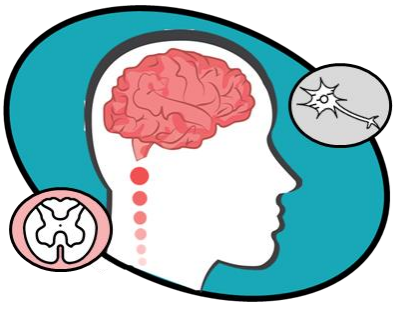


CNS

Biochemistry

Sheet Slide

Number

-2

Done by:

-Ahmad Rawajbeh

Corrected by:

-مَها أبو عجميّة

Doctor:

-Mamoun Ahram

Neurotransmitters

Corrector's note: the doctor has stated that the questions will be easy, don't let the number of pages freak you out, the sheet is long because it is illustrated with graphs taken from the slides and there are many skippable notes, best wishes!

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 biochemistry of neurotransmitters as the doctor said.

Definition:

Neurotransmitter: a **chemical** substance that is synthesized in a **neuron**, then, it is released in a **synapse** following depolarization of the nerve terminal (usually dependent on influx of calcium ions), and binds to receptors on the postsynaptic cell inducing a signal transduction in that cell. Some of neurotransmitters can bind to receptors on the presynaptic cell as well. Eventually, 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 (**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- ability to **bind to receptors** on the postsynaptic cell and/or presynaptic terminal,
4- eliciting **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:

A)-<u>Small-molecule neurotransmitters</u>

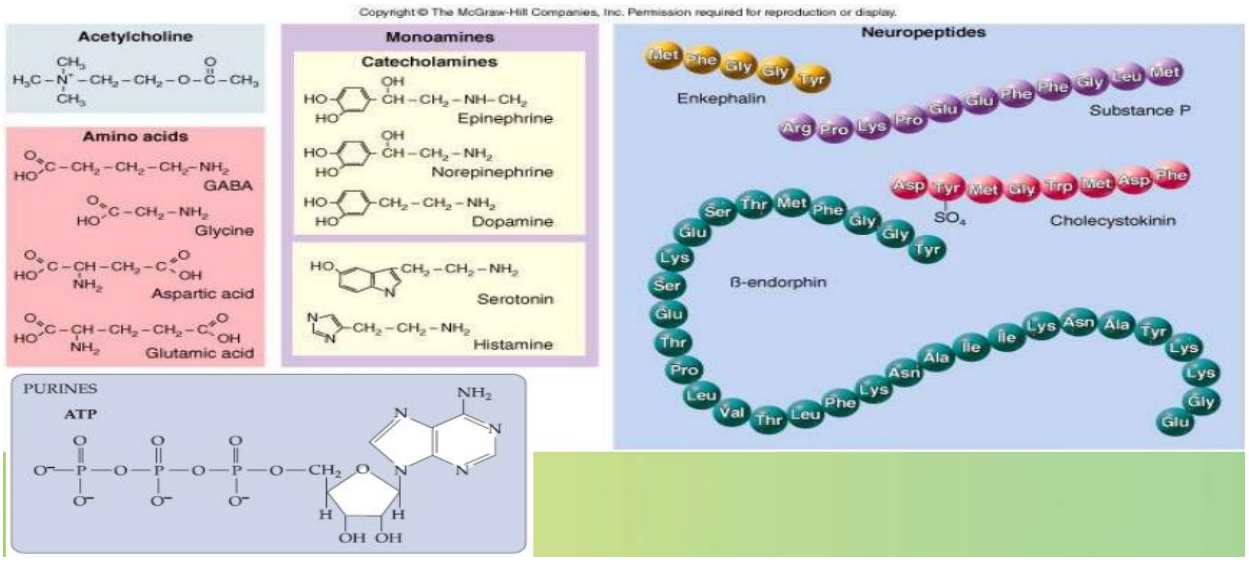
1- Biogenic amines (monoamines): histamine, serotonin and catecholamines (which are epinephrine, norepinephrine, dopamine)
2- Amino acids (<u>GABA</u>, glutamate, aspartate, and glycine)
3- Acetylcholine
4- Purines (ATP)

B)-<u>Neuropeptides</u>, which are **large neurotransmitters** and composed of very short sequences of amino acids which are combined with each other.

C) <u>Gases</u> (nitric oxide NO, carbon monoxide CO), very **small** and **short lived**.

sometimes, the neuron might contain **more than one neurotransmitter (two or more), usually a combination of a <u>small-molecule transmitter</u> and a <u>neuropeptide</u> that coexist in the same neuron. (this can be seen in many **mature** neurons)

e.g: Most spinal motor neurons contain acetylcholine and calcitonin gene-related peptide.



Take a look at the neuropeptides on the right.

NEUROPEPTIDES

Not all of them have been discovered, more than 50 neuropeptides have been identified so far. They are responsible for different **responses** like: behaviour, pain perception, memory, appetite, thirst, temperature, homeostasis, and sleep.

**Is the neuropeptide a neurohormone or a neurotransmitter?

There is a distinction between these two terms. The same molecule can act as a neurotransmitter and as a hormone. It depends on **where** it acts:

- it is a **neurohormone** if it is released by certain cells (neurons) into the haemolymph (blood or lymph) and travels a long distance, then, exerts its effects on **distant peripheral** targets.
- it is a **neurotransmitter** if it functions at the same region of release, in other words, 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 (each one of these families contains different types of neuropeptides)

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, 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. ((+) remember that these hormones are released by neurons from the pituitary gland, so they are neuropeptides. Also, they can act as neurotransmitters in the brain).

• 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.((+)regardless if they are considered neuropeptides or not, the thing is, they contain peptides in their structure and these peptides share a common subunit, but they have another variant subunit that makes the hormones functionally different)

Stages of action:

1- Synthesis and modification of neuropeptides: starts in the rER (**rough ER)** and **Golgi** apparatus
   Neuropeptides are inserted into ER where they get modified, then travel to Golgi for further modification.
2- **Packaging** 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).
3- **Transport** (via fast-axonal transport): along microtubules to cell body, and then from cell body to neuronal terminals, they stay in terminals waiting for a **stimulus** to be released.

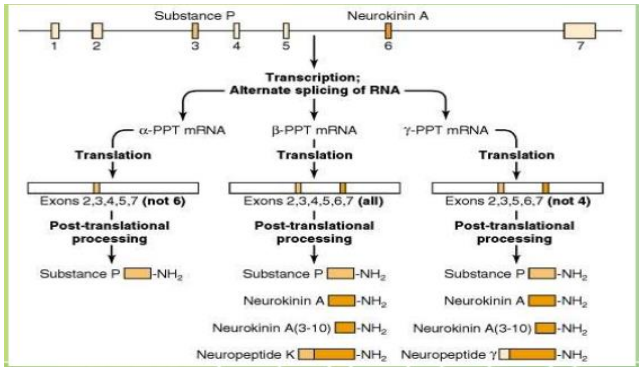
4- **Release (exocytosis)**: They are released **gradually** over time in response to general increases in the level of intracellular **calcium** introduced by a stimulus (in the presence of calcium, they are released by the fusion of the vesicle to the membrane).
5- **Action** (prolonged). They are somehow stable, so they can travel in the blood or the lymph.
6- Termination by **diffusion** and **degradation** (enzymes, found outside the cell, degrade these peptides)

Diversity:

There is diversity in these peptides and this diversity can stem from two mechanisms:

1- **Alternative splicing**: leads to translation of distinct precursors, and subsequent processing leads to unique mature peptides. ((+) remember once an RNA is produced, it is processed and during this processing certain exons are kept. Depending on the selected exons, different mRNAs can be produced from a single gene).

Example: **substance P** and other neuropeptides like n**eurokinin A** are produced from the **same gen**e. And this gene, through alternative splicing, produces **different mRNAs** that will be translated into **different neuropeptides**.



Slides:(substance P mRNA normally includes mRNA encoding substance K)

2- **Proteolytic, differential and sequential processing**: Neuropeptides are produced from a longer precursor protein, in other words, one long polypeptide is expressed and this polypeptide can be degraded in different manners generating different types of neuropeptides. This processing depends on:

a-Type of proteases

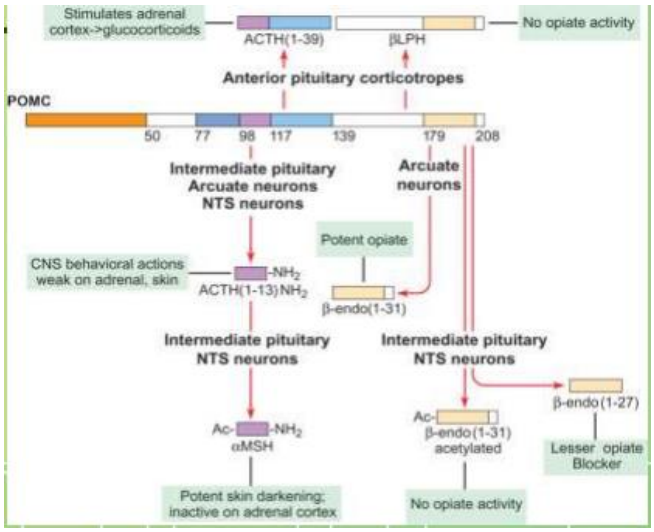
b-Vesicular packaging of different proteases in different vesicles that recognize different cleavage sequences .

c-Hiding a proteolytic site by post-translational modifications that are controlled by the cell (e.g.: addition of a carbohydrate side chain).

** this process is **tissue-specific**.

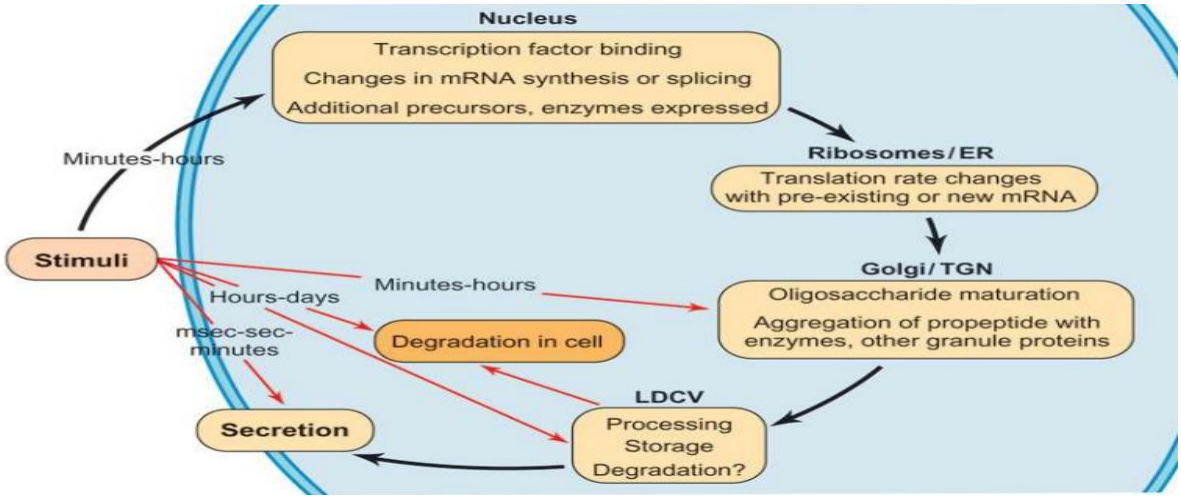
Example:

Processing of the **pro-opiomelanocortin** (POMC) precursor can be degraded into **different** types of **neuropeptides**: **ACTH** (adrenocorticotropic hormone), **CLIP** (corticotrophin-like intermediate lobe peptide), JP, LPH, MSH, and PC. (the doctor didn't focus on these peptides), simply, what you should know is that different neuropeptides can come from the same polypeptide (e.g. POMC).



**In the first mechanism, we get different neuropeptides by different mRNAs from a single gene, while in the second one, different neuropeptides emerge from one polypeptide (one mRNA).

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.

Role of Ca^{2+} ions

*Ca^{2+} ions are important for **inducing fusion** of the vesicles with the presynaptic membrane and the **release** of the neuropeptides

*The source of Ca^{2+} can be **extracellular** as well as **intracellular.**

***Little amount** of calcium ions is needed to induce the release of the neuropeptides, 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.

SMALL-MOLECULE NEUROTRANSMITTERS

They are nitrogen-containing molecules:

- Biogenic amines, modified amino acids and their derivatives
- intermediates of glycolysis and Krebs cycle) (e.g. oxaloacetate and αketoglutarate (intermediates of Krebs) could be converted into aspartate and glutamate (neurotransmitters) respectively.

Extra addition: types of small molecule neurotransmitters:

1-Monoamines (biogenic amines): contain an aromatic ring and one amine group.

histamine, serotonin, catecholamines [dopamine, epinephrine, and norepinephrine], and acetylcholine

(Some references don't consider Ach as a monoamine since it lacks an aromatic ring and doesn't stem from an amino acid like the others)

2-Amino acids and modified amino acids: they have the basic structure of an amino acid (amine group and carboxyl group)

Glutamate, aspartate, GABA, and glycine.

Stages of action

#The differences between neuropeptides and small molecule neurotransmitters:

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, 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**
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

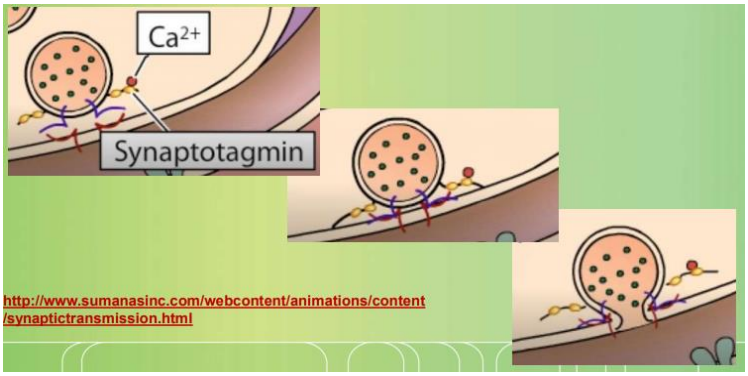
****Termination** of their action occurs by different mechanisms: diffusion out of the synaptic cleft, re-uptake to the presynaptic cell, or inactivation enzymatically.

the differences between neuropeptides and neurotransmitters (very important):

	Neuropeptides	Classical neurotransmitters(small molecule)
Activity	Slow	fast
Response	Slow	Fast
Duration of action	Long	Short
Receptor targets	Each one can bind to different types of receptor	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 Ca^{2+} needed for release	Low concentration	High concentration

Don't forget to compare the synthesis, transport, packaging, modification, action and fate of each one of them, as well.

Role of Ca^{2+} ions influx



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

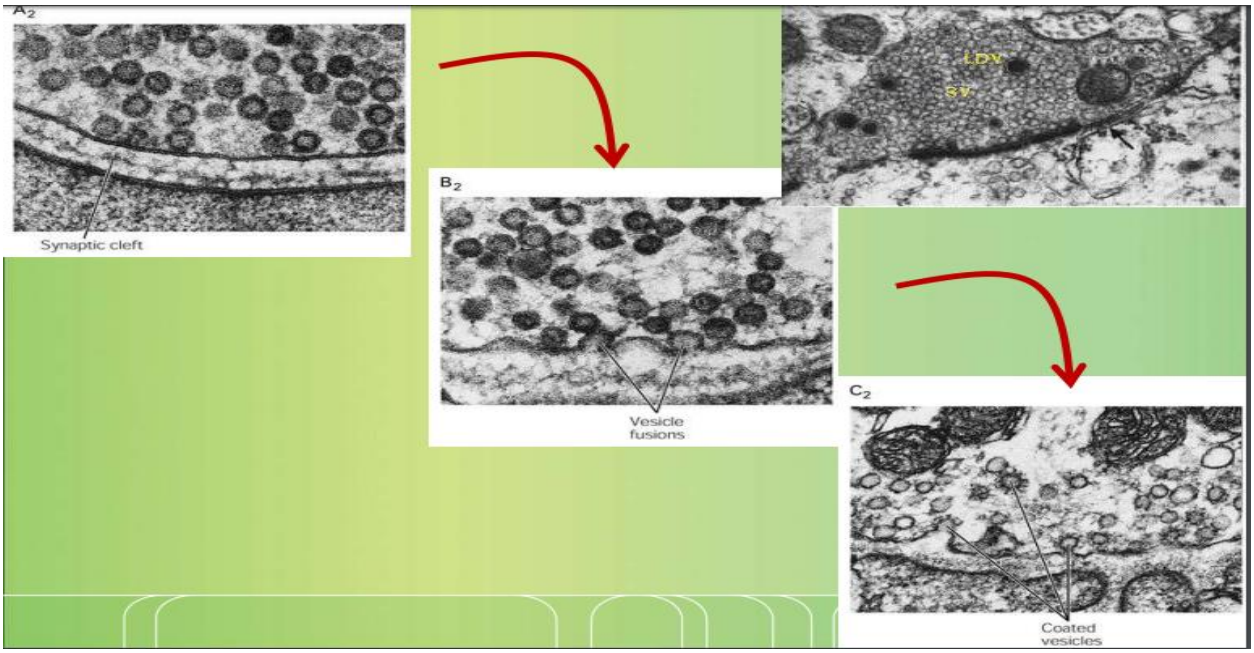
We have talked about the role of these ions in the release of neuropeptides. The same thing happens for the small molecule neurotransmitter except **that higher amount of calcium ions** is needed to induce the release of small molecule NTs and **the site of fusion** of the vesicles with presynaptic membrane must be **near the area of Ca^{2+} entry.**

	Small molecule NTs	Neuropeptides
Action	Short	Long
Synthesis	Occurs in the terminus	Starts in the cell body
Amount of Ca^{2+} needed	High	Small
*The site of fusion	Near the site of Ca^{2+} influx	Away
Recycling	Yes, by reuptake	No, must by synthesized de novo in the cell body

(+)*you may have been confused by the amount of Ca ions needed and the site of the fusion, and according to some references regarding the amount of Ca^{2+}, the opposite is the true. I have written what the doctor said exactly, but what I think is: that the neuropeptides are stored in large dense core vesicles located away from the presynaptic membrane where the Ca^{2+} inflows, in contrast to the classical small molecule neurotransmitters that are mainly stored in smaller synaptic vesicles located near the membrane, so a brief influx of calcium ions will induce the fusion of the closer vesicles much more than that of the far ones. But the fusion of vesicles that contain neuropeptides with the plasma membrane can occur at low

concentration of calcium ions and the thing is, to stimulate the fusion further, we need huge influx of calcium ions to elevate the concentration of calcium away from the membrane where the vesicles are generally accumulated. In summary, low level of Ca^{2+} concentrations is needed to induce release of neuropeptides, but a brief influx of Ca^{2+} in response to low frequency impulse will not increase the release of the neuropeptides as much as the small molecule NTs that are stored near the site if influx.

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.



TYROSINE-DERIVED NEUROTRANSMITTERS

Dopamine, norepinephrine, and epinephrine

These neurotransmitters are synthesized sequentially

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)

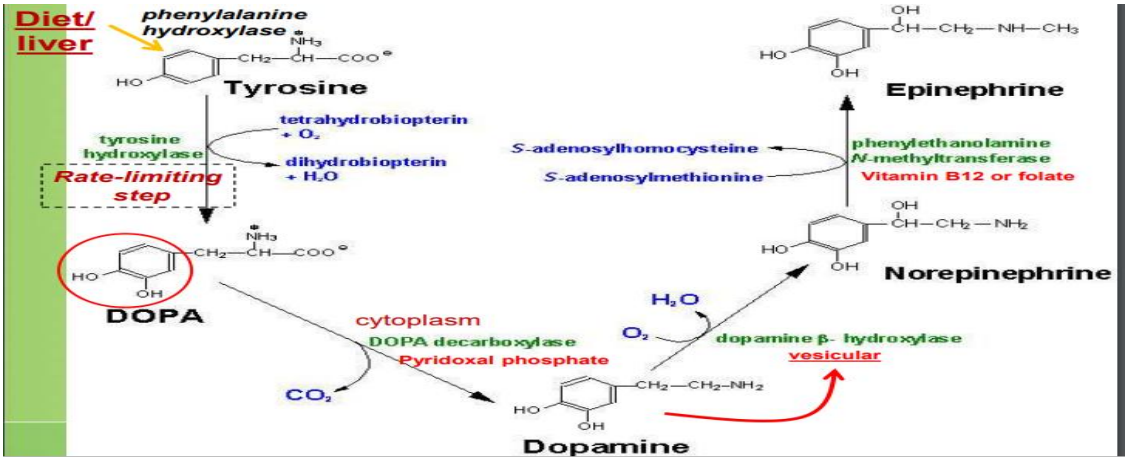
Steps of the synthesis:

1- Tyrosine is converted into DOPA by an enzyme known as **tyrosine hydroxylase**. This step requires **BH4** as a cofactor. The source of tyrosine is either from the diet or synthesis in the liver

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.



the **rate limiting step** in the synthesis of the aforementioned neurotransmitters is the first step (converting tyrosine into DOPA), it is highly **regulated**.

Notice the **ring structure that is called the **catechol** moiety and that is why these molecules are known as catecholamines (catechol represents the ring structure and the amine represents the amine group at the other end of the molecule)

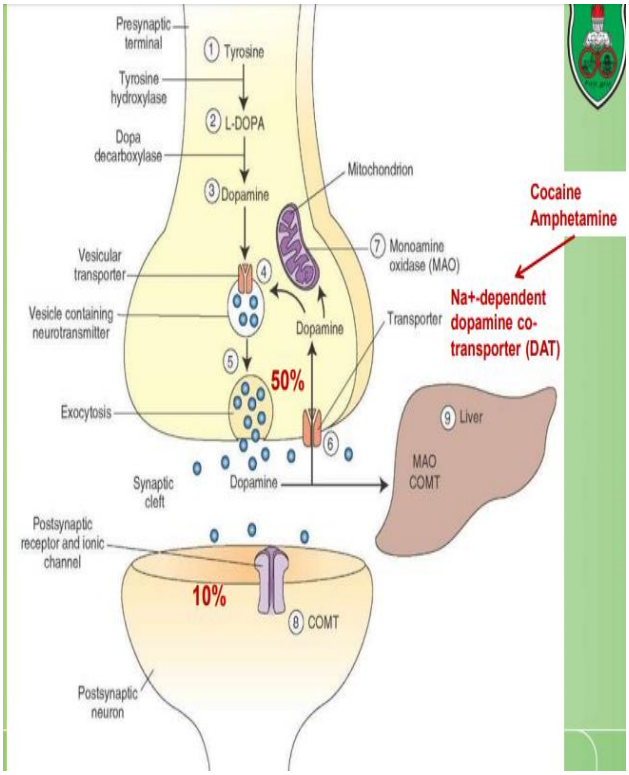
** Notice the differences in synthesis between the following transmitters in terms of required cofactors and enzymes, type of reaction and inactivation, and whether the synthesis is cytosolic or vesicular.

Dopamine

The enzymes are transported from the cell body to the terminus and there they produce dopamine **from tyrosine**. Dopamine is then <u>transported</u> 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: **<u>50%</u>** of the released dopamine can be taken up again by the **presynaptic neuron**, whereas **<u>10%</u>** are used by the **postsynaptic neuron**. The **rest** is either <u>degraded</u> by certain enzymes, removed enzymatically by <u>monoamine oxidase</u> or <u>methyltransferase enzyme</u> or diffuse out of the synaptic cleft to be eliminated by the <u>liver.</u>

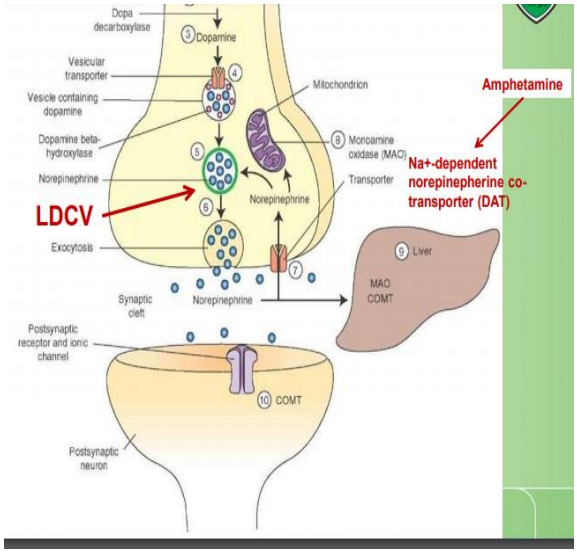
**Effect of cocaine and amphetamine: they prevent the uptake of dopamine, so that it stays for a longer period in the synapse and stimulates the postsynaptic neuron for a longer time. And this explains the feel of awaking and excitement produced by cocaine.



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)

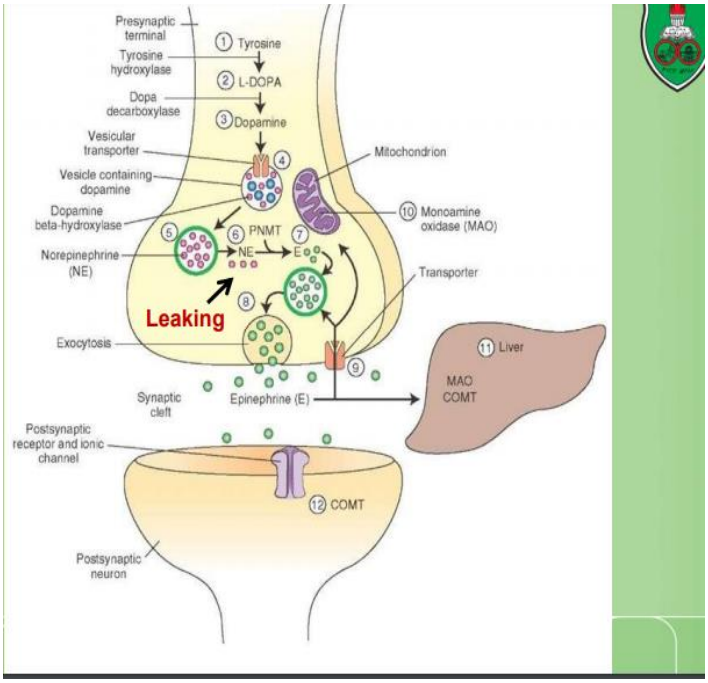
<u>Monoamine oxidase (MAO) or methyltransferase</u>

**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.

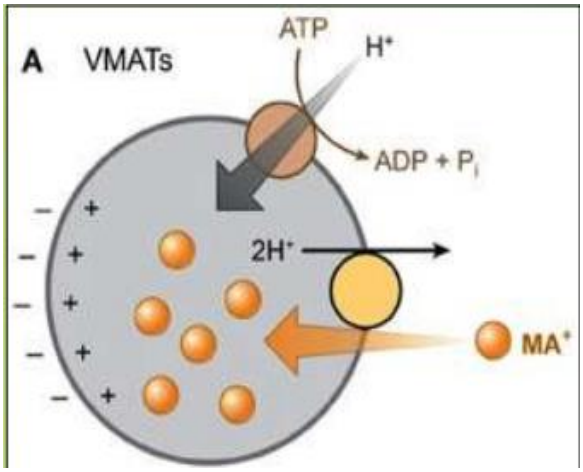


Packaging into vesicles

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 **transporter**s:

1- **the vesicular ATPase** (for protons)
2- **the vesicular monoamine transporter** (VMAT) – (for neurotransmitters)



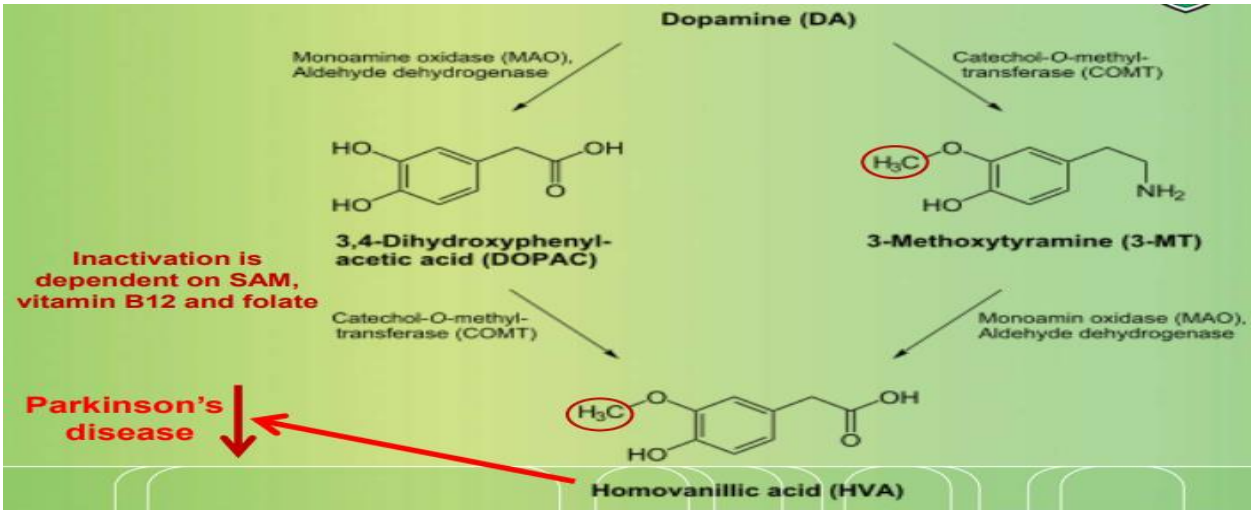
The inactivation

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**)



**Interstingly, the decrease in the level of homovanillic acid is associated with Parkinson's disease.

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) the enzyme that catalyses this step is Tyrosine Hydroxylase, 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)

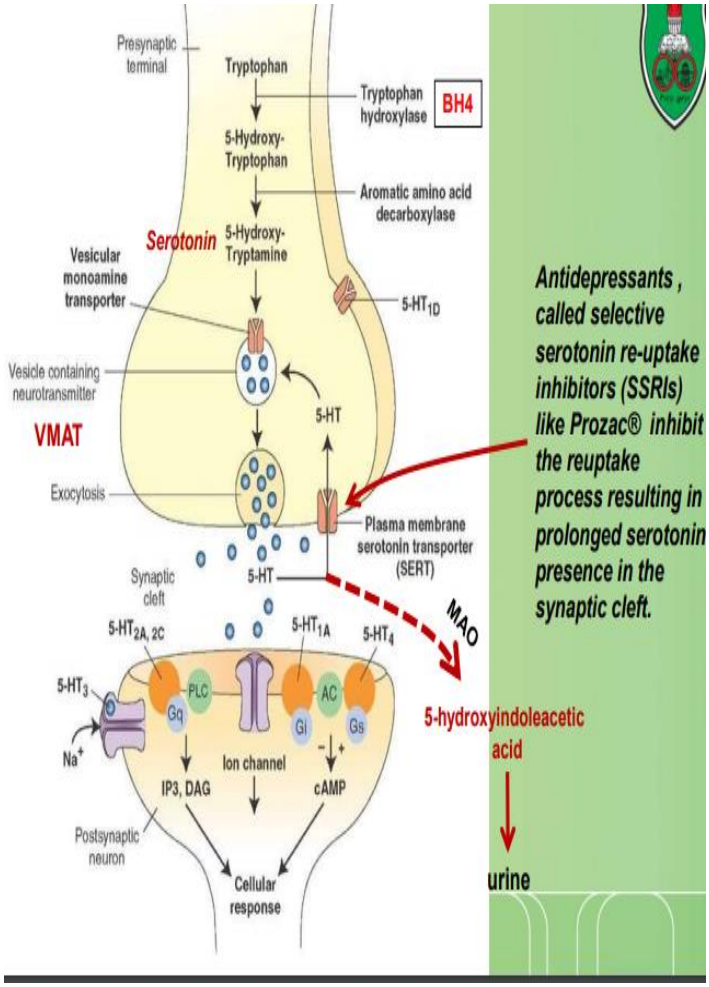
Serotonin which is also known as 5-hydroxy-tryptamine

Synthesis of serotonin:

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

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

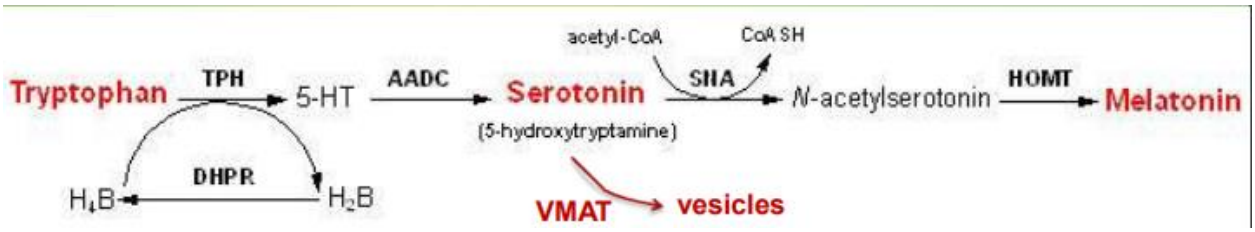
3- Packaging of serotonin into small synaptic vesicles waiting for the Ca^{2+} influx



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**.

Note: Serotonin is packed into vesicles in a similar manner to chachecolamines.



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

GLUTAMATE AND ASPARTATE

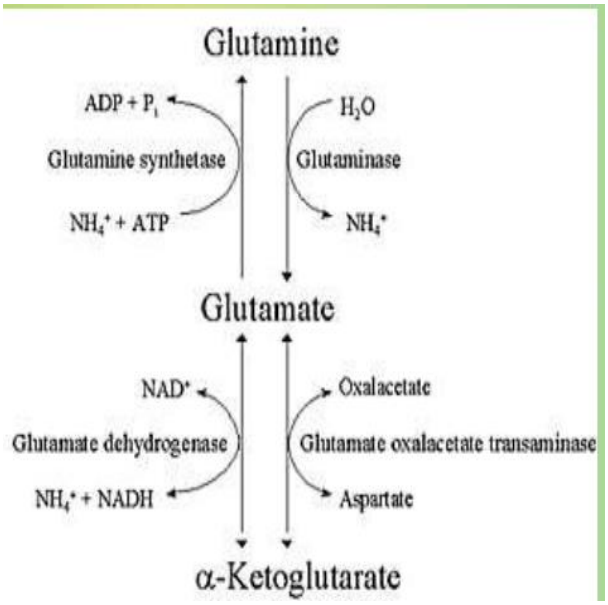
They are **nonessential** (can be synthesized in sufficient amount) amino acids that **do not cross Blood Brain Barrier** and must be **synthesized in neurons**.

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

**Both are excitatory neurotransmitters.

Glutamate

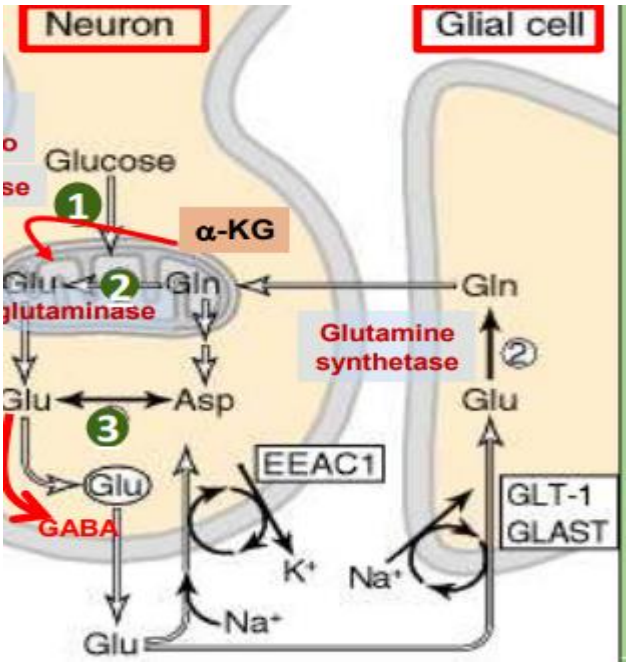
Sources of glutamate:



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

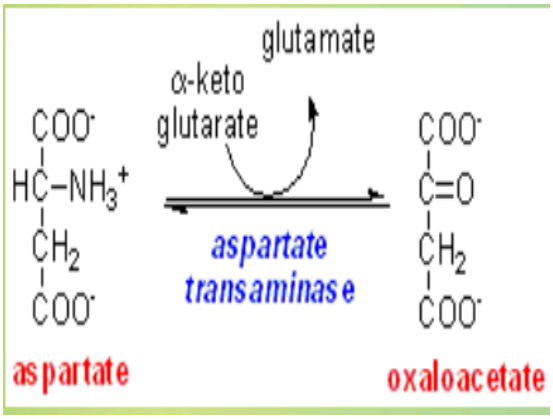
b- Aspartate, to be converted back into glutamate as well.

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



Notes:

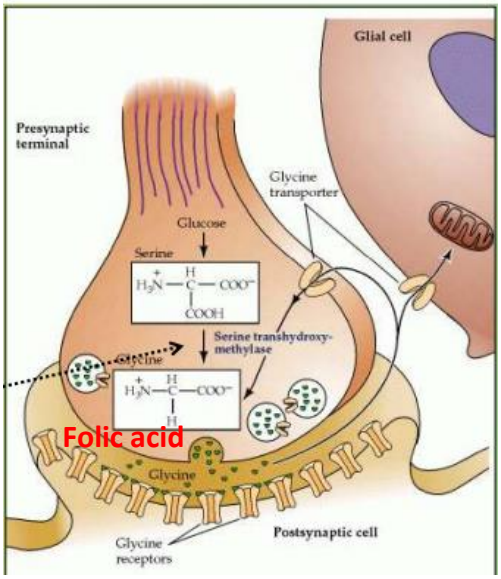
- aspartate becomes oxaloacetate if deaminated, α-ketoglutarate becomes glutamate if deaminated.
- The vesiculo-reuptake mechanisms are not shown because there is a controversy whether aspartate is a neurotransmitter or not.

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

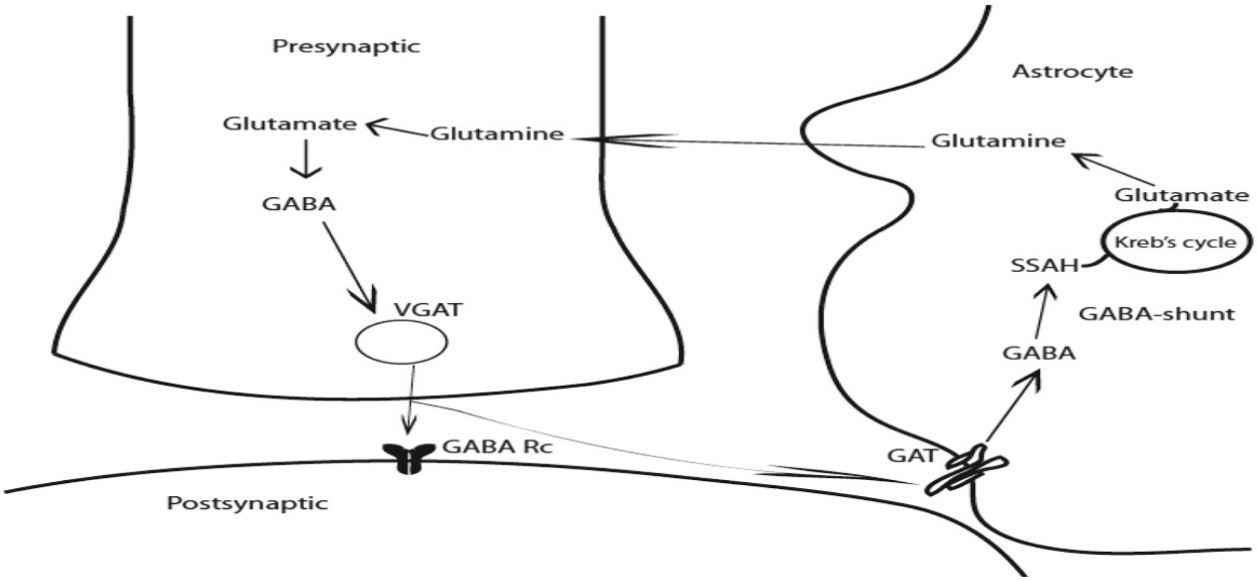
monoamine neurotransmitters in the same regions. This high concentration requires a pathway known as the GABA shunt to preserve the NT and prevents its removal

The GABA shunt is a closed-loop process with the dual purpose of producing and conserving the supply of GABA.

In this pathway:

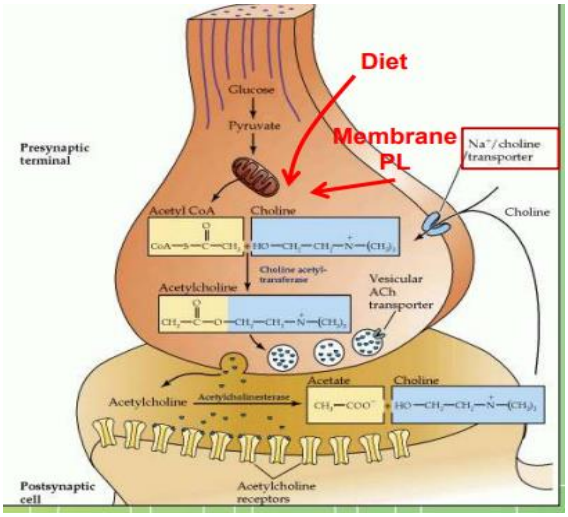
1- Glutamine is converted into glutamate by glutaminase in neuron itself.
2- 2-Glutamate is ⟨-decarboxylated forming GABA via glutamate decarboxylase (GAD), which requires pyridoxal phosphate (vitamin B6).
3- GABA is stored in vesicles until released in a similar mechanism as all of the other small molecule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



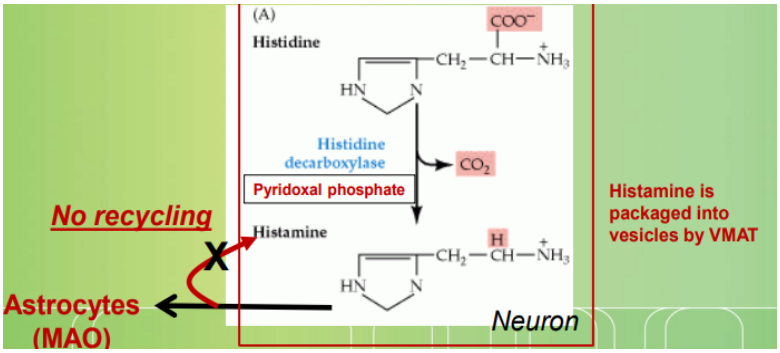
There are different sources of choline: diet or phospholipids in the plasma membrane

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

(+) sarin is a gas used as chemical weapon, it targets **acetylcholinesterase.**

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).[(+) monoamines are molecules with an aromatic ring and an amine group, so these molecules are transported by the same transporter which is the vesicular monoamine transporter VMAT].



It can be <u>inactivated</u> **two enzymes** either the histamine <u>methyltransferase</u> or <u>diamine oxidase</u> (aka histaminase). It has an important property that there is no mechanism to reuptake histamine into the presynaptic neuron and by the **MAO** in the neighbouring astrocytes once 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
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)

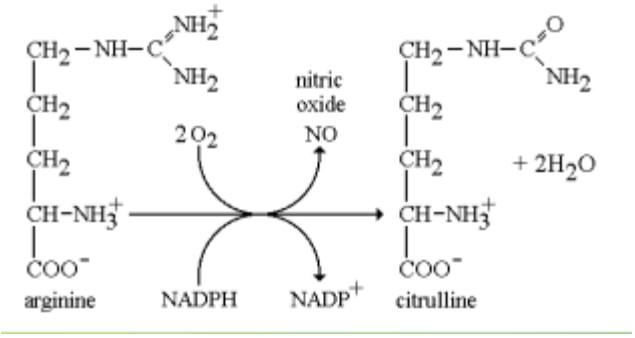
√ It does not interact with receptors on target cells, rather it **diffuses** and **interacts** with **enzymes** inside the postsynaptic cell ‘s cytosol.

√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): E**ndothelial cells lining blood vessels activated by the influx of extracellular