Molecular basis of neurotransmission biology essay

Brain is one of the most important organs of the body with continuous network connecting each cell physically with the help of neurons the building blocks of nervous system. Neurons transmit their signal to other cells in the form of electrochemical waves through their fibres called axons. Signal is transmitted in the synaptic gap with the help of chemical substances called Neurotransmitters. These signals are important in order to coordinate organ functions, smooth, skeletal and cardiac muscle actions and bodily secretions for the long time survival of mammals. The current topic depicts the understanding of the molecular mechanisms of neurotransmission with particular emphasis on the neurotransmitter release, action and inhibition.

Background Information:

Neurons are the building blocks of nervous system transmit information by electrical and chemical signalling. These neurons consist of mainly three parts they are cell body, dendrites and an axon. The gap between the two neurons is called synapse. The chemical substances which transmit impulses through the gap are called Neurotransmitters.

Neurotransmitter release occurs by the regulated exocytosis of vesicles containing the transmitter. As transmitters are released by a process of fusion of vesicular membrane with plasma membrane. The way of release of transmitter is not identical for all neurotransmitters and all synapses. The rate of release of different vesicles varies because small scale vesicles (SSVs) lie close to the synaptic membrane at specialized areas called active zones release faster where as large dense core vesicles (LDCVs) which are present at the body terminal release slowly.

Quantal release of Neurotransmitters:

Neurotransmitters are stored in special membrane enclosed organelles called synaptic vesicles and packed as discrete packets called Quanta. At normal conditions a huge number of vesicles are released simultaneously leading to depolarization of the postsynaptic membrane and the generation of an action potential. Each vesicle contains approximately the same amount of neurotransmitters, since each quantum released produces approximately the same postsynaptic depolarization. The depolarizations are observed in small amounts of 0. 5mv and they are called Miniature end plate potentials. At central synapses one quantum is released on arrival of a single action potential, but with a probability of less than one.

Calcium ions involvement in transmitter release:

External calcium is essential for transmitter release and this calcium enters the nerve terminal through voltage gated calcium channels. The calcium involvement in transmitter release is found by various studies like

Freeze Fraction Studies

Omega Profile and

Cage Molecules

The active zone that is present at the pre-synaptic site contains the Calcium channels and the action potential release transmitter by depolarizing the pres-synaptic membrane and opening calcium channels. The rise in local calcium concentration makes the exocytosis of the docked vesicles with the plasma membrane and release of transmitter into the synaptic cleft. Calcium concentration adjacent to the calcium channels increase from resting level of

0. 2μM to steady state of about 400μM. The concentration at half maximal is 194μM which is a relatively low affinity and the maximal rate of secretion was high.

The active zone contains more than hundred calcium channels all channels do not open for single action potential but at such a site any single vesicle is docked by more than one calcium channel. At CNS synapses N and P/Q type of calcium channels appear to be predominant where as at neuromuscular junction P type channels are responsible for neurotransmitter release. The exocytose trigger must have fast, low affinity, cooperative calcium binding.

Excitation-Secretion coupling:

Calcium concentration is low intracelluraly and both the concentration and electrical gradients provides a strong driving force for calcium entry. Thus when a voltage gated Ca+2 channels open in response to the depolarization of the membrane by an action potential, there is a possibility for the intracellular calcium concentration to increase by large extent. This calcium acts at extremely short distances that is in nanometres in times of microseconds and at very high local concentration of nearly 100 μM.

Calcium dependent steps of Neurotransmitter release:

Synaptic vesicles are tethered to cytoskeletal proteins some distance from the active zone. Vesicle recruitment is a calcium dependent step which frees the vesicles and then moves to the active zone on the presynaptic membrane. Once the vesicle is released from cytoskeleton it binds to the presynaptic membrane a process called Docking. The next step is priming

which is an ATP dependent process and after this calcium stimulus in which there is a rapid fusion of the primed vesicles and exocytosis of the neurotransmitter. Every step requires different amounts of calcium and the final step requires very high local calcium concentration.

Anchored vesicle

Recruitment Ca+2 = 0. 5µM

Docking

Docked vesicle

ATP

Priming Ca+2 = 0. 3µM

ADP+Pi

Primed vesicle

Fusion Ca+2 > 100µM

Exocytosed vesicle

The diagram represents the various steps involved in neurotransmitter release.

Protein involvement in Transmitter release:

There is large number of proteins present on the vesicular membrane and these are involved in the neurotransmitter release and in neurotransmission

process. These proteins perform a general functions that are not restricted to a single class of transmitters. Transmitter release depends not only on the vesicular proteins but also on the proteins of the plasma membrane and cytoplasm. The various proteins involved in neurotransmission are depicted below.

Protein Function

Vesicular transmitter transporter Taking of transmitter into vesicles

Synaptotagmin Trigger for vesicle fusion and docking

Synaptobrevin Acts in a late step of vesicle fusion

Rab3 Regulating vesicle targeting and availability

Synapsin Tether vesicle to actin cytoskeleton

Syntaxin Essential for late step in fusion

NSF Disrupt complexes after exocytosis

The various proteins and their actions are outlined below SNARE complex: The three synaptic proteins Synaptobrevin or vesicular associated membrane protein, Syntaxin and Synaptosomal associated protein of 25KDa form tight 20S complex called as core complex or the SNARE receptor complex. These form a four stranded coiled coil. These coils make the fusion of the membranes of the vesicular membrane and the plasma membrane. These are mainly involved in docking and priming steps of vesicular release.

NSFprotein: N-Ethylmaleimide sensitive factor, an ATPase involved in membrane trafficking. NSF hexane bind a cofactor α-SNAP and this complex in turn binds to SNARE complex this leads to disassembly of the complex and this action of NSF might catalytically rearrange the SNARSEs so that the membranes were brought together.

Calcium binding proteins:

These proteins are candidates for coupling the action potential to exocytosis. Synaptotagmin an integral membrane protein of the synaptic vesicles contains two calcium binding C2 domains called C2A and C2B. These domains interact with SNARE complex proteins and with phospholipids in a calcium dependent manner. These interactions are the triggering events for fusion.

Synapsin:

The cytoskeleton to which vesicles attach contains actin and fodrin. Vesicles are attached to these actin and fodrin by proteins called synapsins. Synapsin binds to vesicles by interaction with the phospholipids and vesicle associated CaMK2 which allow the vesicles to move to the active zone.

Synaptophysin and Physophilin: A vesicular protein Synaptophysin and a plasmembrane protein Physophilin form a pore called fusion pore by their interaction and these fusion pores later expands to allow the release of vesicular contents.

Rab3A:

It is one of the cytosolic small G protein involved in neurotransmitters vesicle fusion and recycling by the help of GTP. It first binds to GTP and then to vesicles, which move the vesicles to the active site and after exocytosis GTP is hydrolysed to GDP and which results in recycling of vesicles.

Nurexins:

Nurexins are the family of brain specific proteins involved in neurotransmitter release.

Molecular basis of synaptic action:

Chemical synaptic transmission is one of the most important ways of communication from neuron to neuron and neuron to muscle. This transmission results in the carrying of impulses from the pre synaptic membrane to the post-synaptic membrane. At the post synaptic site the neurotransmitters binds to macro molecular substances called receptors. This receptor action results in opening of an or alter the concentration of intracellular metabolites. The response may be either excitatory or inhibitory. The magnitude of response depends on the state of the receptor and the amount of transmitter released. Type of receptors present on the post-synaptic site depends on the neurotransmitter. There are two main classes of receptors involved in neurotransmitter action.

They are

1. Ionotropic Receptor and

2. Metabotropic Receptors

1. Ionotropic Receptors:

Ionotropic receptors are multisubunit membrane bound protein complexes composed of proteins that combine to form an ion channel through the membrane. There are two distinct families of ionotropic receptors one consists of Ach, nAch, receptor for gamma-amino butyric acid, glycine receptors and 5HT3 receptors and the other class consists of many types of ionotropic glutamate receptors.

Its structure consists of 5 subunits designated as α, β, γ and δ which are about 290KDa. These subunits assemble to form a ring like structure enclosing a central pore. Each subunit at the outer portion form a funnel shaped extracellular domain with an intracellular diameter of 20-25A0 and also consists of intracellular domain. Each subunit of the receptor consists of four transmembrane spanning segments TM1-TM4. Each segment consists of hydrophobic amino acids which stabilizes the domain within the hydrophobic environment of the lipid membrane. It also consists of N and C terminals.

Structure of the channel pore determines ion selectivity and current flow. The amino acids which form the transmembrane-2 contain a negative charge and are oriented towards the central pore of the channel. This negative charge ensures passage of cations only with prefarability. The physical dimensions of the pore contribute greatly to the selectivity for particular ions. Cytoplasmic portion contains narrow openings made up of α-helical

rods which regulate the flow of ions. Thus these physical characteristics of the pore along with the electrochemical gradients determine the possibility of ionic movements.

TM2 segments are helical in shape and exhibits a kink in their structure which forces leucine residues from each segment such that it effectively blocks the flow of ions through the central pore of the receptors. When the transmitter binds to specific domains on the receptor causes rotation of the TM2 segments which results in the flow of ions.

2. Metabotropic receptors:

Metabotropic receptors are single polypeptides that exert effects not through opening of ion channels but through binding and activating GTP-binding proteins. So these receptors are also called as G-protein coupled receptors. The various receptors comes under this category are α, β-adrenergic, muscarnic, dopamine, GABAergic and glutaminergic.

Its structure consists of a single polypeptide with seven membrane spanning helical segments associating with 24 hydrophobic amino acids. In the centre of the seven membranes spanning segments a pocket is formed which provides the neurotransmitter binding sites. The N-terminal is towards extracellular where as C-terminal is towards cytoplasm.

GPCR activation causes the isomerisation of the receptors spontaneously between active and inactive states. Only the active state of the receptor interacts with G-proteins when the agonist binds and when there is absence of agonist the inactive state of the receptor is favoured. Activation of the receptor causes coupling of G-protein initiating the exchange of GDP for GTP.

This activated G-protein couples to many downstream effectors and alters the activity of intracellular enzymes or ion channels. These G-protein target enzymes produce diffusible second messengers that stimulate further downstream biochemical processes like activation of protein kinases.

Molecular basis of Synaptic Inactivation:

The action of the neurotransmitter in the synapse is terminated by two major mechanisms. They are

1. Diffusion and

2. Uptake processes

1. Diffusion process:

Simple diffusion is the main mechanism of rapidly reducing the concentration of neurotransmitter. The diffusion is mainly affected by the synaptic morphology like geometry of the cleft and adjacent spaces.

2. Uptake process:

Uptake of transmitter from the synaptic cleft is carried out by high affinity sodium dependent transporters. These transporters comes under two families

Na+ and K+ dependent glutamate transporters

Na+ and Cl- dependent transporters

These uptake transporters are inhibited by various uptake inhibitors. For example epinephrine is inhibited by methoxylated metabolites normetanephrine, metanephrine and phenoxybenzamine.

Vesicles are refilled by an antiport mechanism. Inside the vesicles there is high amount of protons produced by the activity of H+-ATPase. Neurotransmitters are transported into vesicles by the antiport of H+ out of the vesicles.

The other mechanisms by which synaptic inactivation occurs are enzymatic inactivation and antagonism. In enzymatic antagonism enzymes inactivate the neurotransmitter for example acetylcholine is inactivated by the enzyme acetyl cholinesterase in which it is cleaved to acetyl and choline groups such that its activity is inhibited and in case of antagonism various drugs and other substances inactivate the neurotransmitter by blocking the receptor on which the neurotransmitter.

Conclusion:

So, I summarise from my essay that in the case of neurotransmitter release from the vesicles, mainly the molecules involve are calcium and specific proteins and in the case of synaptic action of neurotransmitters ionotropic and metabotropic receptors plays an important molecular role and finally in the case of synaptic inactivation of neurotransmitters diffusion, uptake process, metabolism and antagonism form a molecular basis.