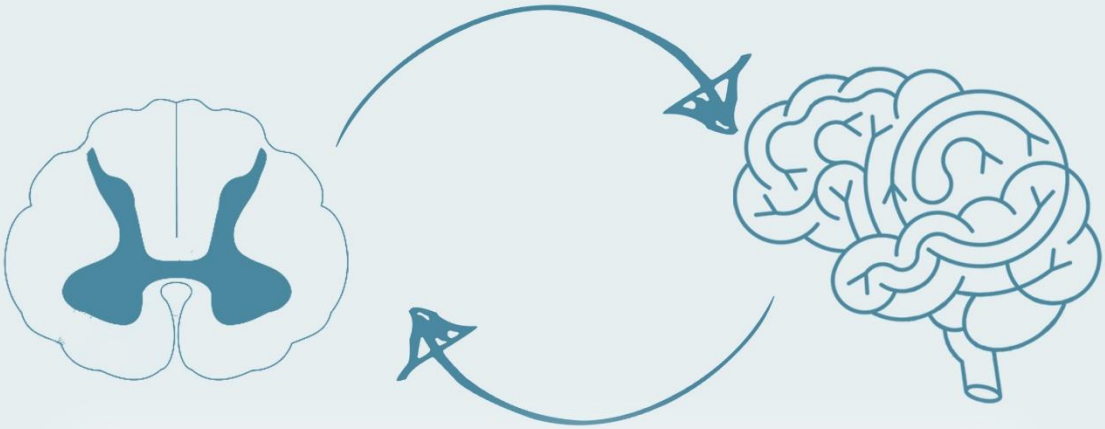

CNS

Biochemistry

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number	1
Done by	Karam Darwish
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Neurotransmitters

Note: anything written with the italic font and present between brackets is from the slides.

A neurotransmitter is a chemical substance that is synthesized in a neuron (all the enzymes needed for the synthesis must be present in the neuron), released in a synaptic cleft following depolarization of the nerve terminal (usually dependent on influx of Calcium ions), then binds to a specific receptor to elicit a specific response and then removed or inactivated to stop its action.

Types of neurotransmitters:-

- Small-molecules
  Could be an amino acid, a derivative of an amino acid or an amine.
- Neuropeptide
  More than one amino acid linked by a peptide bond.
- Gases
  Nitric Oxide is the only example.

Each neuron synthesizes one or more neurotransmitter and is often identified by its neurotransmitter; Cholinergic tracts synthesize and release Acetyl-Choline while Dopaminergic ones use dopamine.

And as you already know about the synthesis of catecholamines, Dopamine is first synthesized then transformed to Norepinephrine then to Epinephrine as a chain of reactions; so a dopaminergic neuron has the enzymes needed to reach dopamine only, while the one which uses epinephrine has the other enzymes needed to complete the chain.

Sometimes more than one neurotransmitter can coexist in one neuron and usually one is a small molecule and the other is a neuropeptide but also

combinations of the same type can exist (*e.g., most spinal motor neurons contain acetylcholine and calcitonin gene-related peptide*).

We said that the neurotransmitter, after releasing, binds to a specific receptor to elicit a specific response; this response can be either excitatory or inhibitory. However, the same neurotransmitter can sometimes elicit an excitatory response and sometimes an inhibitory one; which means that the response doesn't depend on the chemical nature of the neurotransmitter but on the type of the receptor it binds to (*Depends on the type of receptor being activated and the ion species that becomes more permeable*.).

Note*: (The differential release of the various neurotransmitters is the result of the neuron altering its frequency and pattern of firing)*.

Neuropeptides

More than 50 neuropeptides has been described and they affect many functions in the CNS like appetite, sleep, mood and many others. And the main functional difference between them and the small molecules is their longer and delayed action.

Note: (*Usually mediate slow, ongoing brain functions)*.

Neuropeptides in general can act either as neurohormones or as a neurotransmitter. A hormone is chemical substance secreted from a cell then transported by blood to affect a distant target while a neurotransmitter's effect is more local to an anatomically specialized junction.

- *(Neurohormones: a messenger that is released by neurons into the haemolymph and exert its effects on distant peripheral targets, e.g (TSH, GH)).*

- *(Neurotransmitter: a messenger released from a neuron at an anatomically specialized junction, which diffuses across a narrow cleft to affect one or sometimes two postsynaptic neurons, a muscle cell, or another effector cell).*

Neuropeptides are grouped into families which are:

- Tachykinins: substance P, substance K, *bombesin*…
- Insulins: insulin, insulin-like growth factors.
- Somatostatins: somatostatin, pancreatic polypeptide.
- Gastrins: *gastrin, cholecystokinin*.
- Opioids: opiocortins, enkephalins, dynorphin.
  Refer to slide number 12.

Neuropeptides' stages of action:

1) Synthesis (*ER and Golgi apparatus*)
   it is a peptide, which means it is encoded in the DNA → then by transcription mRNA is synthesized → then translated in the ribosomes of the rough ER → then modified by Golgi apparatus.
2) Packaging into vesicle (*large-dense core vesicles*)
   these vesicle contain usually the precursor of the neuropeptide and the enzymes needed for further modifications.
3) Transport
   fast axonal transport on the microtubules to reach the terminals, and during the transport the modifying enzymes (e.g.: proteases) cleave the precursors into the mature form.
4) Release
   mostly associated with calcium influx that triggers the fusion of the vesicle with the presynaptic membrane (*They are released gradually over time in response to general increases in the level of intracellular calcium*.).
5) Action (*prolonged*)
   by binding to their receptors.
6) Termination
   by diffusion and degradation (no reuptake).

** In general neurotransmitters' action is terminated by:

- Degradation by enzymes.
- Reuptake back to presynaptic membrane.
- Diffusion away from the synapse.

- Uptake by other adjacent cells (glial cells).

As we have already said, neuropeptides are encoded by genes then by transcription and translation we synthesize the peptide. Sometimes, we can synthesize different types of neuropeptides from the same gene; this diversity originates from different mechanisms such as alternative splicing.
For example, if there is a gene containing several exons, and then by transcription and translation for all exons we will have a certain peptide. If we remove one exon out, and transcription and translation happened to the remaining exons only, we will have a different peptide and so on.

So, Alternative splicing of mRNA leads to translation of distinct precursors, and subsequent processing leads to unique mature peptides.

Example is the substance P mRNA that normally also includes mRNA encoding substance K.

Diversity is also provided by proteolytic, differential processing; if I had a long peptide and I cut the first 10 amino acids I will have a certain peptide and if I cut the last 10 I will have a totally different peptide. This can happen before packaging or after it by putting the enzymes required for a specific cleavage in the vesicle to yield a specific peptide in a certain neuron

Refer to slide number 16.

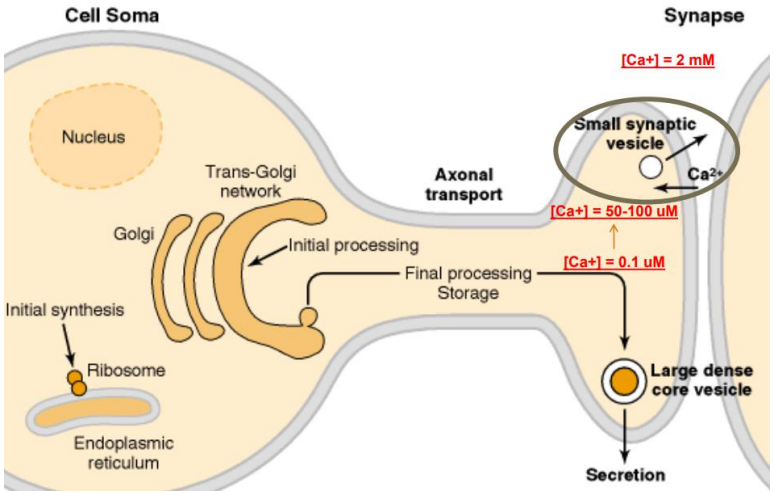
Neuropeptides expression can be regulated by many levels to increases or decreases their amounts; in the nucleus, the presence of transcription factors and activators increases the expression of the neuropeptides and the enzymes into mRNA while some inhibitors decrease it. Also the translation in ribosomes and the modification in Golgi apparatus and in the vesicles can be controlled and also the release is dependent on the stimulus which causes an influx of calcium, and by regulating each step we regulate the amount of neuropeptides expressed or secreted.

Small-molecule neurotransmitter

They are Nitrogen containing molecules and are either amino acids, derivatives of them or amines that are intermediates of Krebs cycle or glycolysis.

Stages of action for small molecules (in general):-

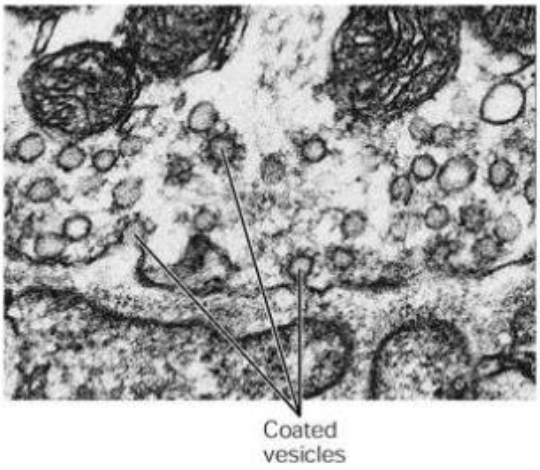
1) Synthesis of the <u>enzymes</u> needed for their synthesis
   - They are not encoded in the DNA as they are small molecules not peptides but their enzymes are proteins so they are the ones encoded
2) Packaging of the enzymes into vesicles
3) Transport of the vesicles containing the enzymes to the terminals (*slow and fast axonal transport*).
   -(*Synthesis in pre-synaptic terminal*).
4) Providing the vesicles with the precursors on which the enzymes work in the presynaptic terminals to synthesize the neurotransmitter (Packaging in synaptic vesicles).
5) Release after an impulse triggers calcium influx *(They are released in brief pulses each time an action potential triggers the infulx of calcium*).
   *Role of calcium:



Note that calcium concentrations are higher in the cleft compared to the terminals, when there is a stimulus there will be an influx of calcium which raise the calcium concentration inside → this calcium binds to fusion proteins such as SNARE proteins present on the vesicles and the

presynaptic membrane inducing structural changes that bring the membranes into closer proximity which enhances their fusion.

After the fusion, the presynaptic membrane is expanded as we've added the vesicle's membrane to it. To readjust its size a process of endocytosis (endocytic budding) occurs to take this extra membrane back to the cytosol, this process is aided by clathrin coating.



Refer to slides 21-24.

6) Action (short)
   they have a faster action compared to neurotransmitters
7) Termination by diffusion, re-uptake, or inactivation

Differences between neuropeptides and small molecule neurotransmitters:

Neurotransmitter	**Neuropeptides**	**Small-molecule**
Onset and duration of action	Delayed and long	Rapid and short
Synthesis	Peptides and enzymes are expressed from the DNA	Enzymes are expressed and provided by the precursors in the terminals
Site of synthesis	In the rough ER	In the terminals
Fate	by diffusion or inactivation (no re-uptake)	Diffusion, re-uptake, or inactivation

(They differ in: -Onset and duration of action - Synthesis, transport, and packaging - Concentration for action and receptor binding - Concentration of [Ca+] for release - Site of synthesis, modification – Fate).

Synthesis of neurotransmitters:

-(Most are synthesized from amino acids, intermediates of glycolysis and the TCA cycle, and O2 in the cytoplasm of the presynaptic terminal).

- (The rate of synthesis is generally regulated to correspond to the rate of firing of the neuron).

Examples of small molecule neurotransmitters:

-Tyrosine-Derived Neurotransmitters

Also called Catecholamines and include: Dopamine, Epinephrine and Norepinephrine.

They contain a Catechol ring which is Benzene ring with two hydroxyl groups attached to adjacent carbons, so they should be derived from tyrosine which has a benzene ring with one hydroxyl group so one step must by a hydroxylation process. They are also amines that contain amino groups without the carboxyl group as for the amino acids so another step must be a decarboxylation on the amino acid to transform it into an amine.

These reactions require different cofactors to occur: A decarboxylation process requires Pyridoxal Phosphate (**PLP**) (Vitamin B6), a hydroxylation requires Tetrahydrobiopterin (**BH4**) while a methylation requires S-adenosylmethionine (**SAM**) as a methyl donor.

Synthesis:-

First, we start with a Tyrosine amino acid which is a non-essential amino acid meaning that it could be synthesized in the body from a Phenylalanine amino acid (which contains a benzene ring) by Phenylalanine hydroxylase. Also, it could be obtained from the diet.
** PKU (Phenylketonuria) patients have a deficiency in this enzyme

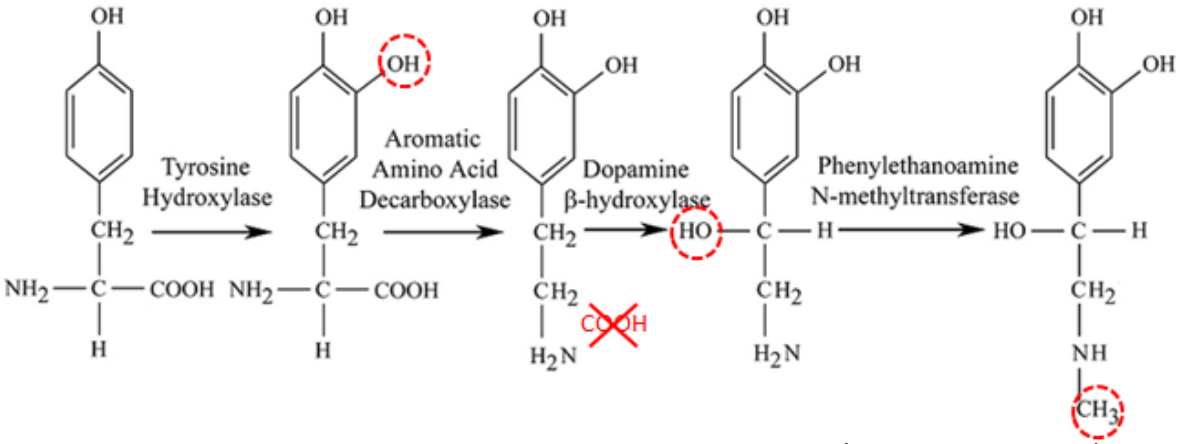
➔ A hydroxylation process occurs on the Tyrosine transforming it into DOPA
** This is the rate limiting step in this pathway and it happens in the cytosol
** It require BH4

➔ A decarboxylation process on DOPA to be transformed into an amine which is Dopamine
** Also happens in the cytosol
** Requires PLP

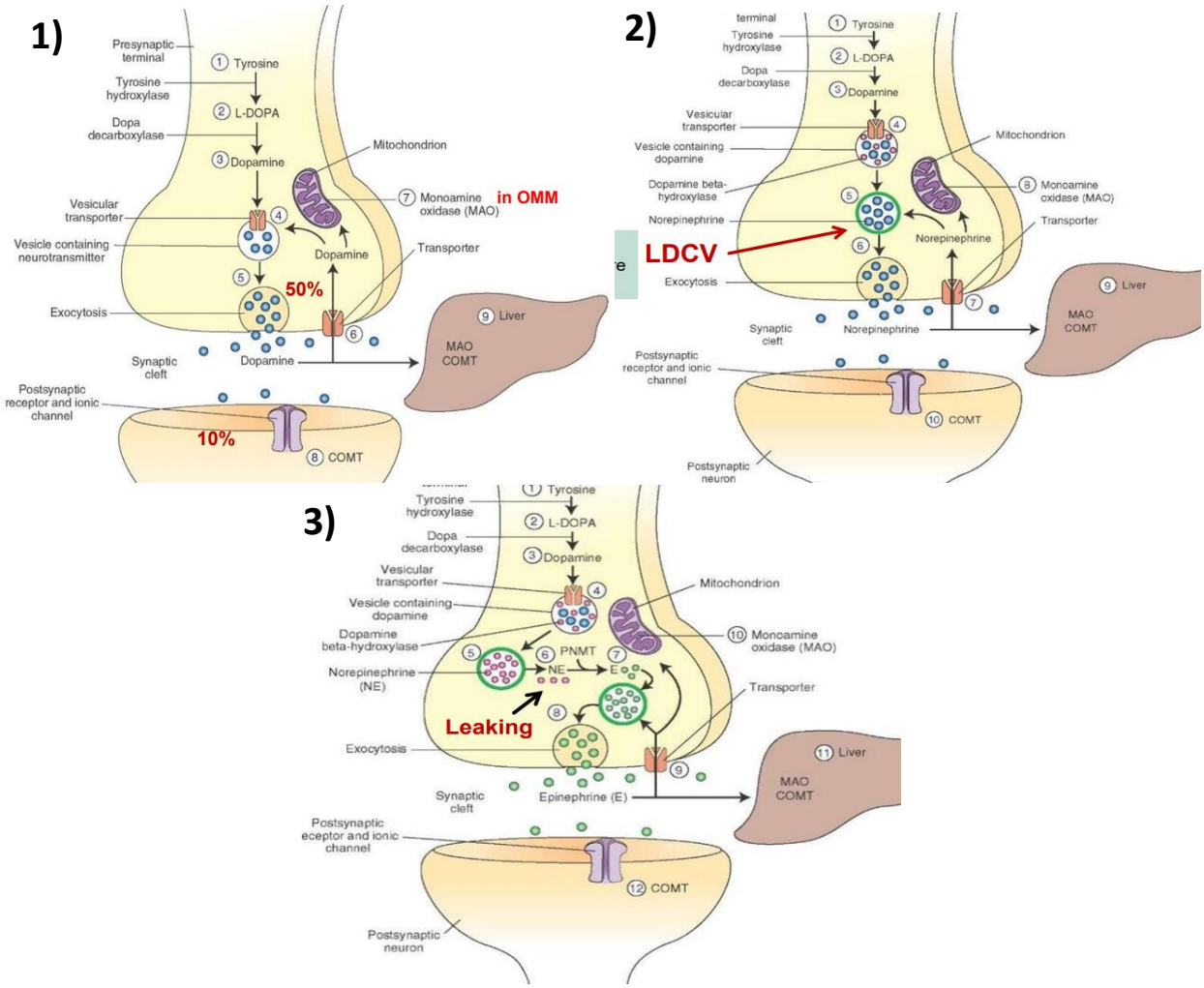
➔ This Dopamine is packages into vesicles which have the enzymes needed to complete the pathway

➔ Another hydroxylation process occurs on Dopamine by Dopamine β-hydroxylase transforming it into Norepinephrine
** Inside the vesicle

➔ A methylation process by a transferase transforms Norepinephrine Epinephrine
** In the Cytosol again
** Requires SAM and vitamin B12 or Folate



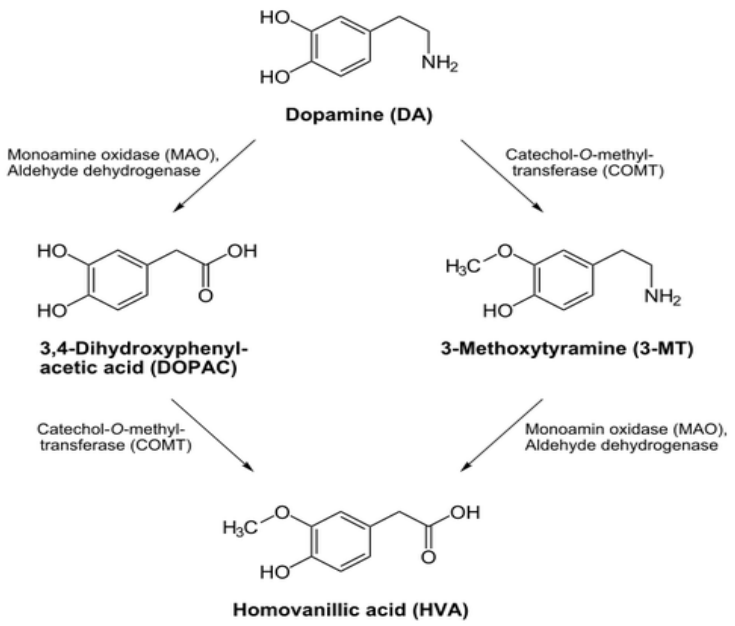
Tyrosine DOPA Dopamine Norepinephrine Epinephrine



- In the first neuron there are only the enzymes required to reach dopamine, and all the process occur in the cytosol but then it must be packaged into vesicles for transport
- In the second one, the enzyme Dopamine B hydroxylase is present in the vesicle to produce Norepinephrine
- In the last one, after the synthesis of NE it leaks out of the vesicle then the methylation occurs transforming it into Epinephrine

The transport of catecholamines (specifically Dopamine and Epinephrine) into vesicles by a secondary active transport process linked to a proton pump (*ATP-dependent process*); the protons are pumped inside the vesicle by an ATPase (*vesicular ATPase (V-ATPase*)), then we use this gradient to transport catecholamines inside and the protons out again by the transporter VMAT 2 (vesicle monoamine transporter2).

They all share the same fate; their action is terminated either by re-uptake (like other small molecules …. Up to 50 % by this way) or degraded in post synaptic cell (10%) or in the liver by the action of **COMT** (catechol O methyl transferase) AND **MAO** (Monoamine oxidase).



Note that either COMT work first and the MAO continue or vice versa, but in both situations, we reach the same final product which is Homovanillic acid HVA.
** COMT requires a SAM molecule and B12 or folate as it is a methyl transferase.
** In Parkinson's disease HVA level decreases because they have less Dopamine and thus less degradation and HVA.

Regulation of the synthesis pathway mainly occurs at the rate limiting step's enzyme (Tyrosine hydroxylase) and is either short term or long term:
- short term: when there are free cytosolic catechol amines that inhibit this enzyme by competition with the cofactor on the binding site, while depolarization causes activation of this enzyme by activation of several kinases (*Tight binding of the enzyme to BH4 following phosphorylation by PKA, CAM kinases, PKC*).
- long term: (also affects Dopamine β-hydroxylase) it happens by an increase of synthesis of the two hydroxylases in the pathway increasing the synthesis.

- (*Alterations (increase) in the enzyme amounts when sympathetic neuronal activity is increased for a prolonged period).*

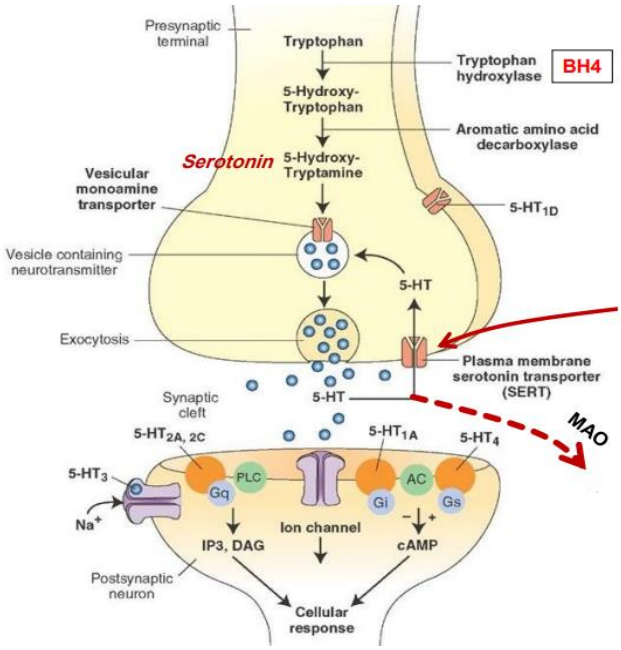
-Tryptophan-Derived Neurotransmitters

Serotonin

Synthesis:-

First we start with Tryptophan which is an essential amino acid obtained from the diet only → A hydroxylation process occurs transforming it into 5-Hydroxytryptophan → A decarboxylation process occurs transforming it into 5- Hydroxytryptamine (Serotonin) → Serotonin is then packaged inside a vesicle

**Remember that hydroxylation requires BH4 while decarboxylation requires PLP.



Action is mediated by different types of receptors on the postsynaptic membrane that trigger several pathways and second messenger.

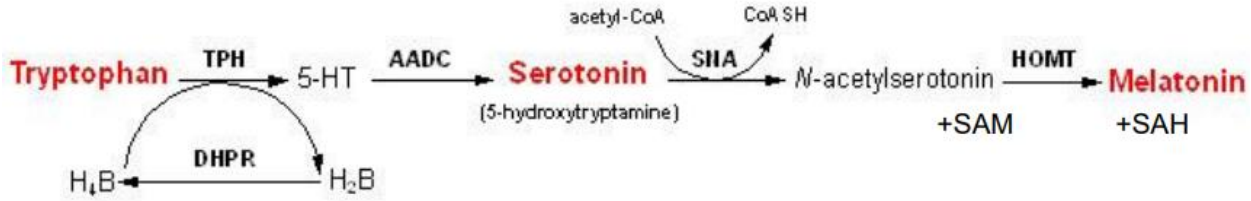
Action is terminated by reuptake by a transporter called SERT, or degraded by MAO producing 5-hydroxyindoleacetic acid that is excreted by the urine.

**Some drugs such as some antidepressant (like Prozac) work on the reuptake process inhibiting it and resulting in prolonged serotonin presence in the synaptic cleft and thus prolonged action to fight depression.
*serotonin does not cross BBB.

Melatonin

It is synthesized from serotonin by acetylation and then methylation (using SAM) and is used for regulation or circadian rhythm and sleep patterns.

- *(Serotonin synthesized in the pineal gland serves as a precursor for the synthesis of melatonin, which is a neurohormone involved in regulating: × sleep patterns × Seasonal and circadian (daily) rhythms × Dark-light cycle).*



- Glutamate and aspartate

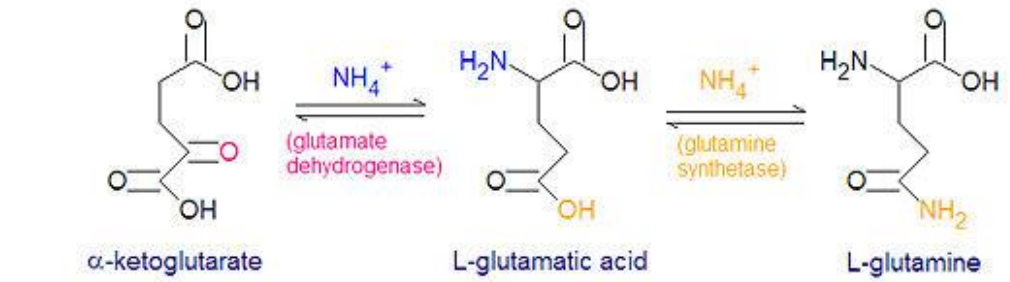
They are non-essential amino acids (amino acids not derivative of them) and they are excitatory neurotransmitters.

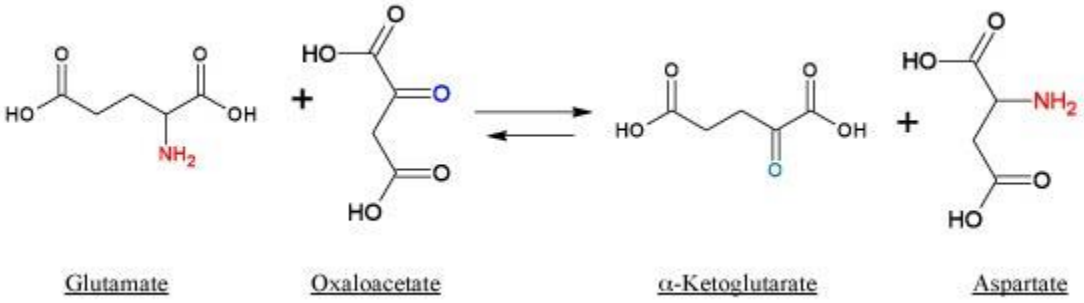
- *(Main synthetic compartments: -neurons - glial cells)*

They don't cross the BBB and must be synthesized in the neuron or glial cells from glucose *(must be synthesized in neurons de novo from glucose rather than taken up from the blood*).

Synthesis of **Glutamate (sources)**:-

- Glycolysis (production of Pyruvate then transformed to acetyl coA) → Krebs cycle (Acetyl coA transformed into α-ketoglutarate) → dehydrogenation of α- ketoglutarate.
  * we didn't mention beta oxidation of fatty acids although it produces Acetyl coA because they don't cross the BBB while glucose does.
- Deamination reaction on Glutamine by Glutaminase.
- Transamination reaction taking amino group from Aspartate and putting it on α- ketoglutarate producing Glutamate.





**Notice here that by transamination we can use Aspartate to make Glutamate or use Glutamate to make Aspartate.

It is stored in vesicles, and its release is Ca2-dependent fashion.

Action is terminated by an uptake process and transformed into Glutamine; the transporters that aid its reuptake include excitatory amino acid carrier-1 (EAAC1), glutamate transporter-1 (GLT-1) and glutamate—aspartate transporter (GLAST). *(Removal by high-affinity uptake systems in nerve terminals and glial cells).*

Aspartate:-

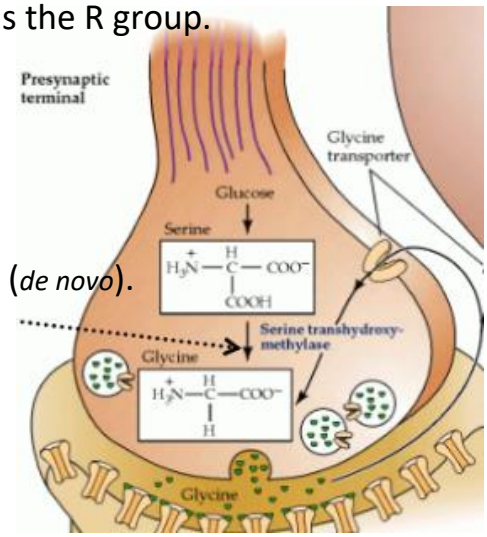
Synthesized by a transamination reaction by transporting an amino group from Glutamate to oxaloacetate as mentioned above.

Its function as a neurotransmitter is debatable and not well demonstrated.

-Glycine

The smallest amino acids having only hydrogen as the R group.

It is an inhibitory neurotransmitter. (*The major inhibitory neurotransmitter in the spinal cord*).



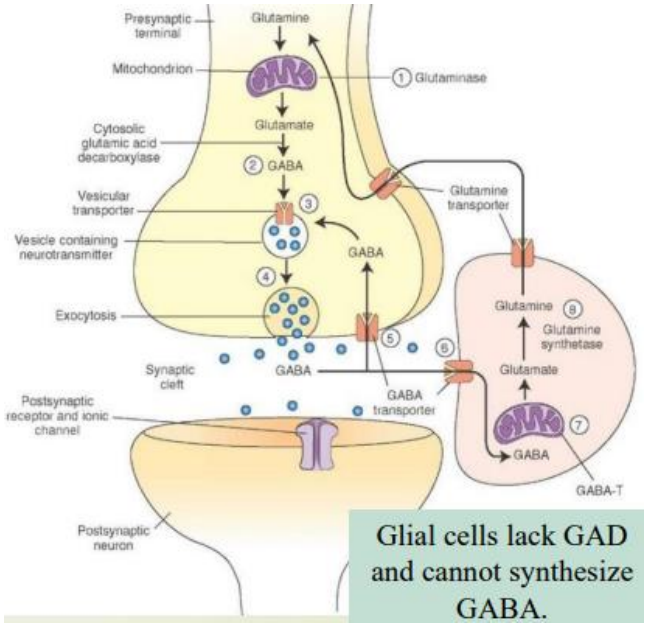
Synthesized from Serine by removing COOH group by serine hydroxyl-methyl transferase (need folic acid), (*de novo*). Serine is synthesized from 3- phosphoglycerate which is a glycolytic intermediate.

-(Removal: high-affinity transporter).

-GABA

Gama amino butyric acid (GABA) is a modified Glutamate (amino acid derivative) and is the major inhibitory neurotransmitter of CNS.

Glutamine is first converted to Glutamate by a Glutaminase, and then Glutamate is α-decarboxylated forming GABA via glutamate decarboxylase (GAD), which requires PLP. GABA is then packaged inside a vesicle and stored until released.



After release GABA enters the presynaptic neuron by the re-uptake process (like other small molecules) or taken by glial cells and in its mitochondria it is transformed back into Glutamate then to Glutamine (this group of reactions is called GABA shunt (work to preserve GABA and its precursor)); Glutamine is then transported to the adjacent terminals to synthesize new GABA molecules back again.

- Refer to slides 45-46.

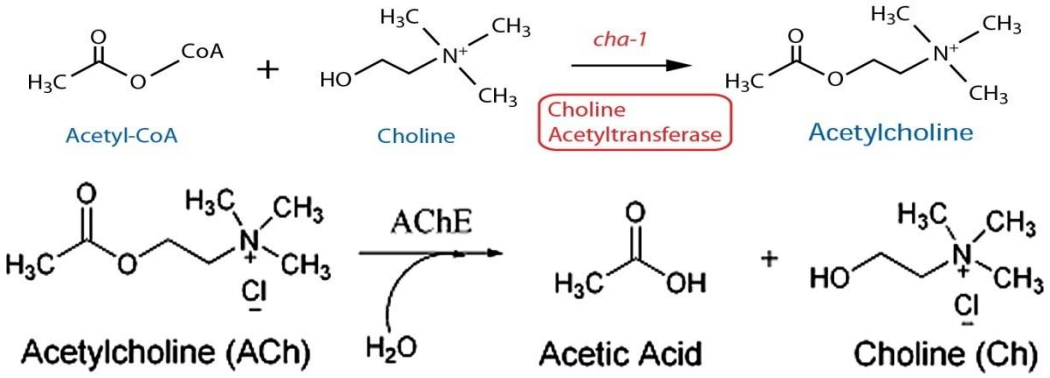
-Acetylcholine

The major neurotransmitter at the neuromuscular junction.

First, Choline is derived from phospholipids or serine amino acids, and then Choline acetyl transferase takes acetyl group from acetyl coA and attaches it to the Choline forming Acetylcholine in the cytoplasm, and then stored into vesicles until release. Its action is then terminated by acetylcholine esterase (removal by hydrolysis).

**Acetyl choline esterase is inhibited by Sarin gas prolonging the action of acetyl choline in the NMJ causing paralysis *(Inability to inactivate AC leads to constant activation of the nerve–muscle synapses, leading to varying degrees of paralysis*.).

- *(The acetyl group used for acetylcholine synthesis is derived principally from glucose oxidation to pyruvate and decarboxylation of pyruvate to form acetyl-CoA via the pyruvate dehydrogenase reaction*.)



-Histamine

It does not cross the BBB and, hence, must be synthesized in specific neurons (remember it's synthesized in mast cells. Also, some neurons use it as neurotransmitter).

An amino acid derivative synthesized from Histidine amino acid in a single step reaction which is a decarboxylation process requiring PLP.

Although it is a small molecule no reuptake and recycling processes occur; instead, it is taken by astrocytes and inactivated by MAO.

Refer to slide 49.

Nitric Oxide (gas neurotransmitter)

The only neurotransmitter that present in the form of gas. So, a lot of the rules we discussed doesn't occur here.

It doesn't have a receptor.

It's a small molecule so it diffuses through the membrane.

Synthesized from arginine stimulated by Glutamate:

- After exocytosis of Glutamate to the cleft, it will bind to NMDA receptors located on the post-synaptic neuron, this going to cause calcium influx which will lead to activation of NOS enzyme that has many isoforms.

- This enzyme will convert arginine to citrulline and produce NO as a product from the reaction.

- NO will affect signaling pathways leading to activation of guanylate cyclase forming cGMP which will work on the downstream targets.

Notes:

- Has **A PROLONGED ACTION RELATIVE TO SMALL MOLECULES**.

- We can't control its inactivation and degradation processes because it's a gas.

- Differences between NO and other neurotransmitters:

1. It is not stored in vesicles.
2. It is not released by calcium-dependent exocytosis (it diffuses).
3. Its inactivation is passive (there is no active process that terminates its action). It decays spontaneously.
4. It does not interact with receptors on target cells. (diffuse through membrane).
5. Its sphere of action depends on the extent to which it diffuses, and its action is not confined to the conventional presynaptic-postsynaptic direction.
6. NO acts as a retrograde messenger and regulates the function of axon terminals presynaptic to the neuron in which it is synthesized, it may even go back to the cleft that precedes its cleft.

- **NO synthase isoforms:**

1. Isoform I (nNOS or cNOS):
× Neurons and epithelial cells.
× activated by the influx of extracellular calcium.

2. Isoform II (iNOS):
× Macrophages and smooth muscle cells.
× induced by cytokines (its mostly related to damage and inflammation).

3. Isoform III (eNOS):
× Endothelial cells lining blood vessels
× activated by the influx of extracellular calcium (it's mostly related to vasodilation and movement of calcium).

× All three isoforms require BH2 as a cofactor and nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme.

THE END