Introduction

What is a neurotransmitter?

Basically it is a molecule that is **synthesized and stored** in a presynaptic neuron (the enzymes needed for its synthesis must be present in the neuron), then it is **packed in vesicles** with the enzymes and stay there until there is **signal** which is usually **influx of Ca^{++}** and as **Ca^{++}** goes in, these vesicles will **fuse** with the plasma membrane **releasing** the neurotransmitter then the neurotransmitter act on the post synaptic neuron by **binding to a receptor** inducing a signal inside the post synaptic neuron.

So the response is usually **fast** and **reversible** because eventually we want the action of neurotransmitter to be terminated and this is done by different mechanisms.

There are **three** types of neurotransmitters:

1-Small-molecule
– Amines (acetylcholine, epinephrine, dopamine, histamine, etc.)
– Amino acids (glutamate, aspartate)
2- Neuropeptides
3- Gases (nitric oxide, some people consider CO2 as a neurotransmitter

When we study neurotransmitters we should know the differences between them in terms of:

- Onset and duration of action
- Concentration for action and receptor binding
- Concentration of [Ca+] required for release of neurotransmitters
- Site of synthesis (which cell and where exactly inside the cell), modification
- Fate (termination of action)

❖ Neuropeptides

There are numerous molecules that can be considered as neuropeptide {More than 50 neuropeptides have been described} and they have variety of action so they can control things like:

- Behavior
- Pain perception
- Memory
- Appetite
- Thirst
- Temperature
- Homeostasis
- Sleep

Do we consider the neuropeptide as a nuerohormone or a neurotransmitter?

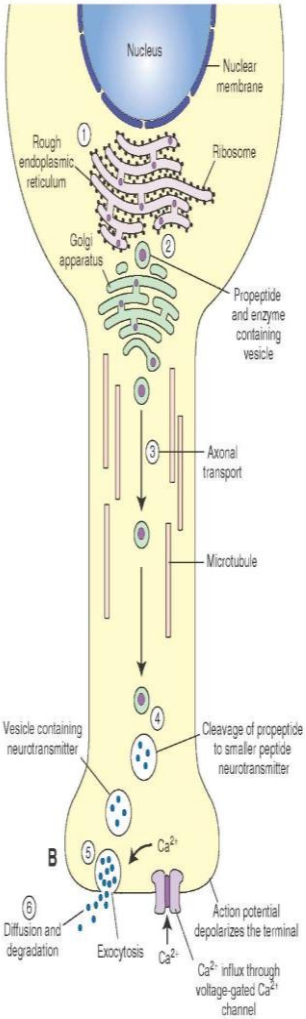
It can be both:

- If it is released in the blood stream and work in a place away from where it is released then it is considered as a neurohormone

- If its action is close to the place it is released from then it is considered a neurotransmitter (as what occurs in the synapse)

Neurotransmitters can be classified in two ways:

1-Families: (Tachykinins, Insulins, Somatostatins, Gastrins, Opioids)
2- Amino Acid sequence: such as Opiate family, which has (**Tyr-Gly-Gly-Phe**-…-OH)



<u>Synthesis:</u> they are synthesized in the **cell soma** (the cell body of the neuron)

1- they are synthesized on the surface of **Rough Endoplasmic Reticulum** (How: they are targeted with a signaled sequence on the N-terminal which indicates that this molecule should complete it's synthesis in RER) as it goes to RER the signaled sequence cleaved off, and the protein goes in the RER and gets modified.

2- Then it goes to **Golgi** where it get packed in vesicles called **Large Dense Core Vesicles LDCV** (they are called large because they are relatively larger than synaptic vesicles) and inside these vesicles we have the polypeptide and we also have the enzymes that are necessary for completing the processing of this peptide.

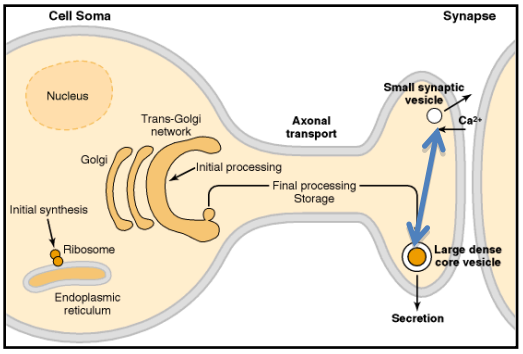
3- The vesicles attaches to microtubules and travels along them to the periphery of the neuron (the presynaptic region) by a mechanism known as **fast axonal transport {During the transport, proteases cleave the precursor neuropeptide into the final mature form.}**

4- as it goes there it gets processed further and stays there waiting for a signal (influx of Ca^{++})

5- fusion of the vesicle with the cell membrane and then the release of the neuropeptide in the synapse, the release of the peptide is gradually and the response is prolonged so they take a long time in the synapse.

6- the signal is terminated by enzymatic **degradation or diffusion** (the neuropeptide travel away from the synapse)

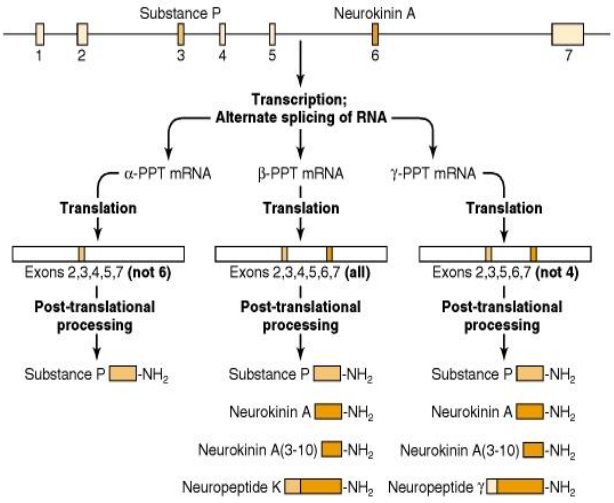
Release of neuropeptide is controlled by influx of Ca^{++}, but we should notice that the **site of Ca^{++} influx** is far away from where the LDCV are and this is different from synaptic vesicles where the site of Ca^{++} influx is close to the vesicles, other difference between the LDCV and synaptic vesicles is that the synaptic vesicle is close to plasma membrane where as the LDCV can be found far from the plasma membrane



Diversity: alternative splicing

From the same gene we can produce different neuropeptides by a number of mechanisms one of these mechanisms is **alternative splicing** (having a different connections between different exons) so depending on how the mRNA get spliced we can have different neuropeptides

What we have to know from this that we can have many different neuropeptides that originates from the same gene such as substance P & Neurokinin



The alternative splicing takes place in different cells and differs from tissue to other (**tissue specific**)

Another level of diversity occurs by **Posttranslational processing** (huge polypeptides can be processed in different ways by proteases to generate different types of neuropeptide and it is tissue specific) so it is controlled by the proteases and how they cleave (process) the polypeptide.

How the cell Packaging the polypeptide as well as the enzymes (proteases) creates another level of diversity also

What also interface with proteases actions are carbohydrates by attaching to the polypeptide and mask the site that proteases work at .

So there is different levels of regulation in terms of neuropeptide synthesis at the RNA level of transcription, at the level of alternative splicing, in ER, in Golgi ,sugar attachment sites, the cleavage of the polypeptide, the packaging and storing of the neuropeptide, the proteases that work in t he Large dense core vesicles, degradation of the neuropeptide in the lysosomes (and that controls how much neuropeptide the cell contains or can be secreted)

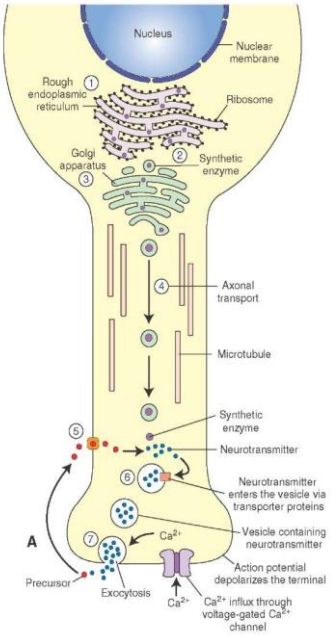
Neuropeptides (the doctor said just read them, the physiology will cover them)

- **The endogenous opiates**
- **Neuropeptide Y**
- **Galanin**
- **Pituitary adenylate cyclase–activating peptide (PACAP)**
- **Melanocyte-stimulating hormone (MSH)**
- **Neurokinin A (NKA)**
- **Substance P (SP)**
- **Neurotensin**
- **Calcitonin-gene–related protein (CGRP)**
- **Vasoactive intestinal polypeptide (VIP)**

❖ Small-molecule neurotransmitters

These molecules are small relative to neuropeptide and they are produced from amino acid or their derivatives or produced from an intermediate of Krebs cycle or Glycolysis.

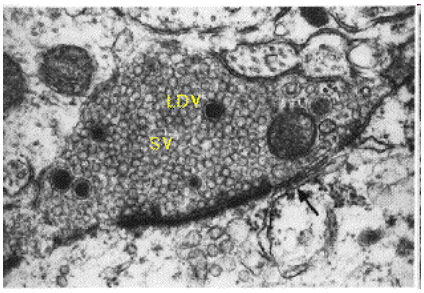
Synthesis :



The enzymes (not the NTs themselves) are synthesized in the **cell soma** and then the enzymes themselves travel along microtubule via **2 mechanisms (Small Axonal Transport or Fast Axonal transport)** " remember for neuropeptide it was **only** Fast axonal transport" "and there what was travelling is the neurotransmitter itself but here **what travels is the enzymes**" so when the enzymes reach the periphery of the neuron then they are packaged inside vesicles along **with precursor of the neurotransmitter** itself, inside these vesicles neurotransmitters are synthesized, after the neurotransmitter is synthesized in the vesicle it become so **close** to the plasma membrane and waits for a signal (Ca^{++} influx) which goes in and vesicles fuses with plasma membrane

[Ca+] = 2 mM in the synapse, **[Ca+] = 0.1 uM** inside the cell and when it's get stimulated and Ca^{++}enters it becomes up to 100uM

which indicates a rapid increase in [Ca^{++}] .



"we should notice here that the site of vesicular fusion is close to the site where Ca^{++} enter that different from neuropeptide and also the vesicles are close to the plasma membrane"

The action here is short and can be terminated by **diffusion**, **reuptake** (can be taken up by the same cell that release the neurotransmitter or by the post-synaptic cell) and **synaptic inactivation**.

Another difference from neuropeptide is that the fusion of vesicles and **release of neurotransmitter is done in pulses** as "Ca^{++} goes in –fusion .. Ca^{++} goes in –fusion".

The role of cofactors:

- **S-adenosylmethionine (methyl transfer) "**whenever we see an enzyme that works as methyl transferase then it depends on this Co factor"
- **Pyrodoxal phosphate (vitamin B6): transamination, decarboxylation**
- **Tetrahydrobiopterin (BH4)**

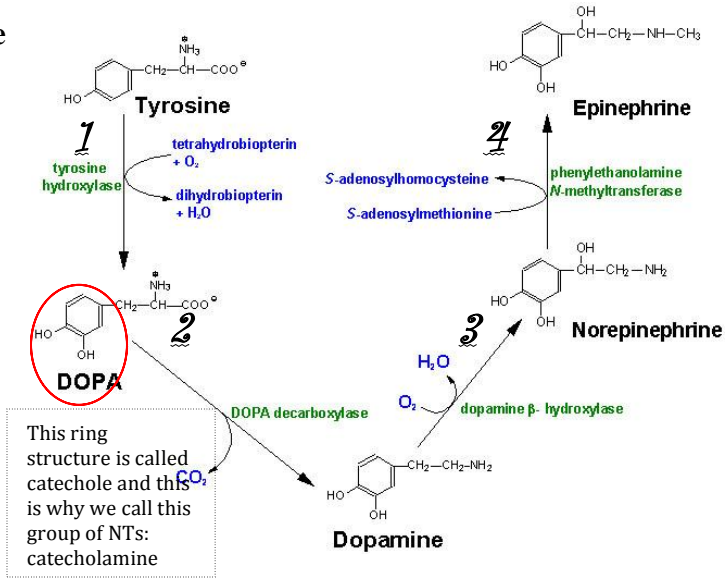
Tyrosine derived neurotransmitters: Dopamine, norepinephrine, and epinephrine

Tyrosine is found in diet as well as it can be synthesized in liver

1-It is modified by **Tyrosine hydroxylase** which add a hydroxyl group to tyrosine producing DOPA

I-This reaction is the rate limiting reaction and under strict control

II-this reaction requires BH4 as a cofactor



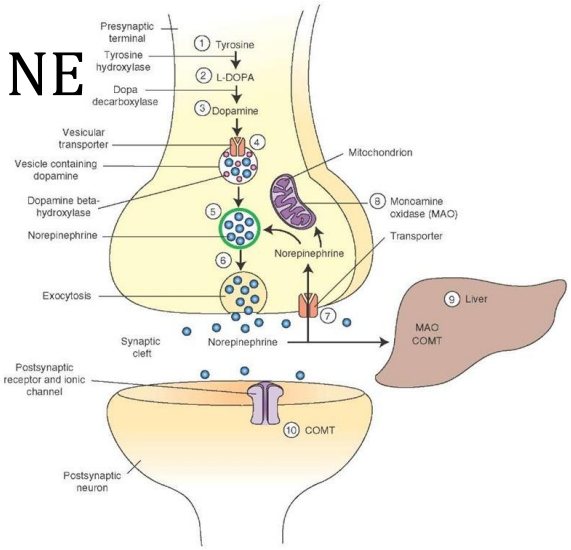
2-DOPA is converted into Dopamine via **DOPA decarboxylase**, **which requires pyrodoxal phosphate**. This reaction takes place in the cytoplasm of the cell's periphery

3-Dopamine goes into vesicles where Dopamine Hydroxylase can convert it into NE

4-NE is converted to Epinephrine and this reaction requires **vitamin B12** as well as **Folate** and **S-adenosylmethionine**

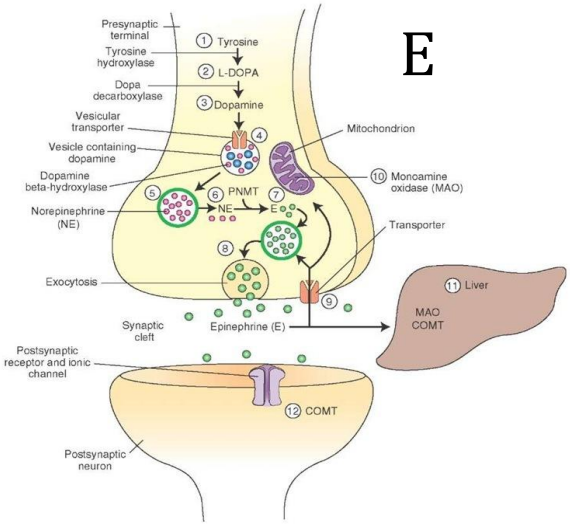
Once dopamine is released most of it is taken up by the pre-synaptic cell and once it is inside 50% of it is repackaged in synaptic vesicle, some of it goes into Mitochondria where it gets inactivated by an enzyme known as monoamine oxidase **MAO**, 10% of the dopamine is taken up by the post synaptic cell and it can be inactivated by another enzyme **COMT** (methyl transferase), if dopamine diffuses away the synapse it gets in the blood stream goes to liver where it can be inactivated by these two enzymes.

NE



Here dopamine is converted to NE and it waits now for a Ca^{++} to influx so it can be released, once it is released it continues it's story as Dopamine "refer to the last paragraph in the previous page"

E



NE leaks out the vesicle and gets converted to Epinephrine and then repackaged again in vesicles and waiting now for a Ca^{++} to influx so it can be released it continues it's story as Dopamine "refer to the last paragraph in the previous page"

"Notice that Dopamine and Epinephrine are synthesized in the cytoplasm whereas the NE is synthesized in the vesicle"

The inactivation of catecholamine takes a place by **MOA & COMT** (methyl transferase) and these tow enzymes can work without specific order so either can work first then followed by the other (i.e.: MOA first then COMT or COMT then MAO) after inactivation we will end up with **HVA (Homovanillic acid)** this product is a biomarker of **Parkinson's disease** where it's **level is reduced** in this patient.

Inactivation is dependent on SAM and vitamin B12 and folate

Regulation of synthesis of catecholamine:

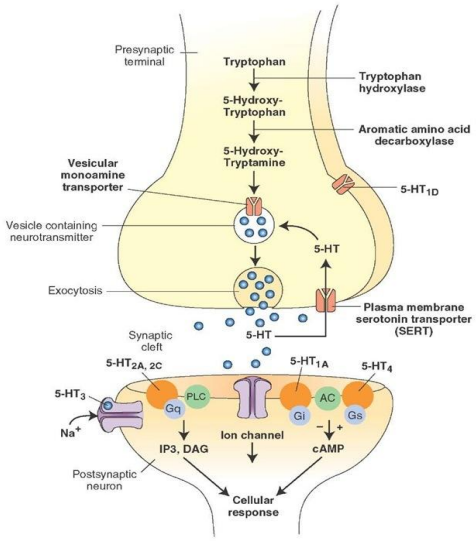
1- long term regulation: is basically controlling the synthesis of the gene and the gene activity itself of the enzymes (tyrosine hydroxylase as well as dopamine hydroxylase)

2- short term regulation:

- Inhibition by free cytosolic catecholamines
  - Catecholamines compete with BH4 binding to enzyme
- Activation by depolarization
  - Tight binding to BH4 following phosphorylation by PKA, CAM kinases, PKC

Tryptophan-Derived Neurotransmitters: Serotonin and melatonin

Serotonin is produced from tryptophan. Tryptophan is **hydroxylated** in a reaction that requires **BH4** and produces **5 Hydroxy-Tryptamine 5-HT** (the scientific name of serotonin) then it is packaged in the cell waiting for a signal.

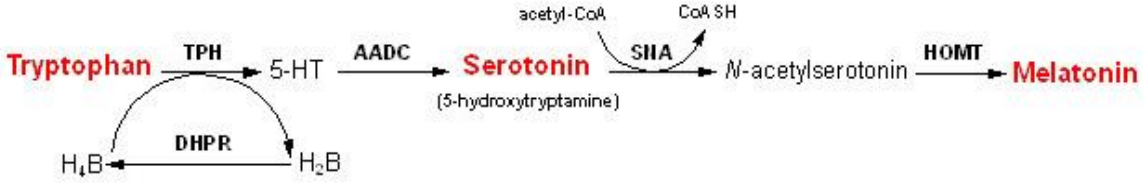


MAO inactivates it, and the signal can be terminated by **reuptake** of 5-HT back to the presynaptic cell and this reuptake mechanism is the main target for **treating depression**, by giving the pt**. serotonin reuptake inhibitor.**

Serotonin is responsible for **feeling happy & energetic** so giving depressed pt. serotonin reuptake inhibitor will keep the serotonin in the synapse for a **longer time.**

Melatonin is a neurotransmitter that is synthesized in **pineal gland** from serotonin, It is responsible for **sleep patterns, Seasonal and circadian (daily) rhythms & Dark-light cycle**.

Infant's produces high levels of melatonin and **the production of melatonin decreases with age**, and that's why infants sleep for a long time Vs. old people who sleep for 4-5 hours.

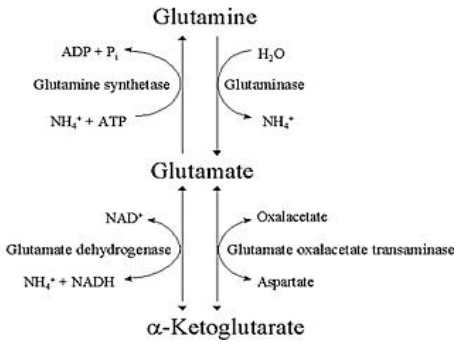


Glutamate and aspartate: Some amino acids can work as neurotransmitters

Both are:

- Nonessential amino acids
- Do not cross BBB
  - Must be synthesized in neurons (CNS)
- Main synthetic compartments
  - Neurons - Glial cells
- Both are excitatory neurotransmitters.

GLUTAMATE:



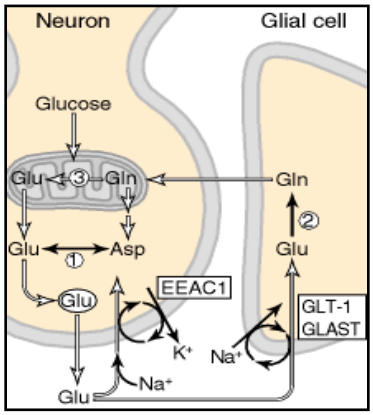
Synthesis:

There are 3 sources of GLU:

1- Glycolysis → Krebs cycle → **α-KG** receives amino group by Transamination reaction and produces GLU

2- **Glutamine** (deamination by removing the amino group of the R group)

3- **Aspartate** transamination reaction



GLU can be released and work as a neurotransmitter or it can be involved in the synthesis of GABA

Once it is outside, it can be inactivated by reuptake in the presynaptic nerve or into glial cells, inside glial cells GLU is converted to glutamine that is released then it goes to the neuron and starts the cycle all over again.

Aspartate

It is controversial whether it is considered as neurotransmitter or not, one reason is that a vesicular uptake mechanism for aspartate has not yet been demonstrated.

It's Precursor: **oxaloacetate (transamination)** from Krebs cycle.

Glycine

Is synthesized from **3-phosphoglycerate** that is converted to **serine** and serine is converted to **Glycine.**
High affinity transporters located in both presynaptic and glial cells can remove it.

GABA

It is present in high concentrations in many brain lesions.

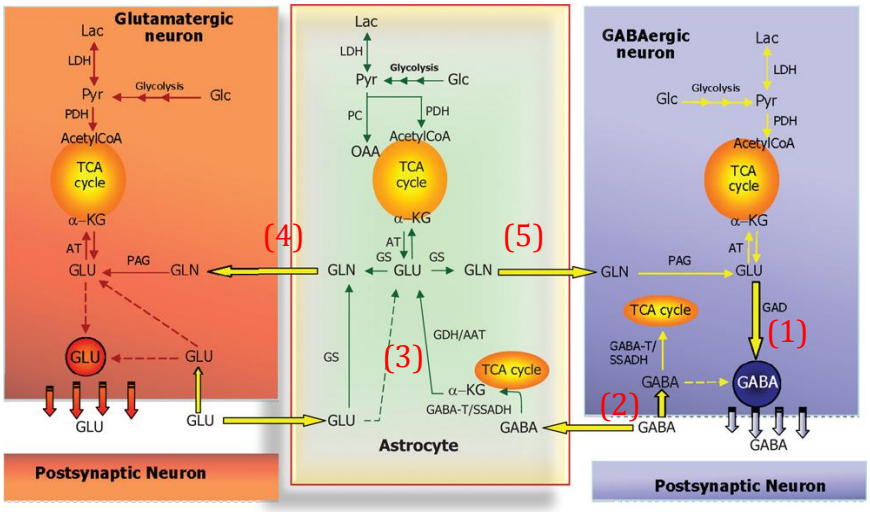
These concentrations are about 1,000 times higher than concentrations of the classical monoamine neurotransmitters in the same regions.

Since GABA is really important and is needed in high concentrations, there must be a mechanism by which we preserve GABA and this done via what is known a **GABA Shunt.**

(1) Glutamic acid is converted to **GABA** in **GABAergic neuron**

(2) GABA once it is releases it is **taken up** by the **pre-synaptic cells or by Astrocytes**

(3) In Astocytes GABA enters Krebs cycle and is converted to **α-KG** which in is converted to **Glutamate** then this Glutamate is converted to **Glutamine**



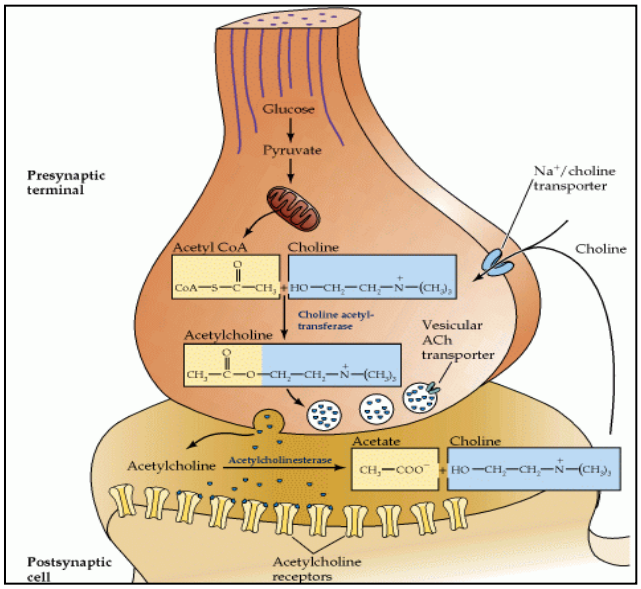
Glutamine has two gates out now either it (4) enters **a Glutamatergic neuron** where it is converted back to Glutamate that is released from the neuron as neurotransmitter **<u>or</u>** (5) it enters **GABAergic neuron** where it is convert to Glutamate then **GABA** and so on …

<u>Ach</u>

Is synthesized by an enzyme called **Choline AcetylTransferase (CAT)**

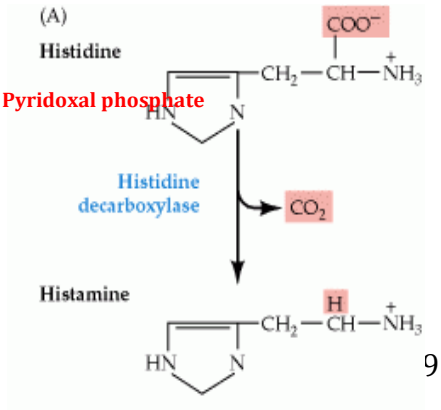
Choline sources:
1- diet
2- **plasma membrane** (one of plasma membrane components is **Phosphatidyl<u>cholines</u>**)

Then it is packaged in vesicles and released, once it is released, Ach must be inactivated (**hydrolysis)** by the enzyme **AchEsterase.**



<u>Histamine</u>

It is synthesized from **Histidine** in a reaction that requires **Pyridoxal phosphate**, once it is released it can be **inactivated in astrocytes by MAO.**
It does not penetrate the blood brain barrier and, hence, must be synthesized in CNS.



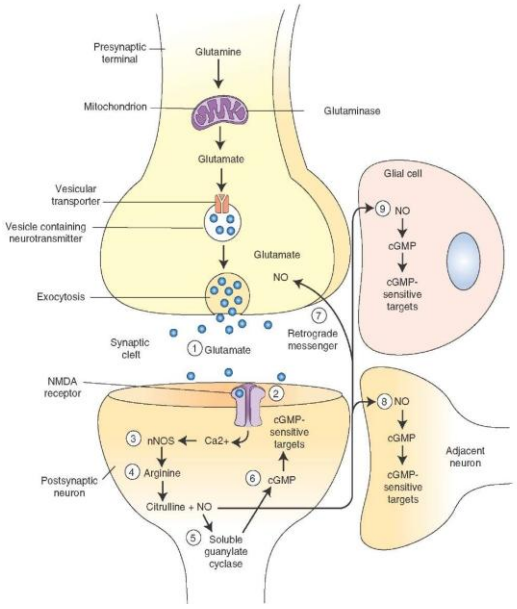
What makes Histamine different from other neurotransmitters that there is no mechanism that takes Histamine back to the same cell, it goes to other cells, SAM can inactivate it in brain but in peripheral tissues it is inactivated by number of enzymes

❖ Gases

NO

It is a gas and it is different from other neurotransmitters in number of aspects one of them that it is not synthesized in the presynaptic cell rather it is synthesized in the post synaptic cell.

(1) Glutamate is **released**
(2) Acts on NMDA **receptors** located on the **post-synaptic neuron**
(3) **Ca2+ enters** the postsynaptic neuron and **binds with Calmodulin activating NOS**
(4) This results in formation of **NO** and citrulline **from L-arginine.**
(5) NO **stimulates guanylate cyclase** forming **cGMP**
(6) This results in a **physiological response**



NO can diffuse out:

a) To the **presynaptic terminal** (***retrograde messenger***) (7) prolonging effect

b) Into adjacent **neurons (8)** and **glial cells (9**) stimulating guanylate cyclase

NO is **inhibited by hemoglobin** and other **heme proteins** which bind it tightly and it's half life is very short **2-4 seconds**

Is NO a neurotransmitter ?

- **Yes, but:**
    - It is not stored in vesicles
    - It is not released by calcium-dependent exocytosis (it diffuses)
    - Its inactivation is passive (there is no active process that terminates its action, there is no enzyme, it just diffuses)

- It decays spontaneously (very short half life 2-4 hours)
- It does not interact with receptors on target cells
- Its sphere of action depends on 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

There are **three** isoforms of **NO synthase**, what we really concern about is **Isoform I** that is **found in neurons**, the other isoforms are also very important as **NO** works as **a vaso-dilator.**

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

Done by: Mohammad Alqaisieh

Sorry for any mistakes ^_^