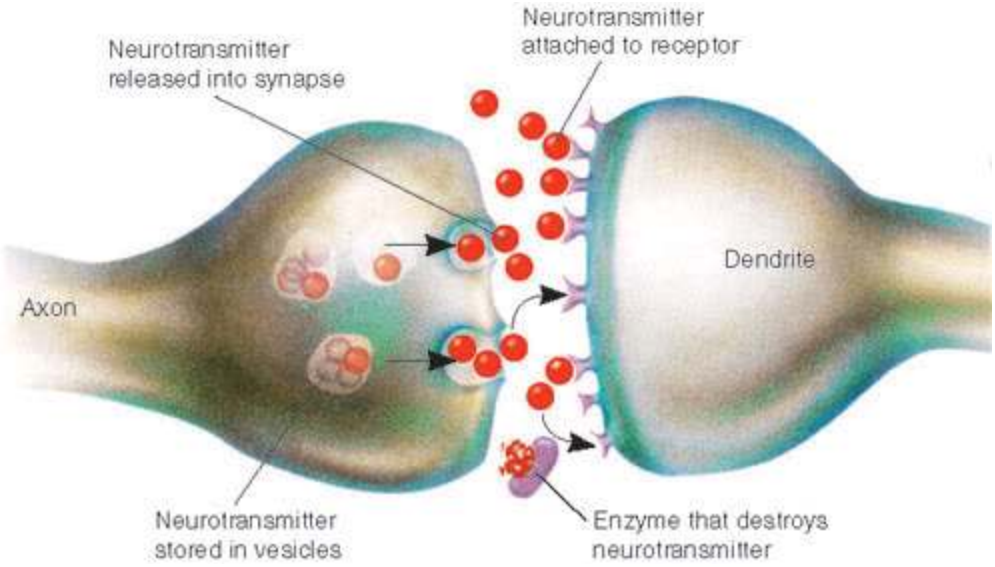
*Acetylcholine* is a transmitter in the CNS, in all motor neurons, in all preganglionic neurons of the autonomic nervous system and postganglionic parasympathetic fibres, and in a few postganglionic sympathetic fibres. The cholinergic receptor subtypes are shown in Table.

## 2. Synapses

Chemical synapses prevail in humans, but we also have electrical synapses in gap junctions.

*A chemical synapse* consists of a neuronal presynaptic terminal, a synaptic cleft and a subsynaptic (or postsynaptic) membrane with associated receptor proteins. The chemical synapse is highly developed in the CNS. It conducts the signal one way only, and has a characteristic *synaptic delay*.

The *presynaptic axon terminal* typically broadens to form a *bouton terminaux* (presynaptic terminal).




1. The action potential, originating in the CNS, *depolarises* the axon membrane by selective influx of $Na^+$, which has a large electrochemical gradient. Repolarization follows rapidly by selective $K^+$-efflux.
2. When the action potential reaches the presynaptic membrane, $Ca^{2+}$ enters the terminal through voltage-gated *$Ca^{2+}$-channels*.
3. Vesicles containing transmitter, fuse with the presynaptic membrane and release their contents of acetylcholine into the synaptic cleft ($Ca^{2+}$-induced exocytosis).
4. Transmitter molecules (acetylcholine, ACh) diffuse across the synaptic cleft and bind to specific receptors, which are located into the postsynaptic membrane. This ligand binding elicits a transient opening of *pores*, which are specifically permeable to small cations. The synaptic cleft of a chemical synapse is about 30 nm.
5. The ACh-receptor opens and allows influx of $Na^+$, whereby the membrane depolarizes and an action potential is generated which propagates along the length of the postganglionic axon. This is an appropriate response of the postsynaptic cell to the received signal.
6. The effect is rapidly terminated by the highly specific enzyme acetylcholinesterase, which hydrolyses acetylcholine into two inactive products (acetic acid and choline).

Influx of $Na^+$ or efflux of $K^+$ through the pores of such receptors changes the postsynaptic membrane potential. If the presynaptic action potential (AP) results in a postsynaptic depolarization, the transient is called an *Excitatory Post-Synaptic Potential* (EPSP). If the AP results in a postsynaptic hyperpolarization, the transient is called an *Inhibitory Post-Synaptic Potential* (IPSP). Excitatory synapses often use *glutamate* as the transmitter. The pores are penetrated mainly by $Na^+$, which enters the cell, depolarizes the membrane, and produces an EPSP.

The axon hillock on the *cell body* has a high density of voltage-gated $Na^+$- and $K^+$-channels. The axon hillock probably integrates the many synaptic potentials, and from here the action potential is generated. The *dendrites* have voltage-gated

channels for $K^+$ and for $Ca^{2+}$. Recent evidence suggests that dendrites also contain voltage-gated $Na^+$-channels, which are involved in *electrogenesis* (ie, movement of charge across the membrane).

Each neuron in the CNS is in contact with up to $10^5$ presynaptic axon terminals. Synaptic inputs are integrated at the axon hillock by either *spatial* or *temporal* summation.

*Spatial summation* occurs when inputs from several axons arrive simultaneously at the same postsynaptic cell. Their postsynaptic potentials are additive. EPSPs summate and move the membrane potential closer to the threshold level for firing. Conversely, EPSPs and IPSPs cancel each other out.

*Temporal summation* occurs when successive APs in a presynaptic neuron follow in rapid succession, so that the postsynaptic responses overlap and summate. Summation is possible because the synaptic potential lasts longer than action potentials by a factor of 10-100 times.

Each individual synapse contains receptors, ion channels, and other key molecules, which are sensitive to the neurotransmitters released at the site. These specific protein molecules are involved in synaptic plasticity and summation.

*Electrical synapses*. A *gap junction* is a transmembrane pathway of low electrical resistance that connects the cytoplasm of adjacent cells. A gap junction allows the membrane potential of the adjacent cells to be *electrically coupled*. Gap junctions form *electrical synapses***,** which differ from chemical synapses in that transmission, is instantaneous.

An electrical synapse consists of several protein pores, which close in response to increased intracellular [$Ca^{2+}$] or [$H^+$] in a cell, thereby increasing their resistance. Open gap junctions exchange ions and small molecules up to a molecular weight of 1000 Dalton.

Gap junctions are found in simple reflex pathways, where rapid transfer of the electrical potential is essential, and between non-neural cells such as epithelial and myocardial cells, smooth muscle cells and hepatocytes.

*Neurotransmitters* are divided into classical, rapidly acting non-peptides and putative, slowly acting neuropeptides.

Here is only described the function of GABA, neuropeptides and dopamine.

The major *inhibitory* transmitters are GABA (gamma-aminobutyric acid) in the brain and glycine in the spinal cord. Binding of GABA to the GABA-receptor opens the pore for $Cl^-$ influx, whereby the subsynaptic cell membrane *hyperpolarises* .The increase in $Cl^-$ conductance stabilises the membrane potential and decreases the efficacy of excitatory transmission. The GABA-receptor pore is permeable to $K^+$ besides $Cl^-$. The GABA-receptor has a major inhibitory role in brain function and is the binding site for barbiturates (used as hypnotics in anaesthesia) and for benzodiazepines (used to relieve anxiety).

The $GABA_A$-receptor shown here is related to *sedation* and *mood*, whereas the $GABA_B$-receptor controls *spasticity*. Picrotin blocks the GABA-channel.

*Glutamate, aspartate* and related acidic amino acids are the most important *excitatory transmitters* in the brain and spinal cord. Excitatory neurons possess *excitatory* amino acid (EAA) receptors. EAA receptors are a family of receptors with at least four different ions channels: The N-methyl-D-aspartate-receptor **(**NMDA), and three so-called *non-NMDA receptors* **-** one of which is the *glutamate receptor*. The NMDA-receptor operates with $K^+$-efflux, while $Na^+$ and $Ca^{2+}$ enters the subsynaptic neuron. $Mg^{2+}$ and many antiepileptic drugs block the NMDA-receptor channel. Opening of $Na^+$- and $Ca^{2+}$-

channels, which allow an increased influx of $Na^+$ and $Ca^{2+}$, cause the membrane potential to approach the threshold level for excitation. Both a reduced $Cl^-$-influx to the neuron and a reduced $K^+$-efflux move the membrane potential towards the threshold level and possible excitation. The NMDA-receptor has a separate glycine site.

*Neuropeptides* have slow excitatory or inhibitory transmitter actions. Peptides cannot be synthesized locally in the axon terminals, because they do not have ribosomes.

Peptides are water soluble, and act as hormones by binding to specific cell-surface receptors. *Cell-surface receptors* are a family of guanosine triphosphate-binding proteins, so-called GTP-binding or *G-proteins,* which control and amplify the synthesis of second messengers. Cell-surface receptors for neurohormones can function as transport protein and possess enzyme activity .

Neuropeptides are build by a sequence of amino acids. Neuropeptides are synthesized in the cell bodies of the neurons and transported to the terminal buttons by rapid axonal transport. Some neuropeptides are released together with a non-peptide co-transmitter.

Some neuropeptides are produced when a large *mother-peptide* is cleaved into several active neuropeptides. Neuropeptides are released from the nerve terminal near the surface of its target cell, and diffuse to the receptors of the target cell. Low concentrations of neuropeptides typically affect the membrane potential by changing the conductance of the target cell to small ions. The action of neuropeptides usually lasts longer than that of enzyme-inactivated transmitters. Following prolonged synaptic transmission, neuropeptides are deactivated by *proteolysis*.

*Dopamine* and other catecholamines derive from tyrosine via DOPA, which stands for the precursor 3,4-dihydroxy-phenylalanine. Dopamine is the actively accumulated into storage vesicles in the nerve endings together with noradrenaline and ATP. Dopamine activates both presynaptic and subsynaptic $D_2$-receptors.

Noradrenaline can be oxidatively deaminated by monoamine oxidase (MAO) located on the external membrane of mitochondria . The enzyme COMT (catechol-O-methyl transferase) can also methylate noradrenaline to nor-metanephrine. MAO and COMT are important in metabolising circulating catecholamines. Re-uptake of noradrenaline is the most important terminator of its actions.

Activation of both $D_2$-receptors opens $K^+$-channels and the increased outflux of $K^+$ hyperpolarizes the membrane. Blockage of the presynaptic $D_2$-receptors in substantia nigra with antipsychotic drugs reduces $K^+$-outflux and increases dopamine production and release.

Loss of *dopamine-containing neurons* in substantia nigra results in the lack of dopamine at the $D_2$-receptors of the striatal neurons. These neurons degenerate in Parkinson's disease causing *muscular rigidity* and *hand tremor* .