NEUROCHEMISTRY, THE MOLECULAR BASIS FOR NEUROTRANSMISSION

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Contents

Summary

The nervous system controls all our functions and movements. Activity of the nervous system depends on a mixture of chemical and electrical signals that moves between neurons and between neurons and effector cells. In the present chapter we have concentrated on the chemical signals. In this chapter the reader will be introduced to the properties of these chemical signals, their synthesis and how they conduct their function. The molecular background for some neurological diseases is included. By taking one region of the brain which has been thoroughly investigated, the basal ganglia, it is possible to give an example how the different chemical signals are integrated. Learning and memory are important functions in the brain. We are still early in our understanding of this phenomenon, but some molecular and anatomical basis how to understand this is included.

1. **Introduction**

Neurochemistry can be defined as the chemistry of the nervous tissue. The main emphasis of the subject has been to describe the molecular basis of the function and dysfunction of the nervous tissue. Neurotransmission, the signal transfer in the brain, may be regarded as the main task of the nervous system. A major part of this chapter will deal with the biochemical basis of this information transfer. Dysfunction of the nervous system may throw some light on the function of cell groups and neurotransmitters, and the molecular mechanisms for some of the well-known neurological dysfunctions are therefore discussed.

2. The Structural Elements in the Nervous Tissue

The nervous tissue is separated into the central and the peripheral nervous system. The peripheral nervous system connects all the functions of the peripheral organs. The nervous tissue consists of several different cell types and subcellular structures that contribute in different ways to its function. The central nervous system is very vulnerable because the most important cells, the neurons, do only to a very limited extent regenerate after lesion. It is therefore important that it is protected from toxic substances in the blood stream. The transport of chemical substances from the blood into the brain is restricted by the so-called Blood Brain Barrier (BBB). This barrier is formed by endothelial cells which are linked together by tight junctions and hinders the penetration of large and small solutes (See figure 1). This barrier is different from that of other parts of the body in that endothelial cells around the blood vessels in other parts are not tightly linked. Only lipid soluble compounds and water can diffuse across the BBB. Important nutrients such as glucose, is transported across the BBB by a glucose carrier named GLUT-1. There also exist carrier systems for other essential nutrients such as lactate and essential amino acids such as the aromatic and long chain amino acids. In this respect it is important to note that DOPA, an important remedy for Parkinson's disease is actively transported into the brain. In contrast, the transmitter amino acids glutamate, GABA and glycine are poorly transported and must be synthesized locally in the brain. This barrier plays an important role in regulating the entry of pharmaceutical and of toxic compounds into the brain.

There are approximately 100 billion neurons in the brain and these can be regarded as the most important cell type in the nervous tissue. The neuronal cells consist of 4 parts namely dendrites, cell body, axon and the nerve terminal (See figure 2). The neurons take up many forms and shapes ranging from the globular form of cerebellar granule cells, the star shaped cells in the anterior horn and the Purkinje cells with its beautiful tree of dendrites. The cell bodies range in size from 6 to 80 μm. The prototypical picture of a neuron is a cell body with a dendritic tree emerging from one end and a fine net of axons at the other end. The dendrites form a net of connections with nerve terminals from other neurons. The degree of the branching of the dendrites can be regarded as a measure of its importance.

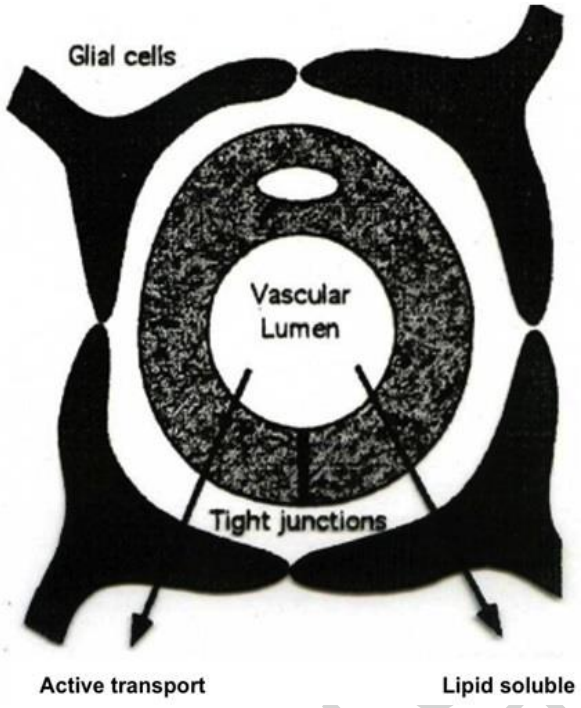

Figure 1. Shows a drawing of the Blood Brain Barrier. Notice that the endothelial cells form a tight junction and that they are surrounded by the endfeet of glial cells.

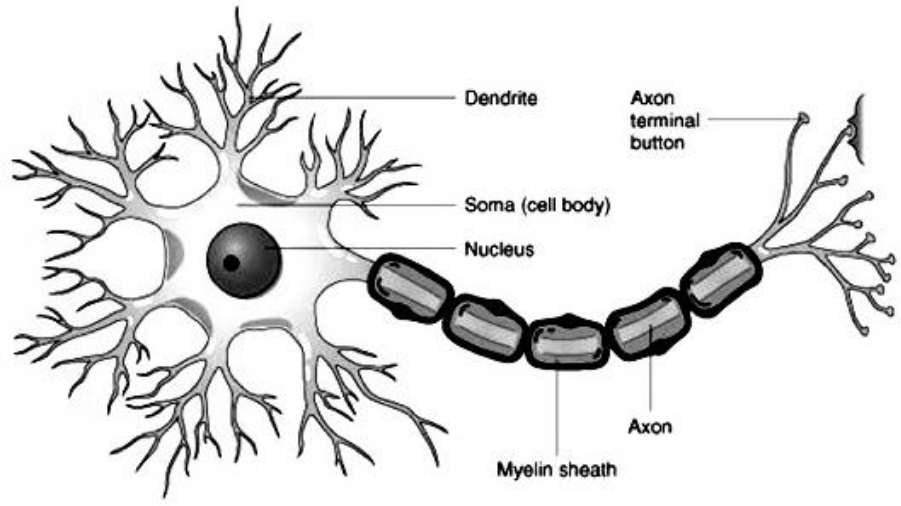

Figure 2. Shows a neuron with dendrites, cell body, axon surrounded by oligodendroglia (myelin) and nerve terminals

The cell bodies contain the DNA and all protein synthesis of the cell occurs there. The axons are surrounded by oligodendroglia, which form an insulating layer. In between the adjacent oligodendroglia are the nodes of Ranvier, which are packed with sodium channels and through which the electrical impulse is rapidly regenerated and transported down the axon. In diseases such as multiple scelrosis, this insulating layer has disappeared and the consequence is that the electrical impulse travel with a lower speed. Since protein synthesis only takes place in the cell bodies, the proteins must be

transported to the dendrites and the nerve terminals. The axon is therefore packed with neurotubules and neurofilaments, which are involved in the transport of proteins to and from the terminals. The transport takes place at three different rates. The cytoplasmic constituents including the cytoskeletal elements move at a slow rate of 2-4 mm pr day. The cytoskeletal components are transported in their polymeric form. The slow transport occurs only in the anterograde direction from the cell body to the terminal. The mitochondria are believed to be transported at an intermediate rate of 10-20 mm pr day. The vesicular organelles are transported by the so called fast transport of the order of a few 100 mm pr day. This transport also involves constituents of organelles to the axolemma such as Ranviers nodes on the axons. Whereas the driving force for the slow axonal transport is unknown, the anterograde fast transport is driven by members of the family of motorprotein called kinesin. The kinesins are long rod shaped proteins which are bound to microtubules by adenyl-iminodiphosphate, a nonhydrolysable analogue of ATP. In neurons kinesins are associated with a variety of membrane-bound organelles such as mitochondria, lysosomes and synaptic vesicles. The variation in transport rate seems to depend on the fact that some particles such as the mitochondria drops off during the transport down the axon. There is also a retrograde transport of particles from the nerve terminal back to the cell body. This retrograde transport is driven by dynein, another important motorprotein which is also associated with microtubules.

The axon ends in a so-called nerve terminal. The nerve terminals are characterized by having synaptic vesicles of several different types. Firstly, there are small and large vesicles. The small vesicles store the so-called classical neurotransmitters whereas the large vesicles store the neuropeptides. The small vesicles can be subdivided into clear round, clear elliptical and round granular vesicles. The different vesicle types can to some extent be used to differentiate whether the terminal is involved in excitatory, inhibitory or modulatory transmission. When an electric impulse reaches the terminal, neurotransmitters are released from synaptic vesicles into the synaptic cleft. The terminal forms a specialized junctional complex known as the synapse. The vesicles are gathered in large numbers in front of this junction. EG Gray suggested to separate synapses in the cerebral cortex into two different types. Type 1 synapse is axodendritic, has membranes that are closely apposed for a long distance, has a large amount of postsynaptic densities and is excitatory. Type 2 is axosomatic, shows less apposing membranes, has smaller postsynaptic densities and is inhibitory.

The other important cell type in the brain is the neuroglia. These cells do not have synaptic contacts and retain the ability to divide throughout life, particularly in response to injury. There are three forms of neuroglia cells namely astrocytes, oligodendrocytes and microglia. The astrocytes sustain a packing around the neurons. They may be involved in maintaining the correct pH and the ionic environment around the neurons. There are some suggestions today that the astrocytes may release transmitters. During injury they proliferate, swell and accumulate glycogen and increase their content of glial fibrillary protein. In case of injury they take over the area where neurons are lost and leave a glial scar. The astrocytes are also involved in the BBB, where their endfeet surround the epithelial cells. It is, however, not known whether they really contribute to the impenetrability of the BBB. To study the contributions from astrocytes, it is possible to inactivate the cells. Fluorocitrate, an environmental toxic compound, which inhibit the enzyme aconitase has been used for this purpose in a series of experiment. The cell

specificity of fluorocitrate depends on the fact that it is transported intoastrocytes and not into neurons.

Oligodendrocytes play an important role in myelination both in the PNS and CNS. In the PNS they are called Schwann cells and are the axon-ensheathing cells of PNS. Along the myelinated fibers of PNS each internode of myelin corresponds to one Schwann cell in contrast to the CNS where one oligodendrocyte is able to proliferate to a large number of internodes. Another distinction is that Schwann cells always remain in intimate contact with its myelin internode, whereas the oligodendrocytes extend processes towards it internodes. In addition the Schwann cells differ biochemically and immunologically from the oligodendrocytes. The oligodendrocytes are slow to respond to injury, whereas the Schwann cells are able to phagocytose damaged myelin and are able to restore lost myelin.

The microglial cells represent the immune system of the nervous system. They have a role in the phagocytosis and inflammation. In the normal state they are resting, but during injury they become very active. The microglia cells produce a large number of proinflammatory cytokines with known effects on T-cells.

3. Neurotransmission

The transfer of impulses between neurons or between neurons and effector cells occur mainly by chemical substances, called neurotransmitters. This transfer takes place at the synapse. During the last 60 years there has been a continuous search for identification and characterization of the neurotransmitters.

The best known transmitters today are glutamate, gamma-aminobutyrate (GABA), glycine, acetylcholine, the biogenic amines 5-hydroxytryptamine (serotonin), dopamine, adrenaline, noradrenaline and histamine as well as purines. In addition there are a number of neuropeptides such as enkephalins, substance P and neuropeptide Y, which may function as neurotransmitters. In the brain it is the amino acids glutamate and GABA which dominate quantitatively whereas in the PNS acetylcholine and noradrenaline dominate.

There are several criteria that must be fulfilled before a compound can be defined as a neurotransmitter:

1. The neurotransmitter should be synthesized in the nerve terminal. In the case of peptides they are synthesized in the form of their precursors in the cell body and transported by axonal flow into the terminal where they are modified to their active form.
2. The neurotransmitter should be released in a calcium dependent manner. Neurotransmitters are released after transport of calcium ions into the nerve terminal.
3. The neurotransmitter candidates should reproduce the specific events of the naturally occurring transmitter. This includes reproducing membrane changes, ionic conductance and reversible potential. It should also be blocked by known antagonist in the same manner as the natural transmitter.

4. The neurotransmitter should be removed from the receptor to terminate its action. This may take place by enzymatic hydrolysis as for acetylcholine or by transport of the transmitter back to the terminal or into a neighboring cell structure.
5. Although it was not one of the original criteria, transmitters should be stored in and released from the synaptic vesicles.

When a neuron receives a signal through its receptor, the signal is transferred as an electrical impulse through its dendrite and down the axon until it reaches the nerve terminal. At the nerve terminal this leads to depolarization of the plasma membrane, which leads to the entrance of calcium ions through voltage sensitive calcium channels. This in turn activates a protein or a series of proteins, which makes a vesicle fuse with the preterminal membrane and release its transmitter content into the synaptic cleft. The vesicles localized close to the presynaptic densities of the plasma membrane are in a favorable position to release neurotransmitters. They contain the immediately available pool of transmitter for release. The release process is called synaptic vesicle exocytosis. The membrane fusion mediating synaptic exocytosis and other intracellular membrane traffic is affected by a universal machinery that includes SNARE (for "soluble NSF-attachment protein receptor") and SM (for "Sec1/Munc18-like") proteins. During fusion, vesicular and target SNARE proteins assemble and forces the two membranes tightly together. After fusion, SNARE complexes are dissociated by the ATPase NSF (for "N-ethylmaleimide sensitive factor"). Fusion-competent conformations of SNARE proteins are maintained by chaperone complexes. The synaptic membrane-fusion machinery is controlled by the Ca-receptor protein called synaptotagmin, and additionally regulated by a presynaptic protein matrix (the "active zone") that includes Munc13 and RIM proteins as central components.

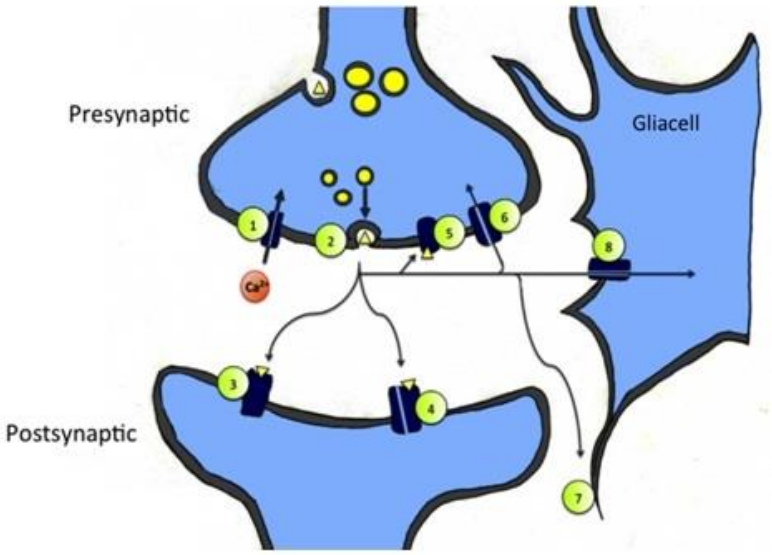

Figure 3. Shows a nerve terminal with vesicles and receptors. 1 entry of Ca ions; 2 vesicle exocytosis; 3 metabotropic receptor; 4 ionophore receptor; 5 presynaptic receptor; 6 plasma membrane transporter on nerve terminal; 7 diffusion of transmitter; 8 plasma membrane transporter on glial cell

Botulinum and tetanus toxins both contain protease activities, which prevent the vesicle exocytosis, and have thrown some light on the release process. Both toxins consist of a large and a small peptide that are linked together by an S-S bridge. The large peptide recognizes the cell structure to be attacked. The two peptides are split after being taken up into the terminal. The light chain has a zinc-dependent endoprotease. There are several forms of botulinum toxin and their endoproteases destroy different proteins involved in the exocytosis. These proteins are part of the SNARE complex which include SNAP-25, VAMP and syntaxin. The two toxins prevent therefore the binding between the vesicle and the plasma membrane and no vesicular exocytosis nor neurotransmission can occur. Botulinum toxin attacks the neuromuscular endplate whereas tetanus toxin attacks inhibitory GABAergic and glyciergic neurons in the spinal cord.

Previously one adhered to the so-called Dale's principle that each neuron used one type of neurotransmitter only. With the discovery that neuropeptides could also act as neurotransmitters, it was realized that a neuron could contain a classical neurotransmitter and a neuropeptide acting as a transmitter. Today we also see that several neurons contain several different neurotransmitters, although only to a small extent are we able to explain how they interact.

4. Membrane Transport

Nutrients and other compounds are actively transported across the plasma membrane in neurons. This transport is dependent on the Na,K-ATP pump. This pump also helps to maintain the ion gradients produced voltages that drive the electrical signals in the neurons across the membranes. There are several Na, K and Ca-channels in the cells.

The transmitter amino acids and amines are transported back into the nerve terminals and into glial cells from the synaptic cleft by transporters located on the plasma membrane. The transporters belong to the solute carrier protein family (SCL). GABA, glycine, catecholamines, serotonin and histamine are all transported by the SLC6 group. They depend on Na,K-ATPase and are inhibited by ouabain. Na ion is transported the same way at the same time as the transmitter and chloride is also co-transported with the transmitter. These transporters are found in high densities in the synaptic gap, but high concentrations may also be found on small endocytotic particles in the terminal.

Similarly the glutamate transporters belong to the group SCL1. Each transporter contains an ionophoric site which binds glutamate and 3 Na ions and one H ion, K ion is transported in the opposite direction. The 5 different glutamate transporters belong to 5 different genes and they have different cellular location. In the glutamate synapse the astroglial transporters play an important role in transmitter uptake and are localized close to the synaptic gap. In contrast, released ACh is not transported back into the nerve terminal, but it is hydrolyzed to choline.. The choline transporter belongs to the SLC5 group. The transporter is located on presynaptic vesicles in the nerve terminal. On release of ACh the vesicles fuse with the presynaptic membrane and thus the choline transporter becomes available to transport choline into the terminal.

The transport systems which pump transmitters into the synaptic vesicles are quite different from the plasma membrane transporters. They are more closely related to bacterial transporters. The vesicular transporters are coupled to an H ion electrochemical gradient (ΔpH) provided by a Mg- ATPase (called the proton pump) whereas the plasma membrane transporters are coupled to Na ions running down the electrochemical gradient. The Mg-ATPase is inhibited by bafilomycine and not by ouabain. There are several different vesicular transporters including one vesicle transporter called VMAT for the catecholamines, serotonin and histamine. There is one common transporter (VIAAT) for the inhibitory amino acids GABA and glycine. There are three distinct transporters for glutamate (VGLUT) and one for Ach (VACh). The transporters depend to different extend on the two component (ΔpH and $\Delta\psi$) of the electrochemical gradient. In the case of the amine and ACh transport ΔpH is more important than $\Delta\psi$, for the inhibitory transmitters they are equally important and for glutamate $\Delta\psi$ is more important than ΔpH.

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Biographical Sketches

Frode Fonnum received his BSc. (Honours) at Heriot Watt University in Edinburgh in 1960 and his Dr.Philos at University of Oslo in 1970. In 1965/66 he worked at Institute of animal physiology, Babraham, Cambridge. In 1971/72 he attended NATO Defence College. He was then appointed as research scientist1961 and from 1971-2004 as research director at division of Toxicology at Norwegian Defence Research Establishment. In 1984 he also became professor of toxicology at University of Oslo. During 1996-2000 he was also VISTA professor (appointed by Norwegian Academy of Sciences and Statoil Co). Since 2004 he has been professor emeritus at University of Oslo. He has been honorary secretary (1987-91) and chairman (91-93) of International Society of Neurochemistry. In 2003 he was awarded the PE Poulsson Award for his contributions in toxicology. He was one of the 10 founding fathers of European Soc of Neuroscience. He has been member and chairman of several research committees in NATO and in Norwegian Research Council. He has been deputy chief editor of J Neurochemistry and member of the editorial board of Brain Research, Neurotoxicology, Environmental Toxicology and Pharmacology. His research projects have been on neurotransmitters in the brain and on the effects of environmental substances on the nervous system.