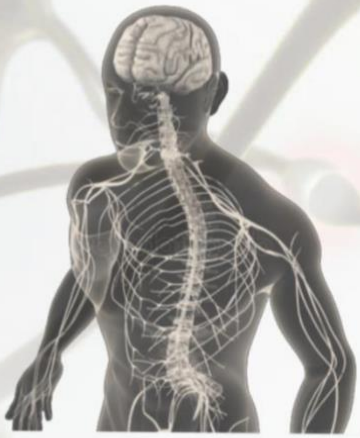
Central Nervous System

Sheet 2



Subject | Biochemistry

Done by | Maryam Ali

Correction | ...

Doctor | Mamoun Ahram

References

- The main source is the lecture, you can find it on the website.
- Mark's Basic Medical Biochemistry, 4th edition, pages: 908-918.
- http://what-when-how.com/neuroscience/neurotransmitters-the-neuron part-1/ this website can help you understand the anatomy and physiology of neurotransmitters as the doctor said.

Let's start by the definition of neurotransmitter:

Neurotransmitter: a chemical substance that is synthesized in a neuron, then, it is released in a synapse following depolarization of the nerve terminal (usually as a result of calcium ions influx), once it releases, it binds to receptors on the postsynaptic cell inducing a signal transduction in that cell or some of them can bind to receptors on the presynaptic cell. Resulting in a specific response is elicited such as muscle contraction.

• Characteristics of a neurotransmitter:

-The conditions of a molecule to be considered as a neurotransmitter are:

1- being a chemical substance that is synthesized and stored in a presynaptic neuron waiting for stimulus (the enzymes needed for its synthesis must be present in the neuron)

2- being released at a synapse following depolarization of the nerve terminal (usually dependent on influx of calcium ions)

3- once they release , they bind to receptors on the postsynaptic cell and/or presynaptic terminal.

4- resulting in activation signaling pathways which are rapid-onset and rapidly reversible responses in the target cell
5- liable to be removed or inactivated from the synaptic cleft, so that the signal is terminated.

* Types of neurotransmitters, there are 3 major types:

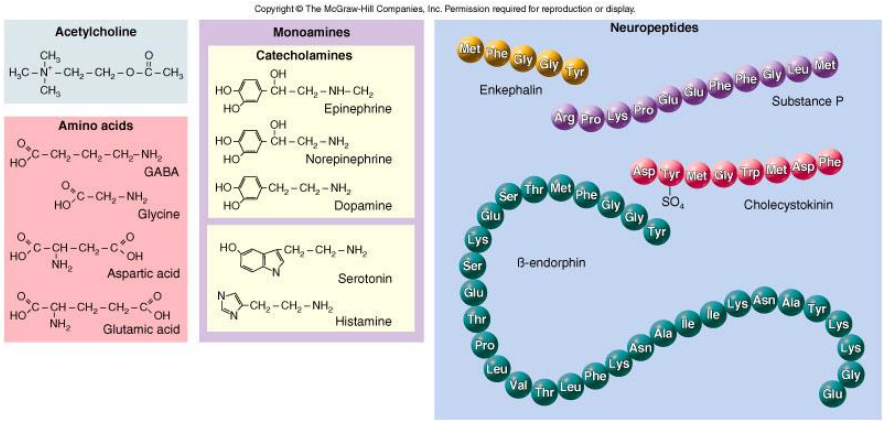
A)-Small-molecule neurotransmitters

1- Biogenic amines: histamine, serotonin and catecholamines (which are epinephrine, norepinephrine, dopamine)
2- Amino acids (GABA(modified glutamate), glutamate, aspartate, and glycine)
3- Acetylcholine
4- Purines (ATP)

B)-Neuropeptides

C) Gases (nitric oxide NO, carbon monoxide CO)

NOTE: Certain neurons might have more than one neurotransmitter (two or more), usually a combination of a small-molecule transmitter and a neuropeptide that coexist in the same neuron.
EXAMPLE: Most spinal motor neurons contain acetylcholine and calcitonin gene-related peptide.



** neuropeptides:
They are more than 50 neuropeptides. They are responsible for different responses like: behaviour, pain perception, memory, appetite, thirst, temperature, homeostasis, and sleep.

*When do say that this neuropeptide is neurohormone or neurotransmitter ?

There is a distinction between these two terms. It depends on the site of action.

- it is a neurohormone if it is released by neurons into the haemolymph (blood system or lymphatic system) and travels a long distance, then, exerts its effects on distant peripheral targets. It has long duration of action

- it is a neurotransmitter if it functions at the neighboring cell (doesn't travel for a long distance), if it is released from a neuron at a specialized junction and diffuses across a narrow cleft to affect one or two postsynaptic neurons, a muscle, or other effector cells.

Classification of neuropeptides

Peptides can be classified according to their structure and function.

We have many neuropeptide families: Tachykinins, Insulins, Somatostatins, Gastrins, and Opioids

From slides but not mentioned by the doctor:
- tachykinins: substance P, bombesin, substance K.
- Insulins: insulin, insulin-like growth factors
- Somatostatins: somatostatin, pancreatic polypeptide
- Gastrins: gastrin, cholecystokinin

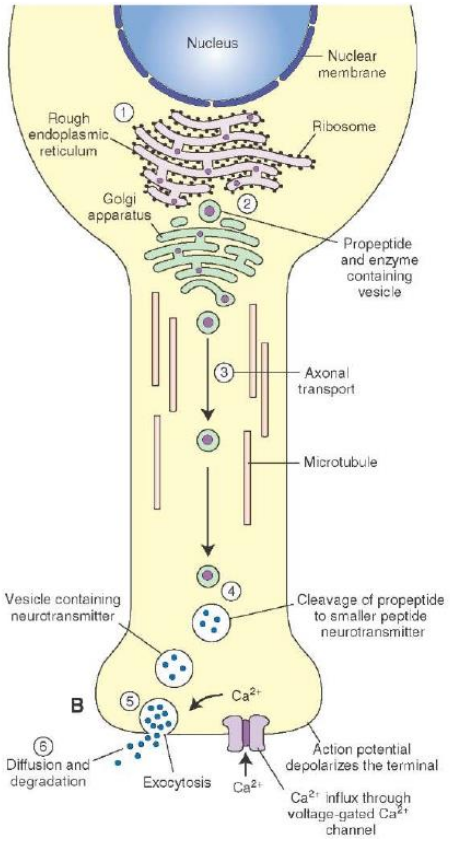
Opioids: opiocortins, enkephalins, dynorphin.

In the opiate family (they are a small peptides that have small number of amino acids), notice that they share common sequence, (i.e.: the same first four amino acids), but they differ in the rest of the amino acids (e.g. Leu-enkephalin and Met-enkephalin differs in the fifth amino acid, methionine and leucine respectively, as can be seen in the table) and bind to different receptors, in other words, although opiate peptides share a common sequence, they are receptor-selective.

Opiate Family	
Name	Amino Acid Sequence
Leu-enkephalin	**Tyr-Gly-Gly-Phe**-Leu-OH
Met-enkephalin	**Tyr-Gly-Gly-Phe**-Met-OH
Beta-endorphin	**Tyr-Gly-Gly-Phe**-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Gly-Gln-His-OH
Dynorphin	**Tyr-Gly-Gly-Phe**-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH

**Vasopressin and oxytocin are neuropeptides that share 7 of 9 amino acids, but have totally different functions regardless where they are.

- The three glycoprotein hormones from the anterior pituitary, TSH (Thyroid-Stimulating Hormone), LH (Luteinizing Hormone), and FSH (Follicle-Stimulating Hormone), share a common α subunit, but have different β subunits, this is why each one of them has distinct structure and function.



***Stages of action:

1- Synthesis and modification of neuropeptides: starts in the rER→through its ribosomes (rough ER) and Golgi apparatus

*It started as prepropeptide then in after the modification in the golgi it will be propeptide

*Neuropeptides are inserted into ER where they get modified, then travel to Golgi for further modification.

2-Packaging the propeptide into large-dense core vesicles. They coexist with modifying enzymes in these vesicles. And during their travelling from cell body toward the terminus, they are modified further inside the vesicles (e.g. proteases cleave the precursor neuropeptide into the final mature form).

The charictertes of these viscles are large and thick membrane

3-Transport (via fast-axonal transport): along microtubules to cell body, and then from cell body to neuronal terminals , at this stage some of the them are being active, they stay in terminals waiting for a stimulus to be released.

4- Release (exocytosis): They are released gradually over time in response to general increases in the level of intracellular calcium introduced by a stimulus (in the presence of calcium, they are released by the fusion of the vesicle to the membrane).

5- Action (prolonged). Once they release they bind to GPCR resulting in the action

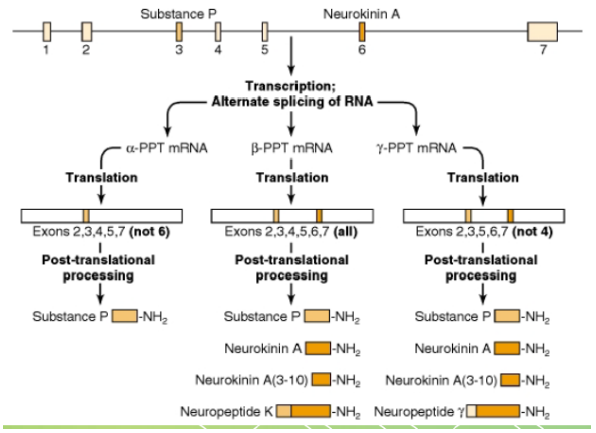
6- Termination by diffusion and degradation (enzymes, found outside the cell, degrade these peptides.

Diversity:

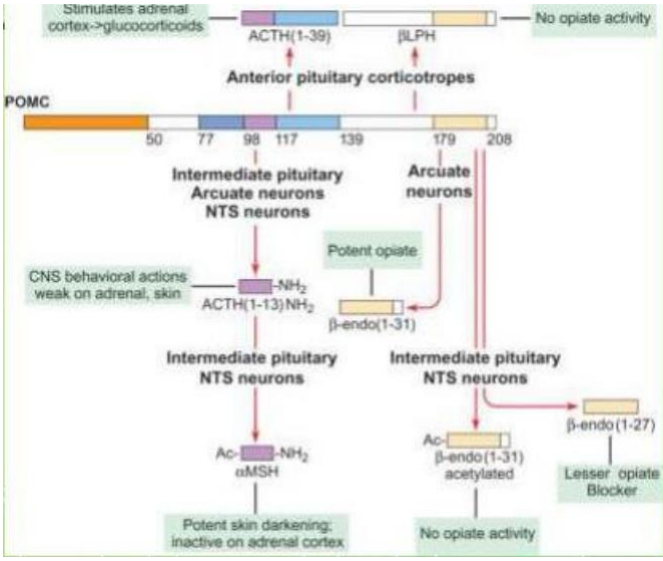
There is diversity in these peptides and this diversity cab=n stem from two mechanisms:

1- Some of peptides are synthesized from the same gene → having mRNA alternatively splice → resulting in different protein isoforms

- Example : substance p



2- Proteolytic processing : once the proteins are post-translation , they can have further processes



This process depends on:

a- Type of protease → different enzyme → different cleavage of peptides → different hormones we will have

b- Vesicular packaging of different protease in different vesicles that recognize different cleavage sequences (this point mentioned in the slide)

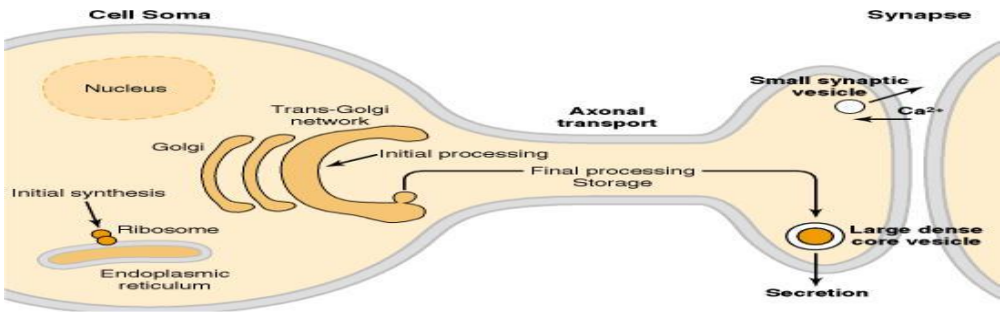
c- Hiding a proteolytic site by post-translation modifications that are controlled by the cell → carboxylation (adding of a carbohydrate side chain) → this process is tissue specific

- Example : pro-opiomelanocortin

The following wasn't mentioned by the doctor

processing of the pro-opiomelanocortin (POMC) precursor proceeds in an ordered, stepwise fashion. Some of the reactions are tissue specific. ACTH, adrenocorticotropic hormone; CLIP, corticotropin-like intermediate lobe peptide; JP, joining peptide; LPH, lipotropin; MSH, melanocytestimulating hormone; PC, prohormone convertase.

***Role of Ca2+ ions



*Ca2+ ions are important for inducing fusion of the vesicles with the presynaptic membrane and the release of the neuropeptides

*The source of Ca2+ can be <u>extracellular as well as intracellular.</u>

*<u>Little amount of calcium ions</u> is needed to induce the release of the <u>neuropeptides</u>, lower concentrations are required unlike small-molecule neurotransmitters.

The vesicles are located far away from the area of calcium ions entry and further away from presynaptic membrane, unlike the small molecules neurotransmitters.

The levels of regulation of neuropeptide expression

The regulation starts with a stimulus that affects different levels in the synthesis of the neuropeptides:

1- Induction of transcription of genes encoding neuropeptides or enzymes (involved in the processing and modification of the immature peptides) in the nucleus and alternative splicing.

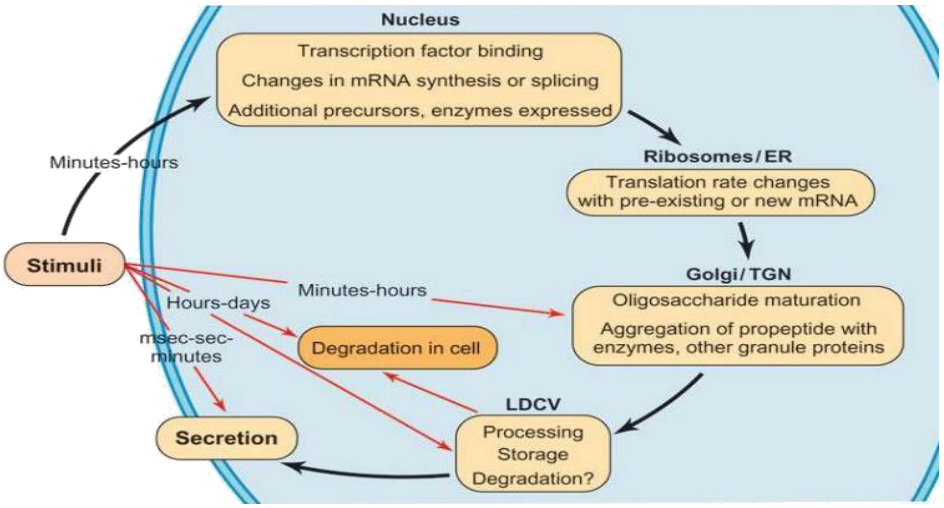
2- Change rate of translation of neuropeptides by the ribosomes on the rER

3- Addition and modification of oligosaccharides and packaging with certain enzymes in the Golgi apparatus.

4- Presence of proteases in vesicles and their processing inside the vesicles

5- Activation or inhibition the degradative enzymes (proteolytic processing takes place)

6- Secretion and Release



SMALL-MOLECULE NEUROTRANSMITTERS

Stages of action

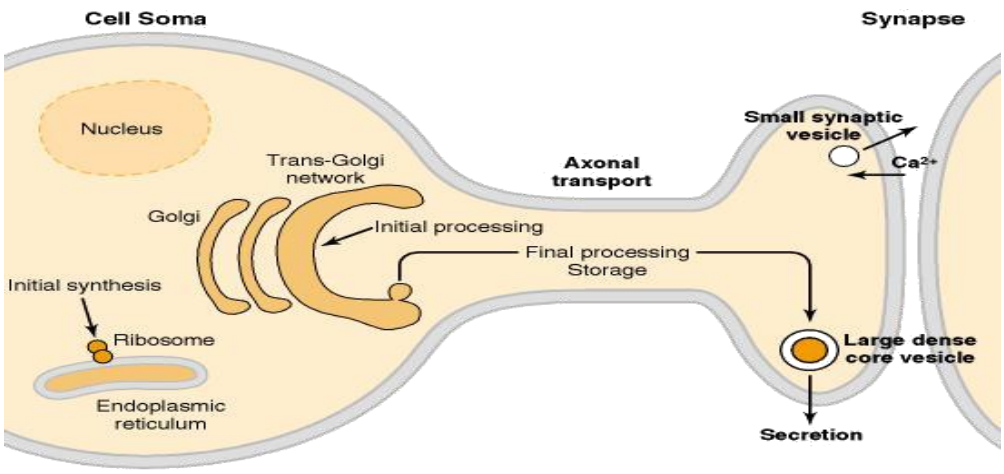
1- Synthesis of enzymes: here, we are talking about the synthesis of the synthesizing enzymes (not the neurotransmitters) that takes place in the cytosol or in the rER-Golgi apparatus (in golgi to get modified) , then they are packaged into large dense core vesicles just like the neuropeptides
2- Transport of enzymes (axonal transport) along the axon to terminals.
3- Synthesis of small molecule neurotransmitters: it takes place in the pre-synaptic terminal either in the cytosol or inside the vesicles depending on the neurotransmitter.
4- Packaging in small synaptic vesicles→ where they can be modified further
5- Release is stimulated by brief pulses each time an action potential triggers the influx of calcium, in other words, a signal triggers the influx of calcium which

will induce the fusion of the vesicles with the presynaptic membrane and release of the small molecule neurotransmitters.

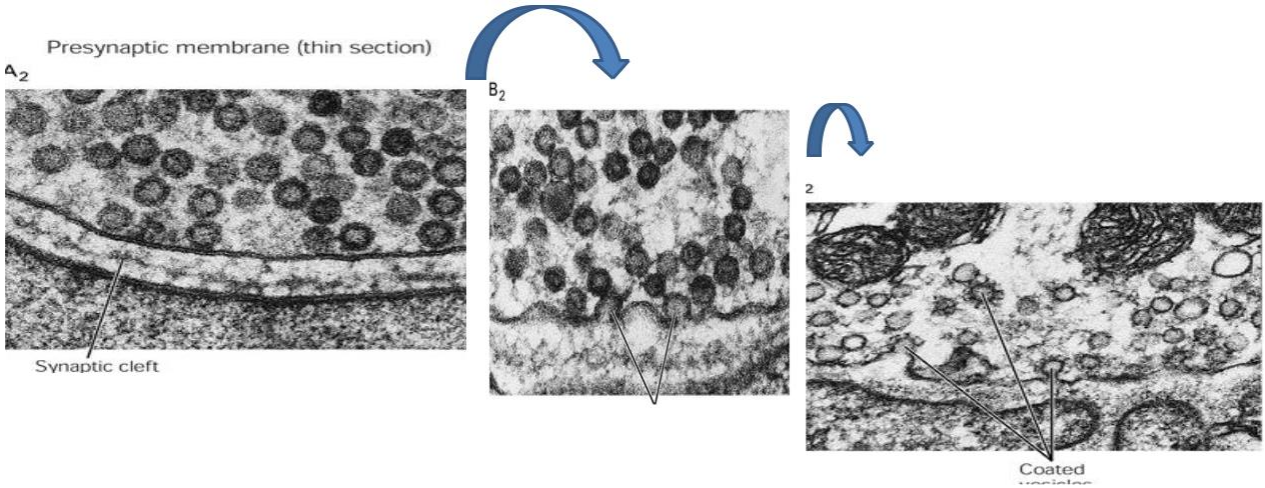
**Their action is short relative to neuropeptides

** we need low numbers of calcium ions influx

6-Termination of their action occurs by different mechanisms: diffusion out of the synaptic cleft, re-uptake to the presynaptic cell by endocytosis , or inactivation enzymatically.



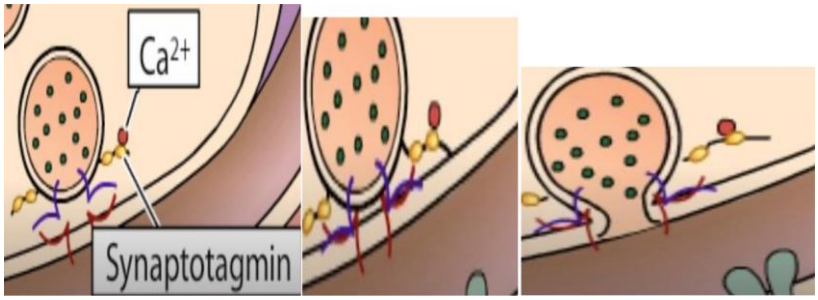
Here are some electron microscopic images of such vesicles fusing with the plasma membranes. You can see the sequence of events that takes place. The small molecule NTs are stored in smaller vesicles, while the neuropeptides and their modifying enzymes are stored in larger vesicles far away from the membrane.



Role of Ca2+ ions influx

that is what let the vesicles released their contains

-Once calcium enters the neuron, it binds synaptotagmin, this influx of Ca2+ ions influence synaptotagmin (protein exists on the vesicular membrane) to interact with other proteins on the plasma membrane and driving the vesicles closer to the membrane leading to fusion of the vesicular and presynaptic membranes (the vesicles will become part of the membrane) and release of the neurotransmitters.



TYROSINE-DERIVED NEUROTRANSMITTERS

Dopamine, norepinephrine, and epinephrine

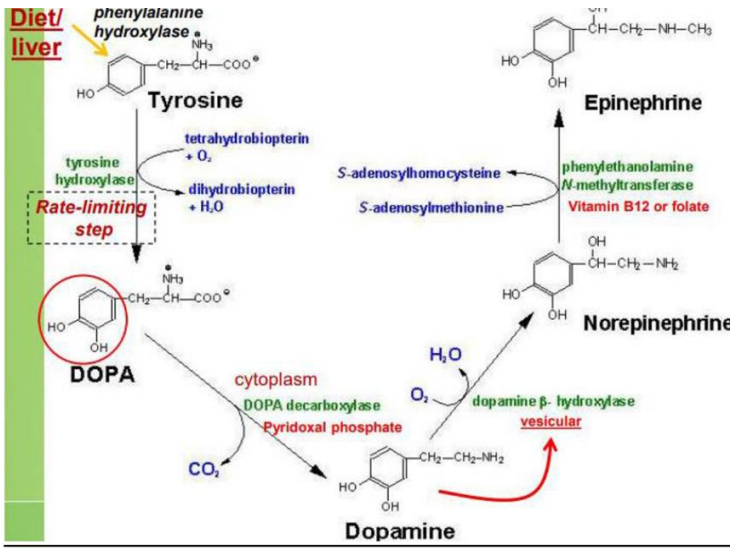
- Pay attention to the role of cofactors in their synthesis:

1- S-adenosylmethionine: it is important for methyl transfer

2- Pyrodoxal phosphate (vitamin B6): important for transamination, decarboxylation.
3- Tetrahydrobiopterin (BH4)

Steps of the synthesis:

1- Tyrosine is converted into DOPA by an enzyme known as tyrosine hydroxylase. This step requires BH4 as a cofactor. (this the rate-limiting step)The source of tyrosine is either from the diet , synthesis in the liver or from hydroxylase phynyalenine
2- DOPA is then converted into dopamine via decarboxylation by DOPA decarboxylase. This reaction requires pyridoxal phosphate (vitamin b6)
3- Dopamine can be converted into norepinephrine by dopamine β hydroxylase. This step occurs in the vesicles
4- Norepinephrine can be methylated at its end to get converted into epinephrine. This step occurs in the cytosol and requires more than one cofactor: S-adenosylmethionine and vitamin B12 or folate(B9), the synthesis of epinephrine from norepinephrine is vesicular.

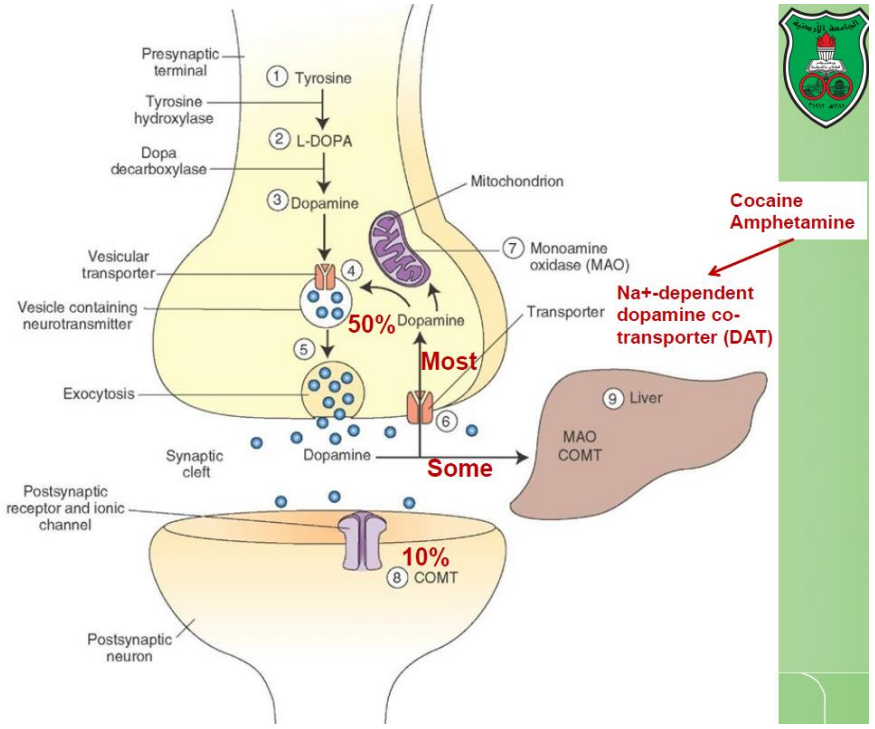


Dopamine

The enzymes are transported from the cell body to the terminus and there they produce dopamine from tyrosine. Dopamine is then transported into the vesicles where it can be converted into the other catecholamines. They are stored in the vesicles waiting the influx of calcium ions to induce the fusion and their release

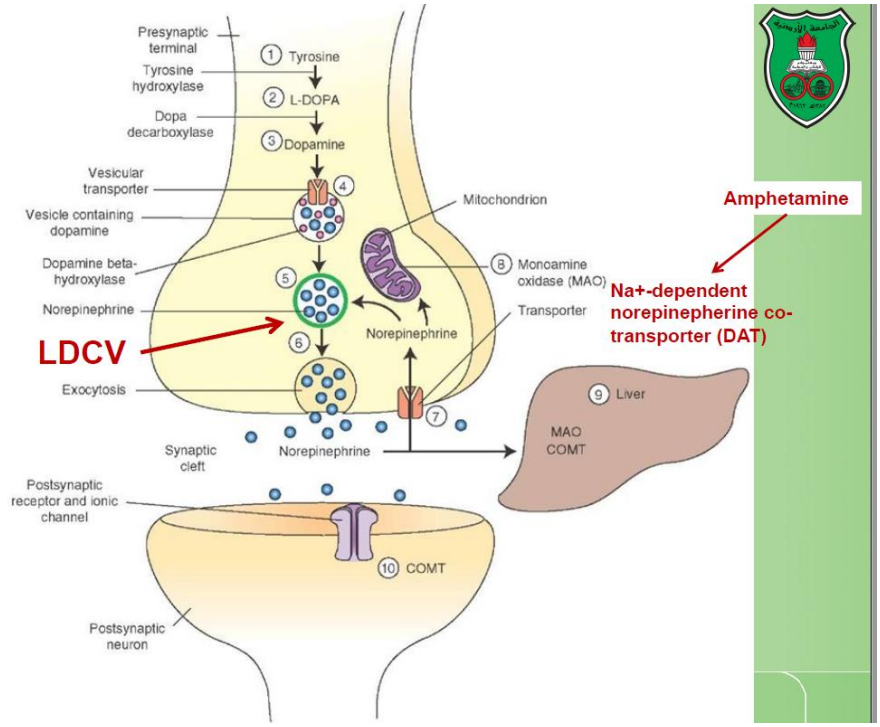
. Fate: 50% of the released dopamine can be taken up again by the presynaptic neuron, whereas 10% are used by the postsynaptic neuron. The rest is either degraded by certain enzymes, removed enzymatically by monoamine oxidase or methyltransferase enzyme or diffuse out of the synaptic cleft to be eliminated by the liver.

**Effect of cocaine and amphetamine: they prevent the uptake of dopamine, so that it stays for a longer period in the synapse and stimulates the postsynaptic neuron for a longer time → resulting in a amplifying the signals. And this explains the feel of awaking and excitement produced by cocaine.



Norepinephrine:

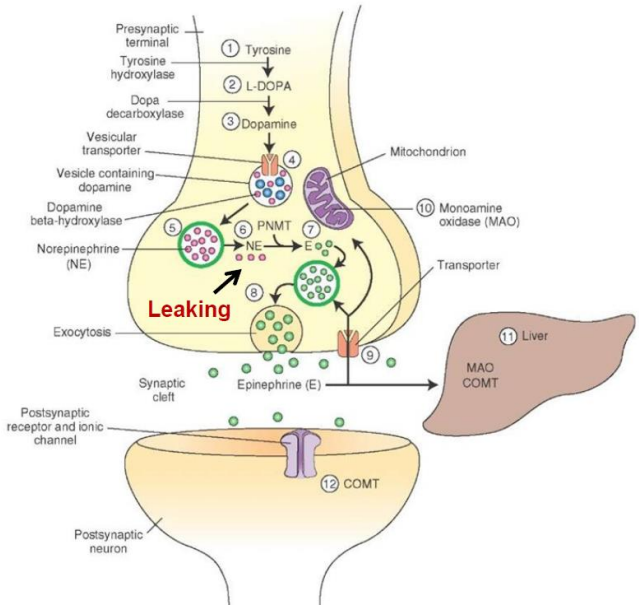
*Its synthesis is vesicular. the dopamine is transported into vesicles that will fuse with other vesicles containing the enzymes required for the conversion into norepinephrine. And these vesicles which are large dense core vesicles (LDCV) fuse with the plasma membranes releasing norepinephrine.
*After their release, the can be either taken up by presynaptic or postsynaptic neurons (predominantly the presynaptic) Monoamine oxidase (MAO) or methyltransferase



**Amphetamine has an effect on the transporter that is responsible for reuptaking the neurotransmitter back into the presynaptic cell.

Epinephrine:

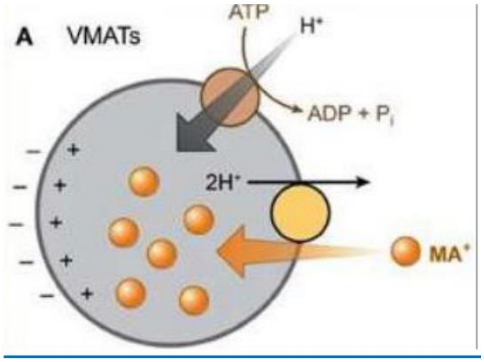
*Norepinephrine leaks out of the LDCVs to the cytosol and then it is converted into epinephrine by a methyltransferase. Then, the epinephrine gets packed into LDCVs, then calcium influx which helps the fusion of vesicles and release of epinephrine.



*Then most of it, is taken up by presynaptic cells, some of it by postsynaptic or become enzymatically inactivated by monoamine oxidase or methyltransferase.

*Packaging into vesicles

The catecholamines (especially epinephrine and dopamine) are transported into vesicles by an ATP-dependent process linked to a proton pump. Protons are pumped into the vesicles by a vesicular ATPase (V-ATPase). The protons then are pumped out in exchange for the positively-charged catecholamine via the transporter VMAT (vesicular monoamine transporter).



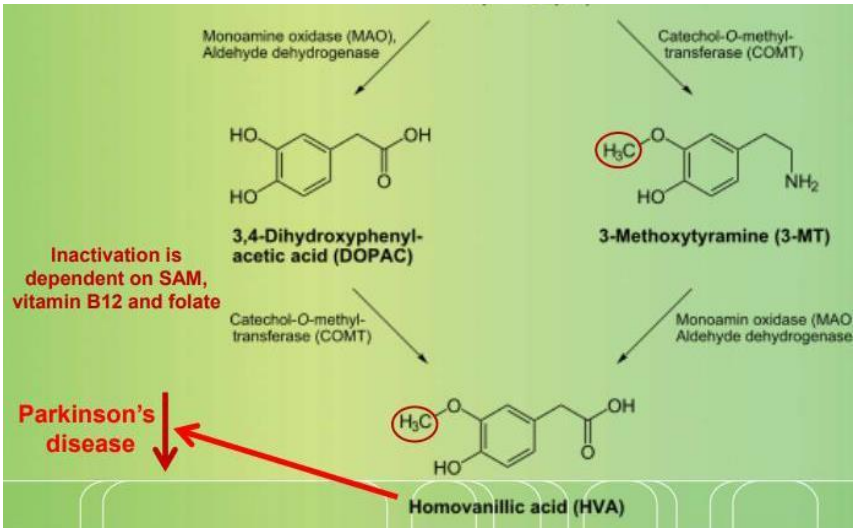
So, this process requires two transporters:
1- the vesicular ATPase (for protons)

2- the vesicular monoamine transporter (VMAT) – (for neurotransmitters)

The inactivation

*It occurs either by reuptake or by the enzymatic conversion:
We have two enzymes:
1- The catechol-O-methyl transferase COMT
2- The monoamine oxidase MAO
*The inactivation requires both of them sequentially regardless of their order (it doesn't matter if MAO or COMT acted first). Eventually, the final product is the homovanillic acid (HVA)

Dopamine



*The inactivation is depedent on SAM, vitamin B12 and folate

* homovanillic acid is a nmarker for parkinson's disease that the ratio of homovonillic acid to dopamine is hig

Regulation of the synthesis

Tyrosine hydroxylation: is the rate limiting step which is the slowest step in a biochemical pathway and it is highly regulated. (note: energy is associated with such reaction but not necessarily) →it can be regulated at two levels:

1-Short term level:

a)-Inhibition by free cytosolic catecholamines: large amount of catecholamines in the cytosol will compete and prevent the binding of BH4 (the cofactor) to the enzyme/ feedback inhibition, in other words, large amounts of catecholamines lead to more inhibition for the enzyme.

b)-Activation by depolarization: different signals activate different kinases (e.g. PKA, CAM kinases, PKC) that will phosphorylate serine residues in this enzyme and make it binds tightly to its cofactor BH4.

2-Long-term level: it takes time because it affects the gene expression of this enzyme synthesis and the expression of dopamine β hydroxylase as well.

TRYPTOPHAN-DERIVED NEUROTRANSMITTERS

*Serotonin and melatonin (derived sequentially from tryptophan)

Serotonin which is also known as 5-hydroxy-tryptamine

**Synthesis of serotonin:

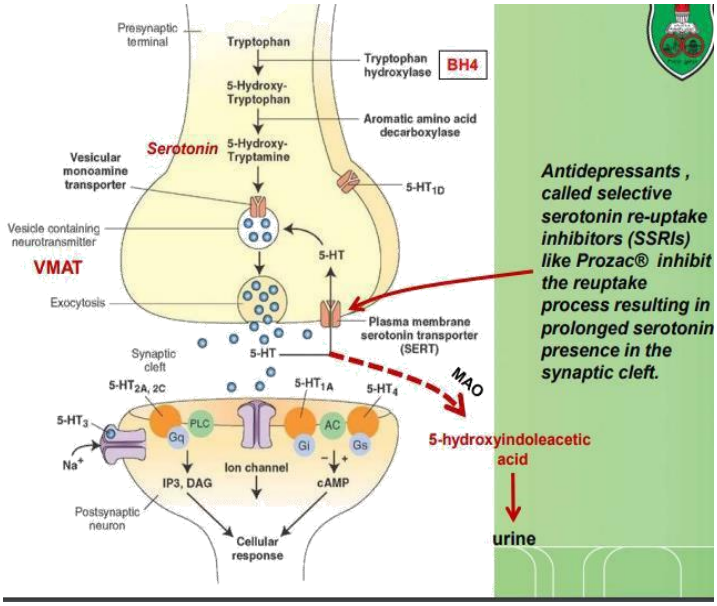
1- tryptophan is hydroxylated and converted into 5-hydroxy-tryptophan by tryptophan hydroxylase. And this step requires BH4 as a cofactor(rate limiting step)

2- 5-hydroxy-tryptophan is converted into 5-hydroxy-tryptamine(serotonin)

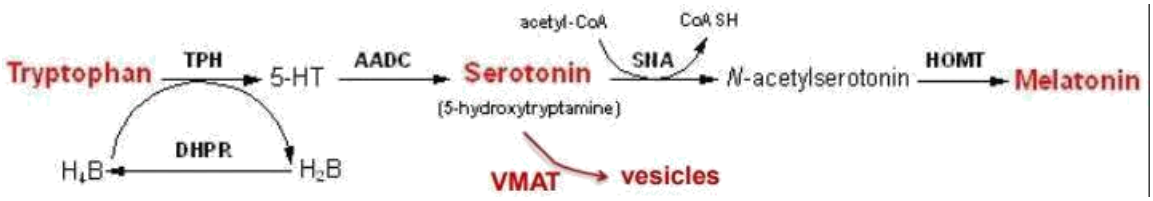
3- Packaging of serotonin into small synaptic vesicles waiting for the Ca2+ influx

4- Once it is released, it can be taken up by the presynaptic cell or the postsynaptic, but most of it is reuptaken by the presynaptic neuron. It also can diffuse away where it can be inactivated and converted by the MAO into a product (5-hydroxyindoleacetic acid) that is eliminated in urine, it is a marker of inactivation.

*This neurotransmitter is responsible for happiness, so prevention of its reuptake prolongs its presence in the synapse and increases the feeling of happiness and comfort. SSRIs (selective serotonin reuptake inhibitors) are antidepressants, they are a group of drugs that target the transporter of this neurotransmitter (e.g. Prozac), they prolong serotonin presence in the synaptic cleft.



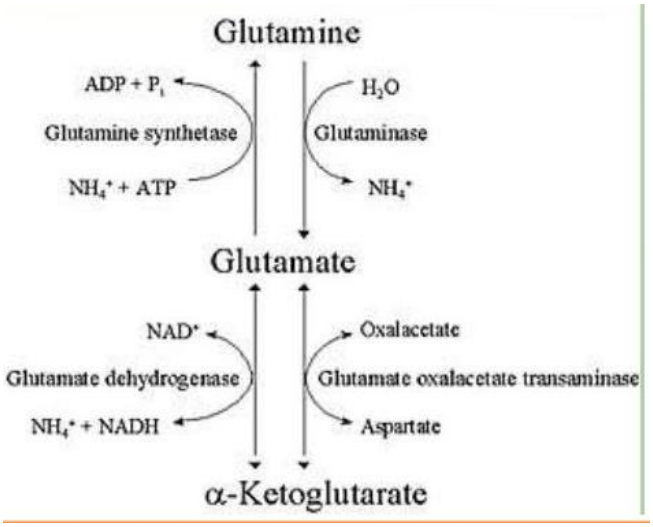
Melatonin



- Serotonin is synthesized in the pineal gland and serves as a precursor for the synthesis of melatonin, melatonin is synthesized from serotonin, initially via acetylation by acetyl-transferase enzyme followed by methylation.

Melatonin is a neurohormone involved in regulating:
1-sleep patterns
2-Seasonal and circadian (daily) rhythms
3-Dark-light cycle
elderly → have less melatonin than babies that why they keep awake in night for long time.

GLUTAMATE AND ASPARTATE



They are nonessential (can be synthesized in sufficient amount) amino acids that do not cross Blood Brain Barrier and must be synthesized in neurons.

There are two sources of these amino acids: neurons or glial cells.

**Both are excitatory neurotransmitters.

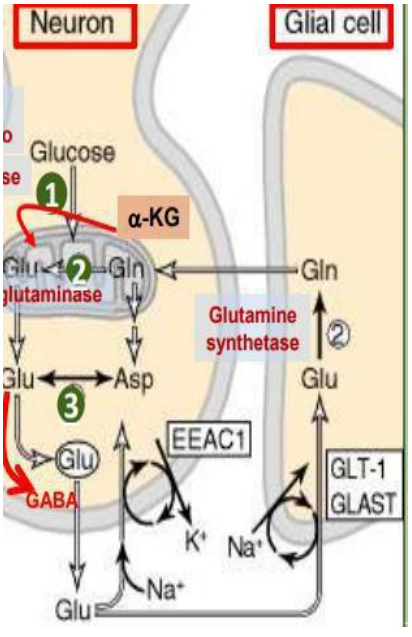
Glutamate

Sources of glutamate:

1. An intermediate in Krebs cycle that is α-ketoglutarate.

Dehydrogenation of α-ketoglutarate or transamination with aspartate both produces glutamate

2. Glutamine by deamination (removal of an amine group)
3. Aspartate by transamination



Removal:

It can be taken up by two different transporters:

1- Excitatory amino acid carrier-1 (EAAC1) which transports glutamate into neurons where it can be packaged again in vesicles.

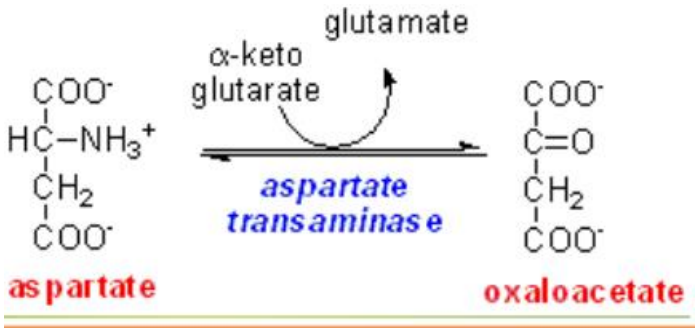
2- Glutamate transporter-1 (GLT-1) and glutamate—aspartate transporter (GLAST) that transport it into glial cells where it can be converted into either:

a- glutamine that is released from the glial cells and enters the neurons where it can be converted back into glutamate
b- Aspartate, to be converted back into glutamate as well.

Aspartate:

*Aspartate is synthesized from oxaloacetate (an intermediate in krebs) by transamination.

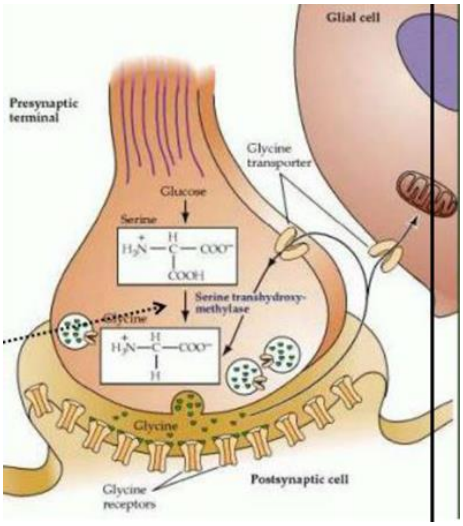
*Notice the involvement of α-ketoglutarate and glutamate.



*The vesicular uptake mechanism for aspartate has not yet been demonstrated, somewhat weakening the case for considering aspartate to be a neurotransmitter

Glycine

*It is the major inhibitory neurotransmitter.
*It is synthesized from serine by serine hydroxymethyltransferase through 3-phosphoglycerate (an intermediate in glycolysis) and this reaction requires folic acid (vitamin B9).
*It can be removed via the high-affinity transporter



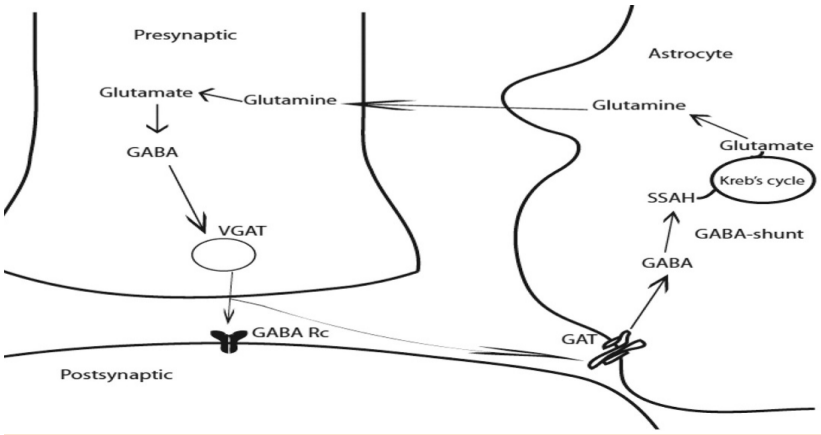
GABA

*Gamma-aminobutyric acid, it must be preserved because of the importance of keeping it in high concentrations (millimolar) in many brain regions. These concentrations are about 1,000 times higher than concentrations of the classical monoamine neurotransmitters in the same regions. This high concentration requires a pathway known as the GABA shunt to preserve the NT and prevents its removal.
*The GABA shunt is a closed-loop process with the dual purpose of producing and conserving the supply of GABA.

*In this pathway:

1- Glutamine is converted into glutamate by glutaminase in neuron itself.
2- 2-Glutamate is →decarboxylated forming GABA via glutamate decarboxylase (GAD), which requires pyridoxal phosphate (vitamin B6).
3- GABA is stored in vesicles until released in a similar mechanism as all of the other small molecule NTs.

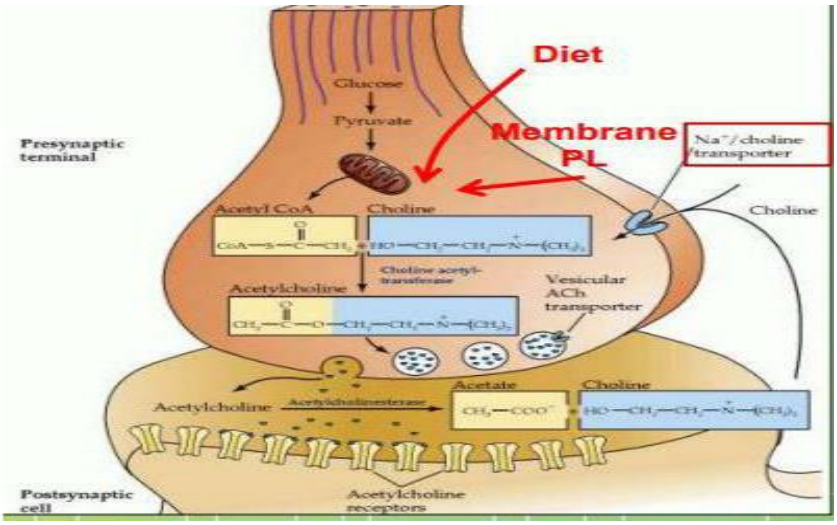
4- Once GABA is released, it is either taken up into presynaptic terminal and repackaged OR goes into the GABA Shunt where it is taken up into the glia and converted to glutamate. Glutamate is converted into glutamine, which is transported from the glial cells into the neighbouring neuron terminals to synthesize glutamate.



*the idea of this shunt is to preserve GABA as much as possible and this takes place in the glial cells.

Acetylcholine

It is synthesized from integration and combination of choline and acetylCo-A by an enzyme known as choline acetyltransferase in the cytoplasm and then, it is stored in vesicles.

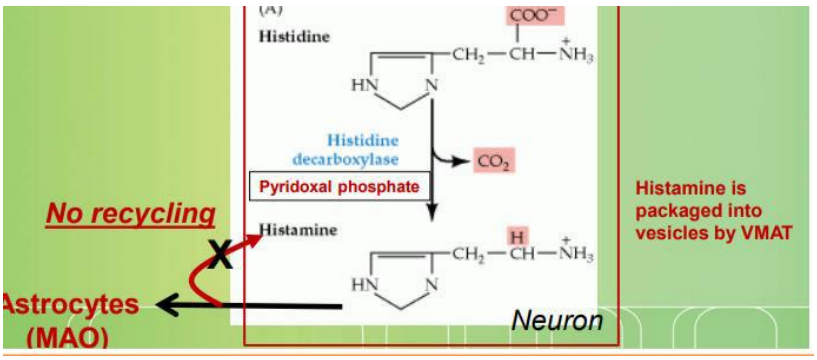


*There are different sources of choline: diet or phospholipids in the plasma membrane

*Once released, It will be hydrolysed by acetylcholinesterase into acetate and choline which will be reuptaken by the presynaptic neuron to produce new Achs.

Histamine

*It is synthesized from histidine through decarboxylation by histidine decarboxylase. And this decarboxylation requires pyridoxal phosphate (vitamin B6). Then, histamine is packaged into vesicles by the same transporter of catecholamines and serotonin (VMAT)



*It can be inactivated two enzymes either the histamine methyltransferase or diamine oxidase (aka histaminase).

It has an important property that there **is no mechanism to reuptake histamine into the presynaptic neuron BUT MAO in the neighbouring astrocytes can uptake histamine once it is released**

*It does not penetrate the blood-brain barrier and, hence, must be synthesized in the brain.

Nitric oxide

It is a gas that is synthesized in the postsynaptic neuron:
1- Glutamate is released and acts on NMDA receptors located on the post-synaptic neuron
2- Ca2+ enters the postsynaptic neuron activating NOS (nitric oxide synthase)
3- NOS forms NO from arginine (NO is generated from arginine)
4- NO stimulates guanylate cyclase forming cGMP from GTP which results in a physiological response in the neighbouring cells by diffusion
5- NO can diffuse out (the postsynaptic) into:
a) the presynaptic terminal (retrograde messenger) inducing an action

b) adjacent neurons and glial cells stimulating guanylate cyclase.

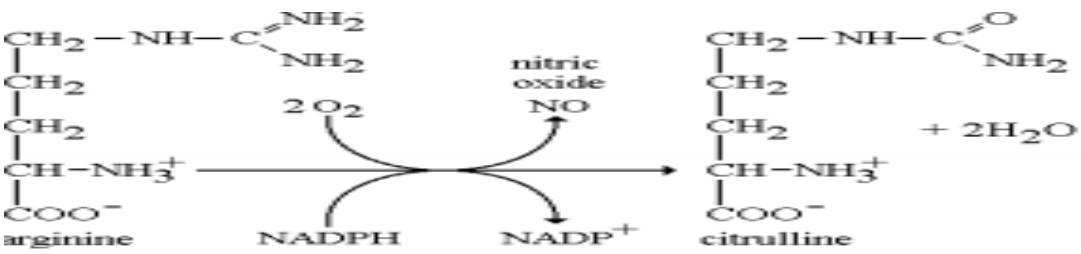
** It has very short half-life: 2-4 seconds NO. It is inhibited by hemoglobin and other heme proteins which bind it tightly. Hb acts as a scavenger for nitric oxide

Is NO a neurotransmitter?

Yes, but it is different from the classical neurotransmitters because:

- It is not stored in vesicles and it is not produced in the presynaptic neuron, instead it is synthesized in the presynaptic cell
- It is not released by calcium-dependent exocytosis (it diffuses)
- Its inactivation is passive, mainly by diffusion (there is no active process that terminates its action) and it decays spontaneously (very short half-life)
- It does not interact with receptors on target cells, rather it diffuses and interacts with enzymes inside the postsynaptic cell 's cytosol.
- Its sphere of action depends on where it diffuses, in other words, the extent to which it diffuses, and its action is not confined to the conventional presynaptic-postsynaptic direction.
- NO acts as a retrograde messenger and regulates the function of axon terminals presynaptic to the neuron in which it is synthesized.

Isoforms of NO



There are different isoforms of NO synthases that exist in different cells. Each one of them has cell-specific localization and different effect. But all the three

isoforms require BH2 as a cofactor and nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme for NO synthesis.

- Isoform I (nNOS or cNOS): Neurons and epithelial cells activated by the influx of extracellular calcium
- Isoform II (iNOS): Macrophages and smooth muscle cells induced by cytokines
- Isoform III (eNOS): Endothelial cells lining blood vessels activated by the influx of extracellular

	neuropeptides	Classical neurotransmitters(small molecules)
Activity	slow	fast
Response	slow	fast
Duration of action	long	short
Receptor targets	Each one can bind to different types of receptors	Single, unique receptor for each one
Effect on gene expression	yes	no
synthesis	Starts in the cell body	Occurs in the cell terminals
Concentration needed for action	low	high
Speed of release	slow	fast
Concentration of calcium ions needed for release	High concentration	low cocentration