CENTRAL NERVOUS SYSTEM

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- ○ Anatomy
- ○ Physiology
- ○ Pathology
- ● Biochemistry
- ○ Microbiology
- ○ Pharmacology
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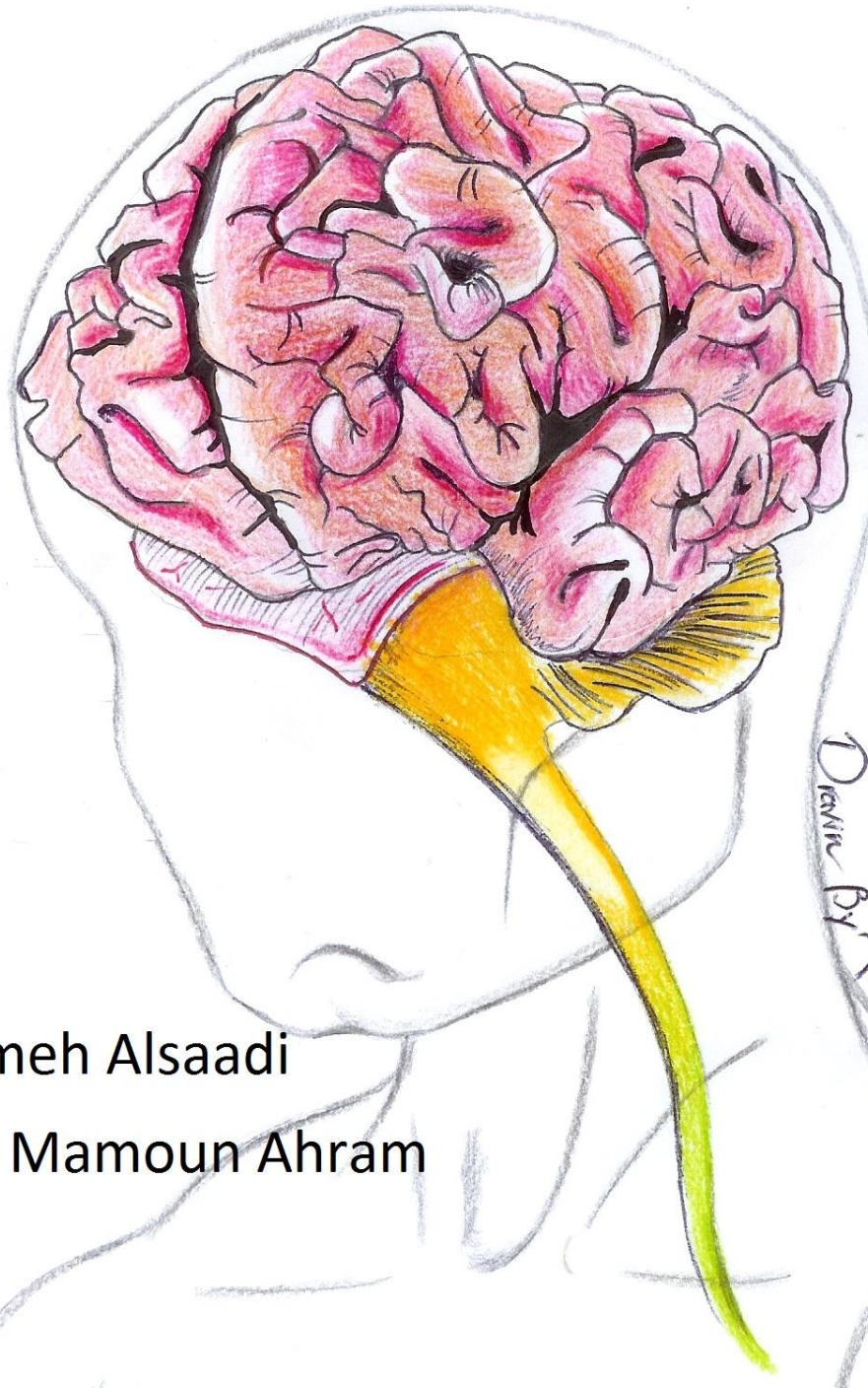

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Dr. Name: Dr. Mamoun Ahram

Lec #: 1

There is absolutely no need for you to refer back to the set of slides provided by the professor. Everything present in the slides was incorporated here, including the figures.

The professor did not read the definition of neurotransmitters or the functions of neuropeptides.

The professor asked us to focus on the differences between neuropeptides and small-molecule neurotransmitters, as well as the needed cofactors for the progression of the mentioned reactions.

SMNTs: Small-molecule Neurotransmitters

According to the professor, these are the references that you should use:

- This lecture
- Mark's Basic Medical Biochemistry, 4th ed, pp. 908-918
- http://what-when-how.com/neuroscience/neurotransmitters-the-neuron-part-1/

This sheet is dedicated to Shosho girls and the two and only Shosho boys out there.

Your feedback is highly appreciated.

The Biochemistry of Neurotransmitters

Neurotransmitters:

A neurotransmitter is defined as a chemical substance that is synthesized in a neuron, released at a synapse following depolarization of the nerve terminal (usually dependent on influx of calcium ions), which binds to receptors on the postsynaptic cell and/or presynaptic terminal to elicit a specific response.

For a chemical substance to be considered as a neurotransmitter, all of the following conditions must be met:

- Is synthesized and stored in a presynaptic neuron (cell) (the enzymes needed for its synthesis must be present in the neuron),
- Is released at a synapse following depolarization of the nerve terminal (usually dependent on influx of calcium ions) [i.e. signal affecting the presynaptic cell that usually results in the influx of calcium and subsequent fusion of the vesicle with the plasma membrane],
- binds to receptors on the postsynaptic cell and/or presynaptic terminal [once it is released or is in the synapse,
- elicits rapid-onset and rapidly reversible responses in the target cell [stimulates signal transduction inside the cell so that the postsynaptic cell would do a certain action],
- Is removed or inactivated from the synaptic cleft [the neurotransmitter is eliminated, thus the signal itself is terminated, and there are different mechanisms by which this takes place].

Neurotransmitters can be grouped into three classes:

- Small-molecule
  - Amine-containing (acetylcholine, epinephrine, dopamine, histamine, etc.)
  - Amino acids (glutamate, aspartate)
- Neuropeptides
- Gases (nitric oxide) [It has been suggested that carbon dioxide acts as a neurotransmitter as well but the professor does not know whether it is true or not]

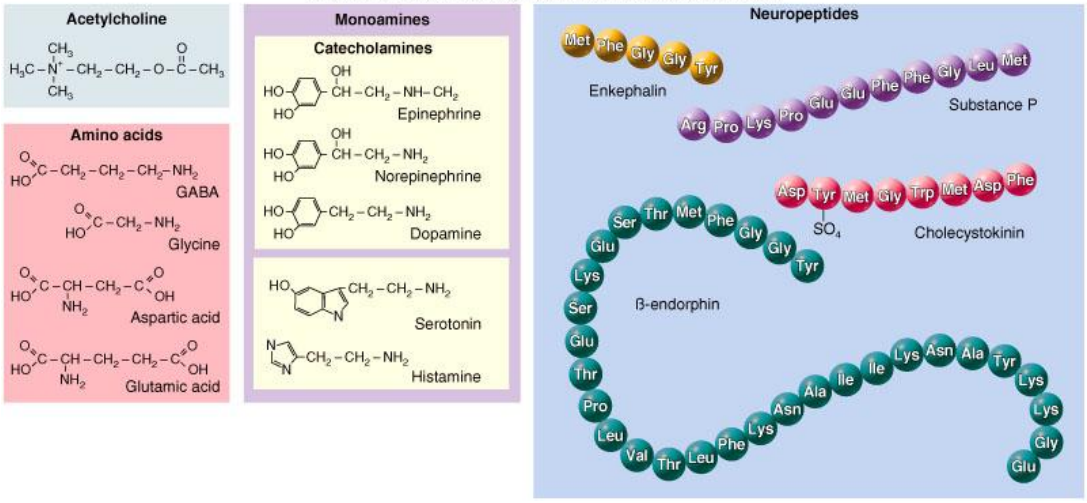
A neurotransmitter,a neurohormone, or both?

- Neurotransmitter: a messenger released from a neuron at an anatomically specialised junction, which diffuses across a narrow cleft to affect one or sometimes two postsynaptic neurons, a muscle cell, or another effector cell (i.e. it has a localized action affecting a neighboring cell).
- Neurohormones: a messenger that is released by neurons into the haemolymph (i.e. lymphatics or the vascular system) and which may therefore exert its effects on distant peripheral targets [acts far away from its site of release].

Some neurotransmitters can also act as neurohormones (e.g. some neuropeptides).

Structures of Neurotransmitters:

These are the different structures of the neurotransmitters. Here you have the single molecule, the small, the amine-containing, the nitrogen containing groups which are really small, then we have the polypeptides and the gas NO as well.



Neuropeptides:

- More than 50 neuropeptides have been described and they have different functions that include regulation of:
  - Behavior
  - Pain perception
  - Memory
  - Appetite
  - Thirst
  - Temperature
  - Homeostasis
  - Sleep

Classification of Neuropeptides:

Neuropeptides can be grouped by structural and functional similarity (according to either their structure or their function).

Sometimes they have similar structures, however, sometimes they look alike but

[illegible]
Tachykinins: substance P, bombesin, substance K Insulins: insulin, insulin-like growth factors Somatostatins: somatostatin, pancreatic polypeptide Gastrins: gastrin, cholecystokinin

have completely different structures.

For example, vasopressin and oxytocin share 7 of the 9 amino acids they composed of (i.e. they only differ in two amino acids), but have different functions.

A closer look at opioids: They have similar functions to some extent.

Looking at their structures, they all share a similar amino acid sequence.

[illegible]	
[illegible]	[illegible]
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

Opiate peptides share a common sequence and all are potent endogenous opiates but with distinct patterns of receptor selectivity.

[Different tissues have different receptors. Since opioids have receptor selectivity, tissue selectivity is present depending on where they are synthesized and produced and depending on the receptor to which they bind.]

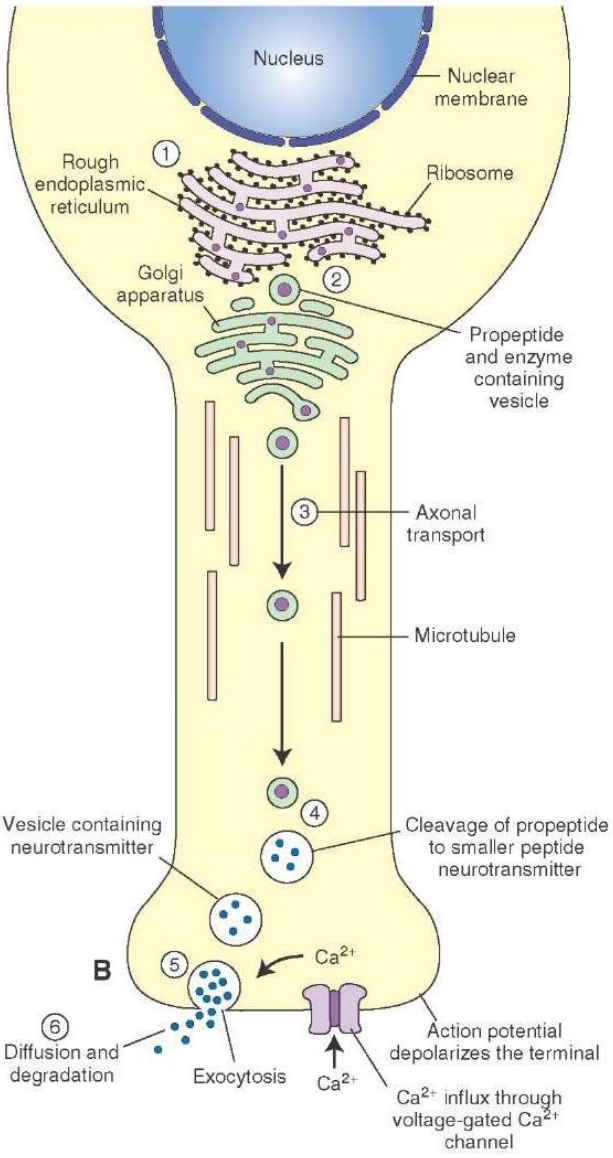
The three glycoprotein hormones from the anterior pituitary, TSH, LH, and FSH, are all dimers and share a common α subunit but have distinct β subunits.

Stages of Action:

- Synthesis (ER and Golgi apparatus)

[They are synthesized from certain genes. They are then inevitably translocated into the endoplasmic reticulum, and then into the Golgi apparatus, where they are modified e.g. by glyclosylation.]

- Packaging into large-dense core vesicles (with modifying enzymes) [They are termed large relative to the synaptic vesicles of small molecule neurotransmitters.The vesicles also contain modifying enzymes.]
- Transport (fast-axonal transport)
  - During the transport, proteases cleave the precursor neuropeptide into the final mature form. [The enzymes work as these vesicles travel from the cytoplasm along the axon into the presynaptic terminal (i.e. the neuropeptides are modified as they are transported) where they wait for an influx of calcium ions.]
- Release
  - They are released gradually over time in response to general increases in the level of intracellular calcium. [Once there is an influx of calcium, the vesicles fuse with the plasma membrane and the neuropeptide is released.]



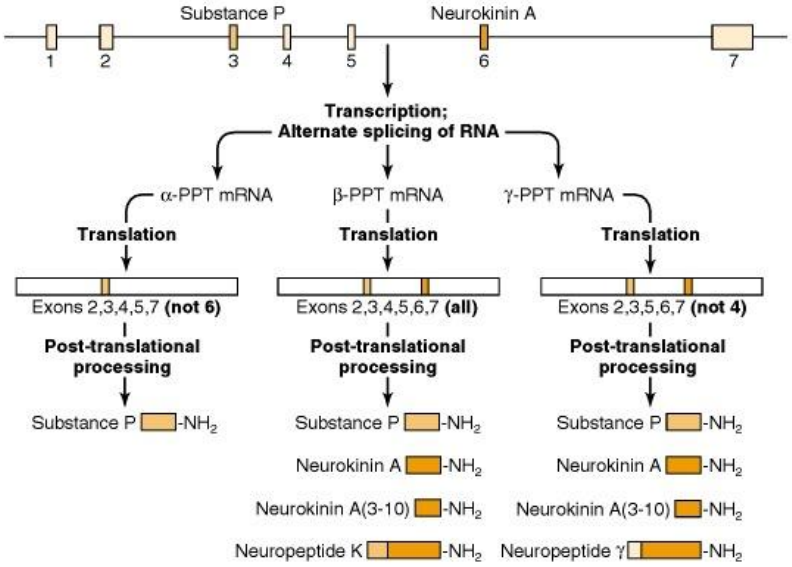
- Action (prolonged) [They act by binding to a receptor in the post synaptic cell]
- Termination by diffusion (away from the postsynaptic cell) and degradation

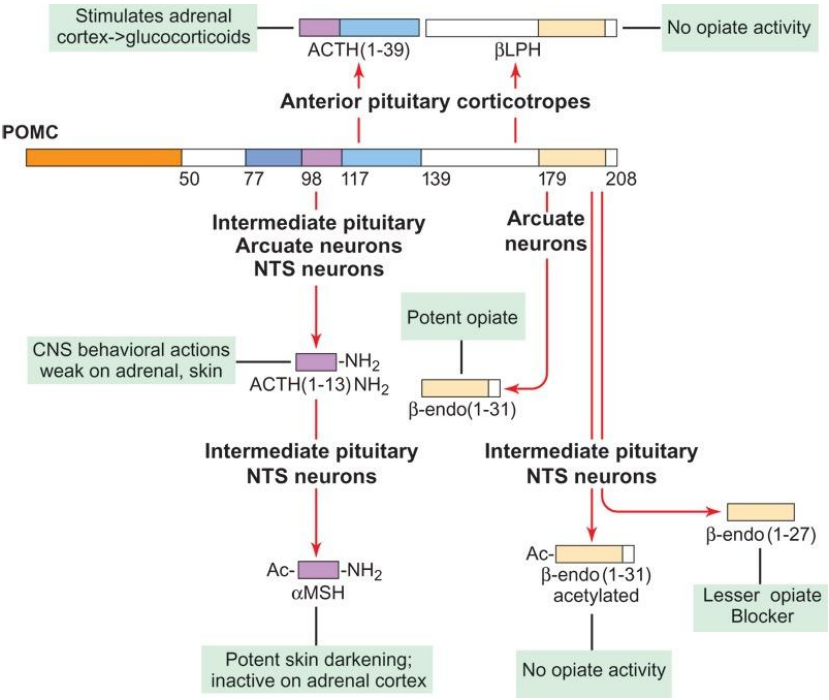
There are different mechanisms by which these neuropeptides are produced:

Diversity: alternative splicing

- Alternative splicing of mRNA results in different mature mRNAs, which 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.
- An example of neuropeptides that are synthesized differently according to alternative splicing is substance P and substance K.
- This alternative splicing is controlled differently in different cells and different tissues and that is why there is a sort of tissue selectivity in the action of these neuropeptides.





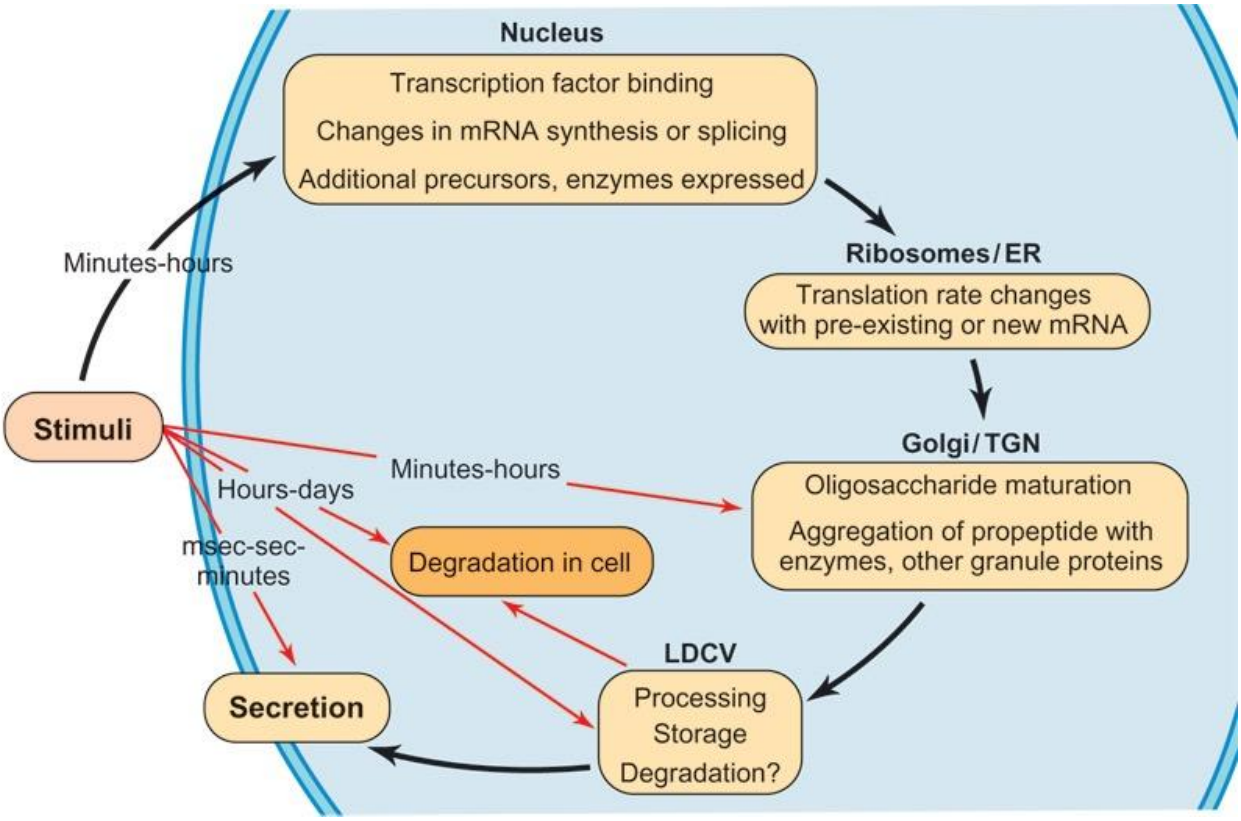
Diversity: proteolytic, differential, sequential processing

- Neuropeptides are produced from a longer precursor protein by :
  - Proteolytic processing. [the protein is cleaved into smaller fragments, each of which is basically a neuropeptide by itself. An example is the pro-opiomelanocortin polypeptide that can produce a number of these neuropeptides.]
  - Vesicular packaging of different proteases that recognize different cleavage sequences. [As mentioned previously, the vesicles contain certain enzymes that can produce two or more of these neuropeptides.]
- Hiding a proteolytic site by post-translational modifications (example: addition of a carbohydrate side chain). [E.g. in glycosylation, the sugar residue that is attached to the polypeptide can mask a proteolytic site. This means that the

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,* melanocyte-stimulating hormone; *PC.* prohormone convertase.

enzyme cannot cut the polypeptide at this site, and that this cell or tissue is unable to produce this specific neuropeptide, but can, however, produce a longer (different) one.]

- Tissue-specific [The resulting neuropeptides differ between tissues because different tissues have different proteolytic enzymes.]



The levels of regulation of neuropeptide expression:

There are different ways by which the production of these neuropeptides is controlled:

-Stimulation: the type of stimulation, the ligand that binds to the receptor, and the presence of a receptor.

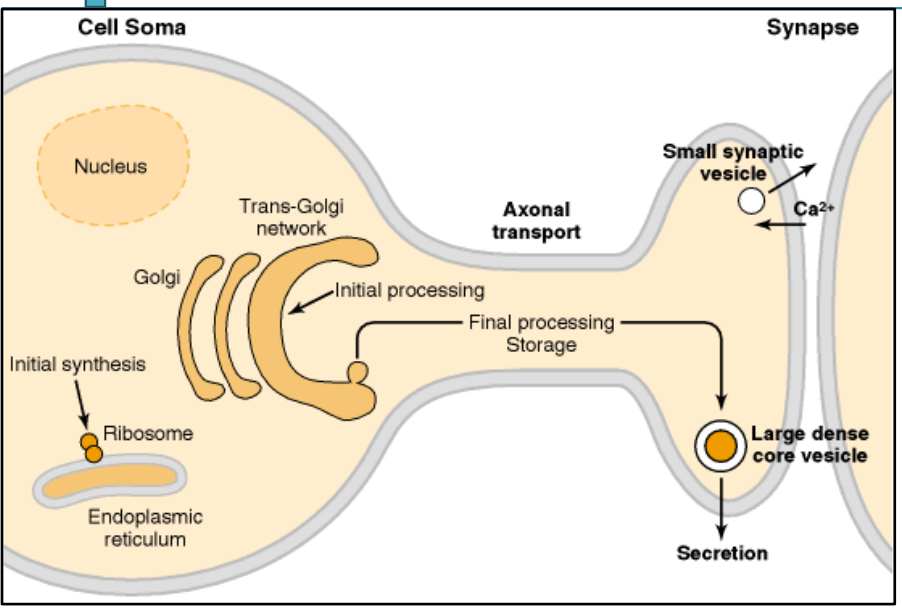
-The expression of certain specific genes.

-The modification in the Golgi apparatus and the endoplasmic reticulum.

-The enzymes that are located along with the neuropeptides inside the vesicles.

-Secretion: the amount secreted and the mechanism by which they are secreted.

-Termination: the mechanism by which they are terminated.



- Vesicles are located further away from the presynaptic membrane and away from place of Ca influx
- Ca influx can be from external of internal sources.

Role of Calcium:

The important difference between the release of neuropeptides and SMNTs is related to calcium.

The release of both is dependent on the influx of calcium ion.

-Magnitude of increase in intracellular calcium concentration:

- Neuropeptides: Low [the release of neuropeptides requires a smaller amount of calcium].
- SMNTs: High [the concentration of calcium that is needed to stimulate the fusion of vesicles with the plasma membrane is higher in the case of SMNTs than neuropeptides].

-Source of calcium:

- Neuropeptides: Either internal or external [be calcium that comes from inside the cell (internal reservoir) or calcium that comes from outside the cell].
- SMNTs: External [the influx of calcium from outside to inside of the cell].

-Distance from the site of entry of calcium ions:

- Large-dense core vesicles containing neuropeptides: At a very far distance
- Synaptic vesicles containing small-molecules neurotransmitter: Very close

Small-molecule Neurotransmitters (SMNTs):

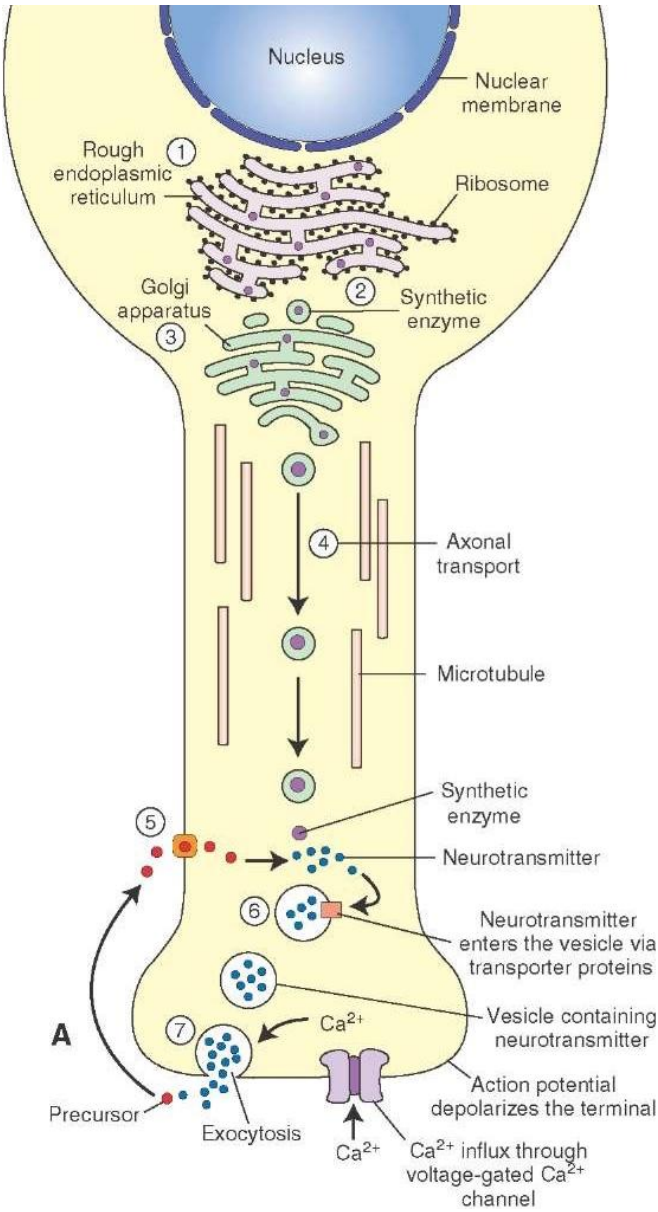
Types of small-molecule neurotransmitter:

Small, nitrogen-containing molecules that can be derived from either:

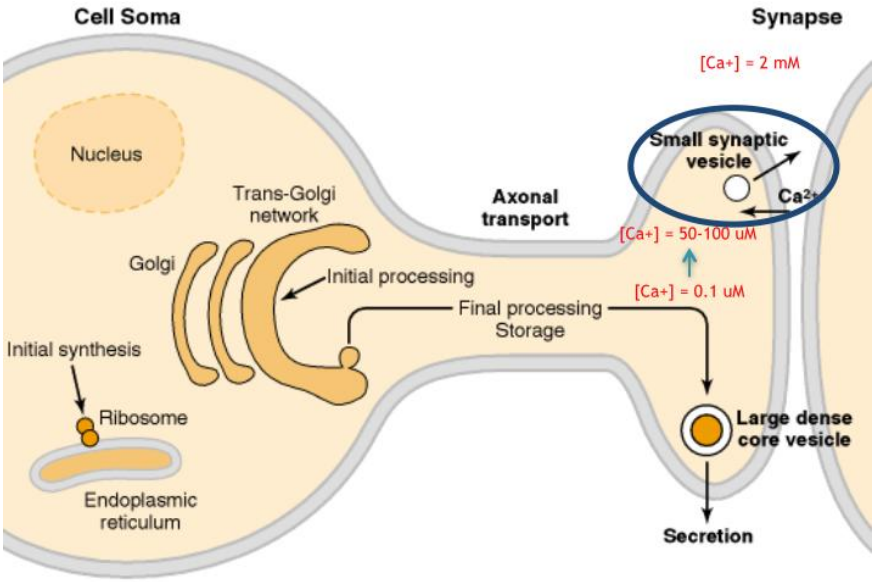
- **amino acids and their derivatives**
- **intermediates of glycolysis and the Krebs cycle (TCA cycle) e.g. α-ketoglutarate**

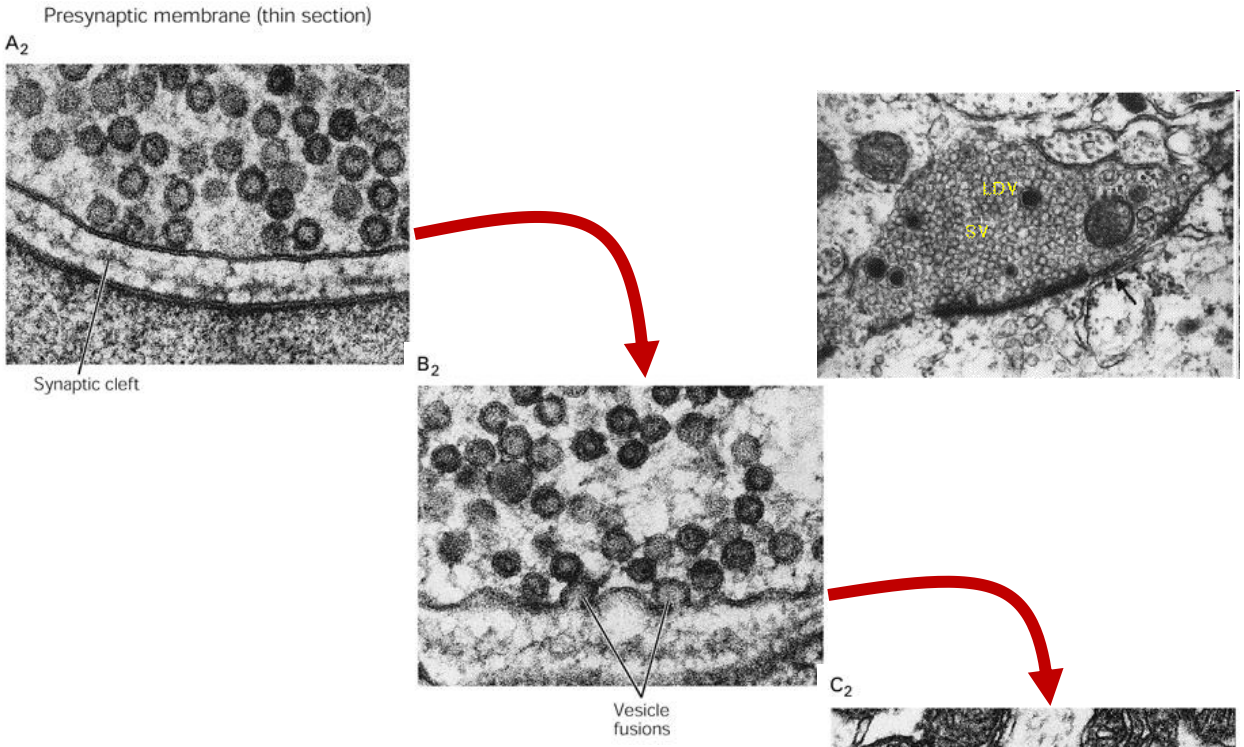
Stages of Action:

- Synthesis of enzymes (from certain genes)
  - Cytosol
  - ER-Golgi apparatus (followed by packaging into different types of vesicles. Some are packaged into large-dense core vesicles)
- Transport of enzymes to the presynaptic terminal (slow and fast-axonal transport) [Neuropeptides (i.e. large-dense core vesicles) are transported via fast-axonal transport.]
- Synthesis of the SMNTs in pre-synaptic terminal
- Packaging into synaptic vesicles (small) that remain near the plasma membrane
- Release
  - They are released in brief pulses each time an action potential triggers the influx of calcium [the vesicles fuse with the plasma membrane releasing their content.]
- Action (short) [They act by binding to a receptor on the postsynaptic cell eliciting a certain signal]



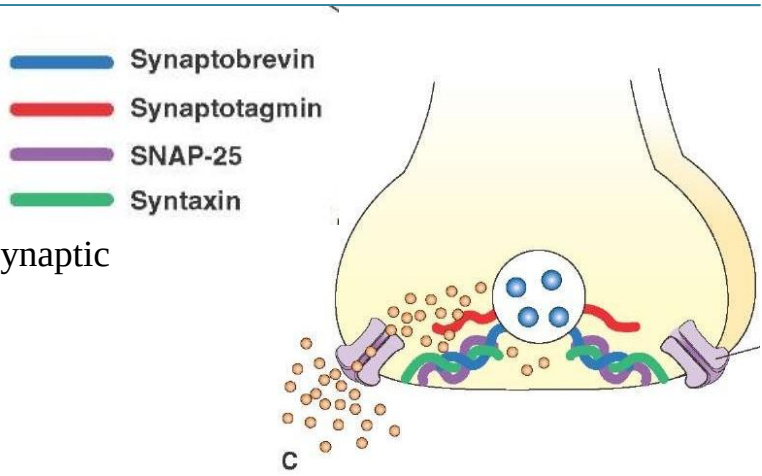
- Termination by diffusion away from the postsynaptic cell, re-uptake [endocytosed into the presynaptic cell where it can be recycled and used again. This is also a different termination mechanism than those of neuropeptides.], or enzymatic inactivation (different from neuropeptides)





Below is a collection of presynaptic vesicles.

Notice the larges vesicles. These are the large-dense core vesicles. Also notice the difference in size between them and the small synaptic vesicles.



These vesicles are coated with clathrin (i.e. these are the endocytosed vesicles that bring back the neurotransmitters into the presynaptic cell).

Proteins and Exocytosis:
The mechanism of fusion, or the question "Why is calcium needed?"

The SNARE proteins in the vesicular and presynaptic membranes form complexes in close apposition of the vesicular and the presynaptic membranes. The influx of Ca2+ ions as a result of depolarization into the terminal allows for calcium ions to interact with **synaptotagmin**, leading to fusion of the vesicular and presynaptic membranes.

In other words, SNARE receptors are present on the cell surface and on the surface of the vesicles. These interact but there is no fusion between them. Calcium is needed to activate a protein known as synaptotagmin. Once this protein is activated, it stimulates not only the interaction but also the fusion of the vesicles with the plasma cell membrane.

Note the differences between neuropeptides and neurotransmitters:

- Onset and duration of action [The duration action of neuropeptides is longer than that of SMNT.]
- Synthesis, transport, and packaging
- Concentration for action and receptor binding [Smaller amounts of neuropeptides are needed to have an action relative to the amounts of SMNTs needed to elicit an action.]
- Concentration of [Ca+] for release
- Site of synthesis, modification [Synthesis of neuropeptides occurs in the cytoplasm while that of the SMNTs occurs in the presynaptic terminal.]
- Fate

The presence of regulatory molecules on each vesicle is the reason behind the vesicles containing small molecules being nearer to the entry site of calcium than those containing larger molecules.

Synthesis of certain SMNTs:

TYROSINE-DERIVED NEUROTRANSMITTERS:

- Below are the most important cofactors needed in these reactions and their roles:
  - S-adenosylmethionine (methyl transfer)
  - Pyrodoxal phosphate (vitamin B6): transamination, decarboxylation
  - Tetrahydrobiopterin (BH4)

Synthesis of Catecholamines:

Catecholamines are called as such because they have a catechol ring (e.g. tryptophan has an indole ring, and histidine has an imidazole ring).

There are three types of such neurotransmitters:

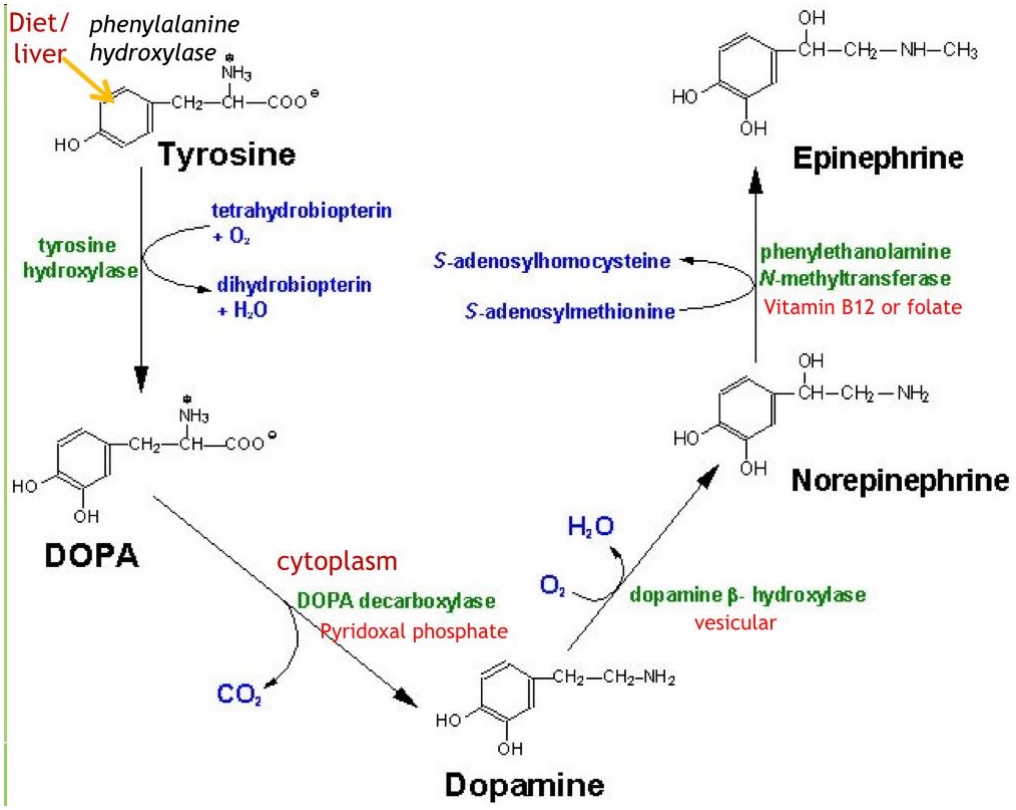
-Dopamine

-Norepinephrine

-Epinephrine

All three are synthesized from the same precursor, tyrosine, and they are synthesized sequentially.

Dopaminergic neurons are neurons that contain enzymes that are necessary for the synthesis of dopamine, but not the enzymes necessary for the synthesis of epinephrine and norepinephrine.



Tyrosine either comes from the diet or it can be synthesized by bile in the liver and then transported to neurons.

The first reaction described here is catalyzed by tyrosine hydroxylase. This is the rate limiting step and the main regulatory step in the synthesis of catecholamines. This enzyme requires tetrahydrobiopterin as a cofactor.

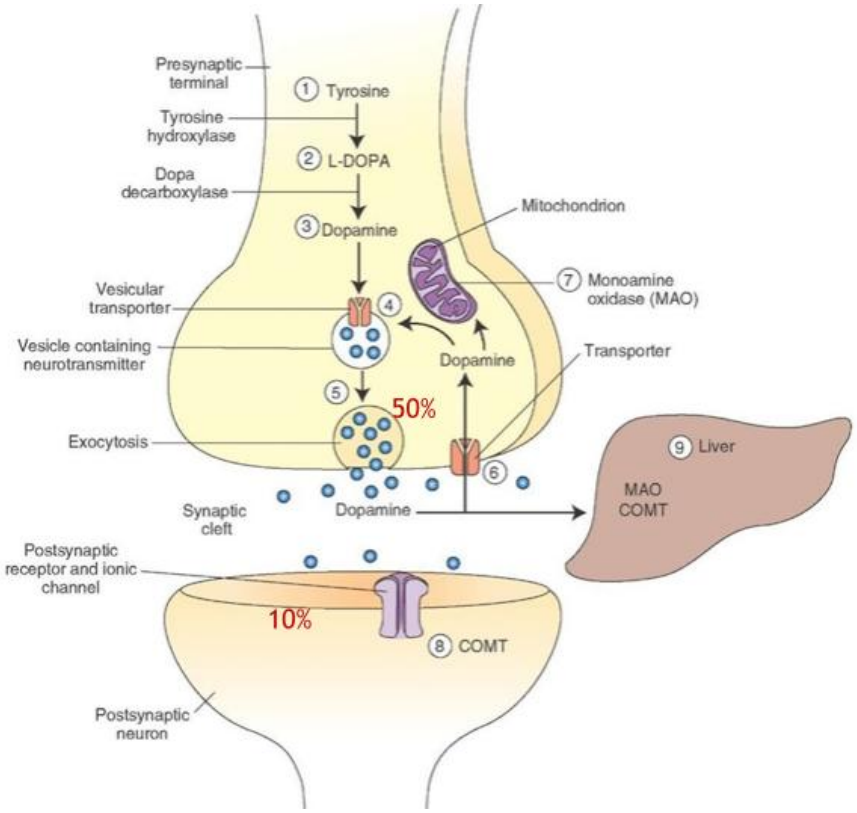
Once dopa is produced, dopamine is synthesized in the cytoplasm by dopa decarboxylase, which requires vitamin B6.

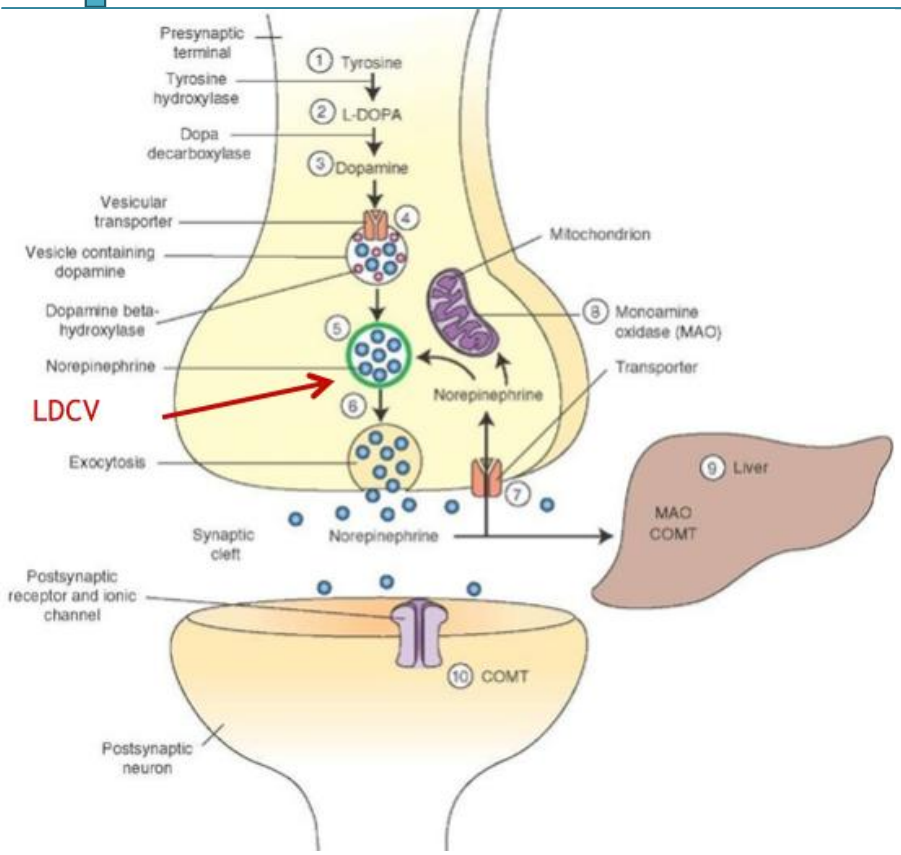
Dopamine is converted into norepinephrine inside the vesicles by a hydroxylase enzyme.

Norepinephrine is methylated by a reaction that requires vitamin B12, folate, as well as S-adenosylmethionine. This results in the formation of epinephrine in the cytoplasm.

Synthesis of Dopamine:

Enzymes that are transported in vesicles are released into the presynaptic terminal. Some of the enzymes can be transported without vesicles by attaching to the microtubules. Once they reach the presynaptic terminal, they start the synthesis of dopamine. Dopamine can then be packaged into synaptic vesicles by mechanisms discussed shortly. Following calcium influx, dopamine is released. To terminate its action, about 50% of dopamine is re-uptaken by the presynaptic cell, 10% is taken-up by the postsynaptic cell, and the rest is transported through the vascular system to the liver, where it can be inactivated by MAO and methyltransferase enzymes.





Norepinephrine Synthesis:

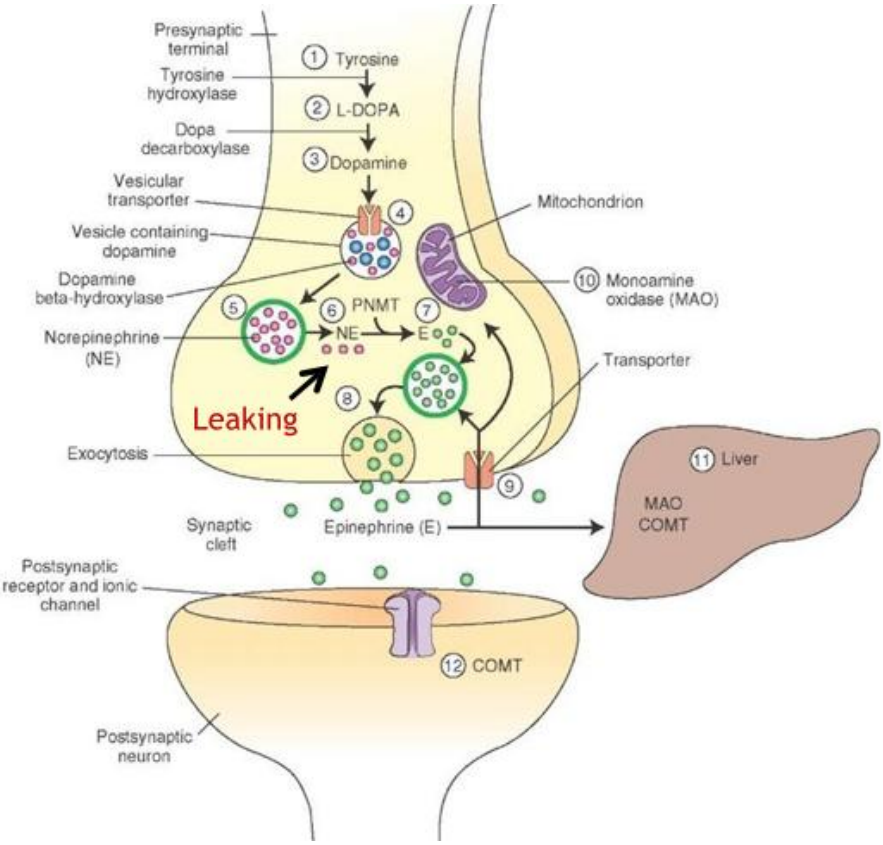
After dopamine is synthesized, it is then packaged into presynaptic vesicles. Inside the vesicles, the synthesis of norepinephrine takes place. These vesicles then become large-dense core vesicles.

There are two of these SMNTs that are not packaged into synaptic vesicles, but rather into large-dense core vesicles. These are norepinephrine and serotonin.

Fusion follows calcium influx. For elimination, norepinephrine is then taken up by the cells or it diffuses away and is then transported to the liver through the vascular system. In the liver, it is inactivated by MAO.

Epinephrine Synthesis:

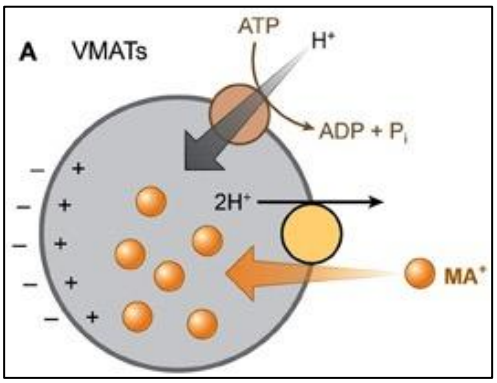
Some of the norepinephrine in the large-dense core vesicles always leaks out regardless of the cell type. In the cytoplasm, norepinephrine is converted into epinephrine, which is then packaged into synaptic vesicles. Fusion and release take place upon calcium influx. The same mechanisms of inactivation apply.

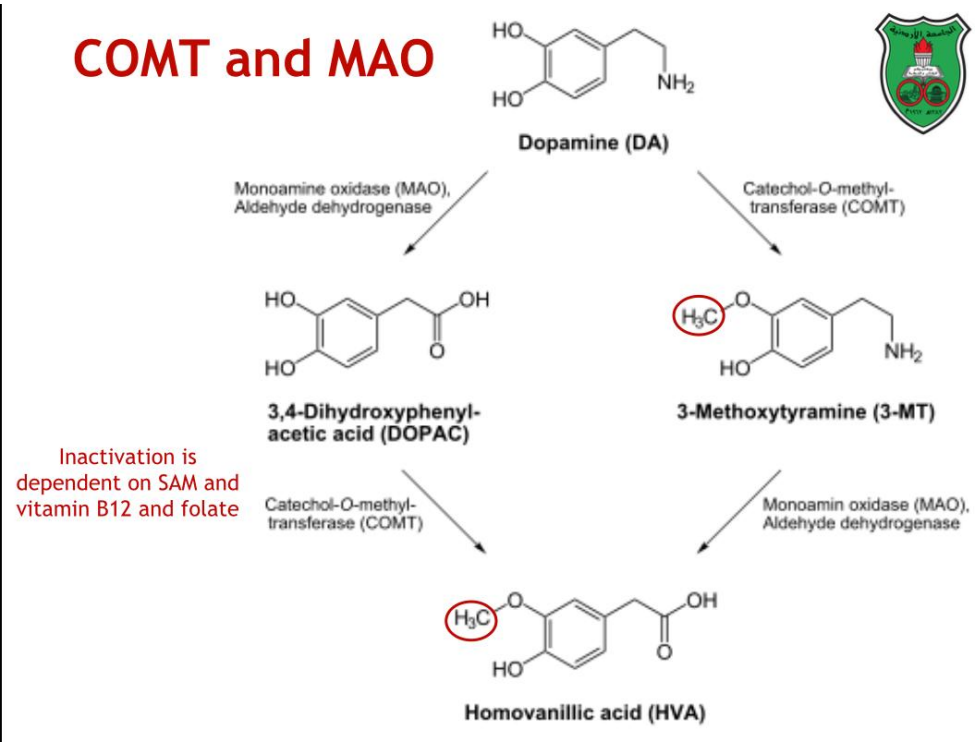


- The presence of enzymes determines which neurotransmitter is produced.
- All of the inactivation mechanisms take place at the same time. The neurotransmitter molecules are inactivated by the previously mentioned mechanisms depending on their location (e.g. those near the presynaptic cell will be re-uptaken by it). The mechanism of inactivation depends on location, and it is a random process that is not regulated.

Packaging into Vesicles:

- The catecholamines (dopamine and epinephrine) 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 are then exchanged for the positively charged catecholamine via the transporter VMAT2 (vesicle monoamine transporter 2).





Inactivation:

Inactivation depends on MAO or the catechol-O-methyltransferase (COMT) enzyme in the liver. Their final product, regardless of the order of action, is homovanillic acid. [Sometimes, the action of COMT is followed by the action of MAO or vice versa.]

Homovanillic acid is a biomarker for Parkinson's disease (i.e. an increase in homovanillic acid blood levels is an indication of Parkinson's disease).

- For methyltransferase to function, S-adenosylmethionine, vitamin B12, and folate must be present.

Regulation:

How is the synthesis of catecholamines regulated?

- As previously mentioned, the rate limiting step is the reaction catalyzed by tyrosine hydroxylase. There are two types of regulation:
- Short term has to do with the activity of the enzyme which is regulated by two mechanisms that regulate the binding of tetrahydrobiopterin to the enzyme.
    - Inhibition by free cytosolic catecholamines
        - Competitive inhibition: Catecholamines compete with BH4 in binding to the enzyme. [An increase in catecholamines will prevent the binding of tetrahydrobiopterin to the enzyme, thus the enzyme is inactivated or inhibited. This results in a decrease in the levels of these catecholamines.]
    - Activation by depolarization
        - Tight binding to BH4 following phosphorylation by PKA, CAM kinases, PKC. [The affinity of tetrahydrobiopterin increases by phosphorylation of the enzyme. This activation takes place by depolarization. Depolarization results in phosphorylation of the enzyme by a number of enzymes. Once the enzyme is phosphorylated, tight binding between the enzyme and tetrahydrobiopterin takes place. So, the enzyme stays active for a longer period of time producing more of these neurotransmitters.]
- Long-term [has to do with the synthesis of the enzyme itself i.e. gene expression which takes time] (plus dopamine β-hyroxylase)

TRYPTOPHAN-DERIVED NEUROTRANSMITTERS:

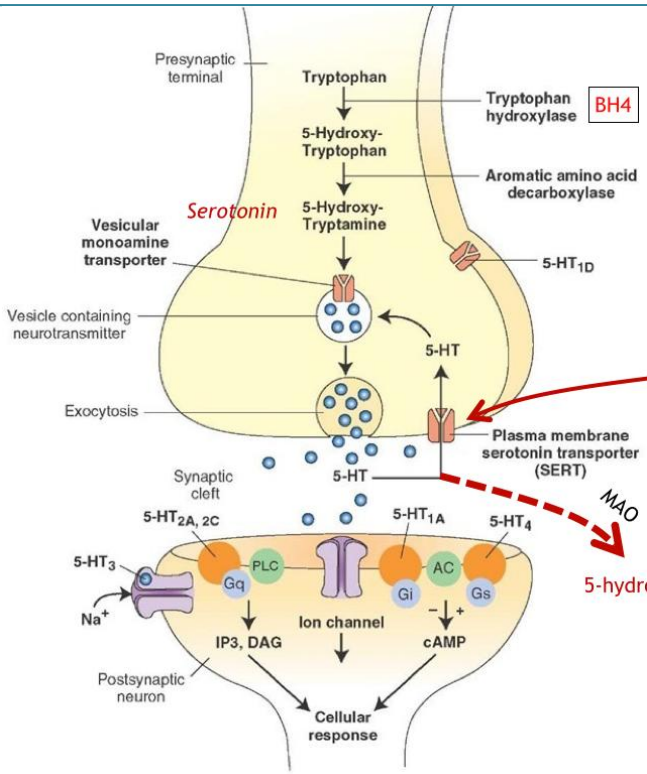
Serotonin and Melatonin:

They are synthesized from tryptophan.

Serotonin

Serotonin is packaged into large-dense core vesicles. Upon calcium influx, serotonin is released. It is then eliminated by either re-uptake or inactivation by MAO. The product will eventually be excreted in the urine.

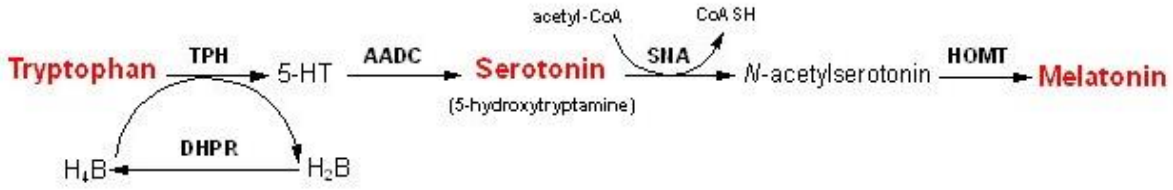
Since the re-uptake mechanism is the main mechanism by which a neurotransmitter signal is terminated, it is targeted by a number of drugs. An example is the serotonin re-uptake inhibitors. These prevent the re-uptake of serotonin, leaving serotonin in the synapse for a longer period of time so that the signal intensifies. Serotonin causes a feeling of happiness. Thus, these drugs are used to treat depression.



Antidepressants , called selective serotonin re-uptake inhibitors (SSRIs), like Prozac® inhibit the reuptake process resulting in prolonged serotonin presence in the synaptic cleft.

Melatonin

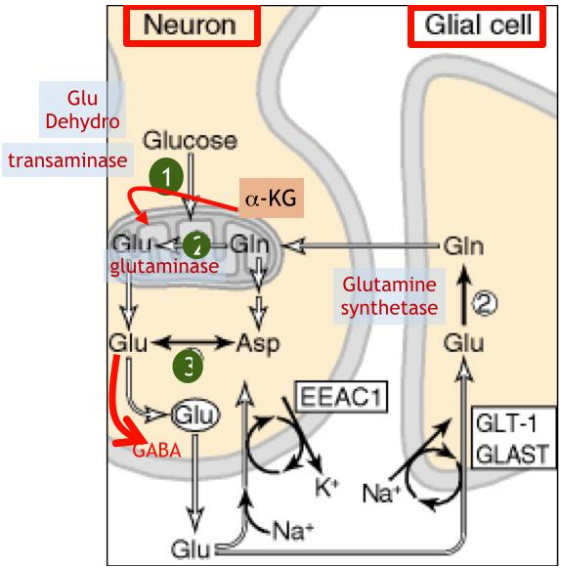
- Serotonin synthesized in the pineal gland serves as a precursor for the sequential synthesis of melatonin, which is a neurohormone involved in the regulation of:
  - Sleep patterns
  - Seasonal and circadian (daily) rhythms
  - Dark-light cycle
  - The higher the amount of melatonin there is in the brain, the longer the person



would sleep. Melatonin allows babies to sleep for a longer period of time than adults. Meaning that, as we age and become older, the amount of melatonin in our brain decreases and that is why we sleep less.

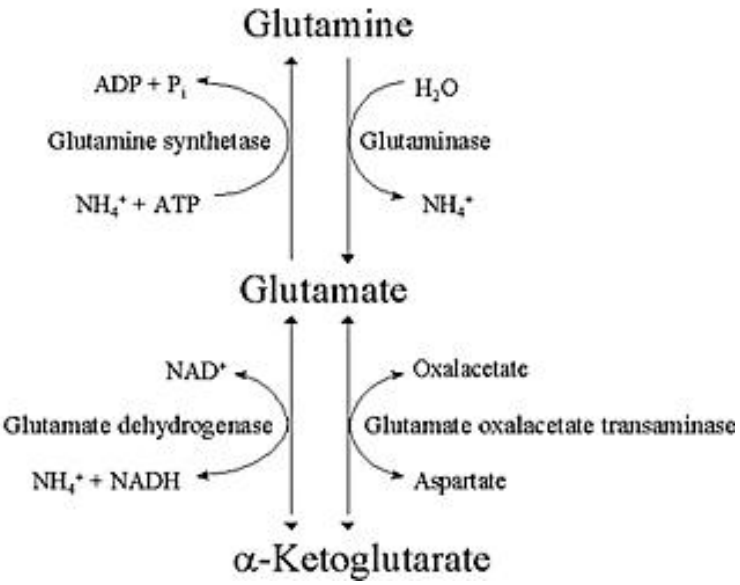
GLUTAMATE AND ASPARTATE:

- Nonessential amino acids
- Do not cross BBB
  - Thus, they must be synthesized in neurons
- Main synthetic compartments
  - neurons
  - glial cells
- Both are excitatory neurotransmitters.



Synthesis of Glutamate

- Sources [Glutamate can be synthesized by three different pathways]:
- Glycolysis → Krebs cycle → α-ketoglutarate → Transamination or dehydrogenation
- Glutamine (deamination)
- Another source: aspartate
- Once glutamate is released, it can be taken up by the glial cells where it is converted into glutamine. Glutamine leaves the cells and goes into the neurons and the cycle continues.
- Removal [It is taken up by three types of transporters. One of them exists in the neurons, and two in the glial cells.]:
  - excitatory amino acid carrier-1 *(EAAC1)*
  - glutamate transporter-1 (GLT-1) and glutamate—aspartate transporter (GLAST)



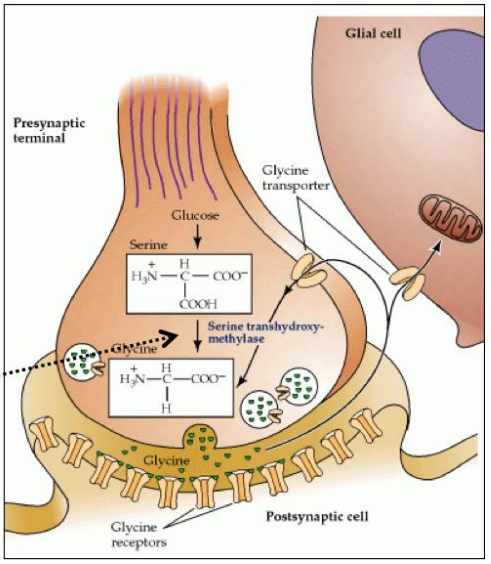
Sources of Glutamate (Supplementary)

Aspartate

- A vesicular uptake mechanism for aspartate has not yet been demonstrated, somewhat weakening the case for considering aspartate to be a neurotransmitter
- Precursor: oxaloacetate (transamination)

GLYCINE:

- The major inhibitory neurotransmitter in the spinal cord
- Synthesized from serine by serine hydroxymethyltransferase through **3-phosphoglycerate** (an intermediate of glycolysis)
- Removal: high-affinity transporter



GABA:

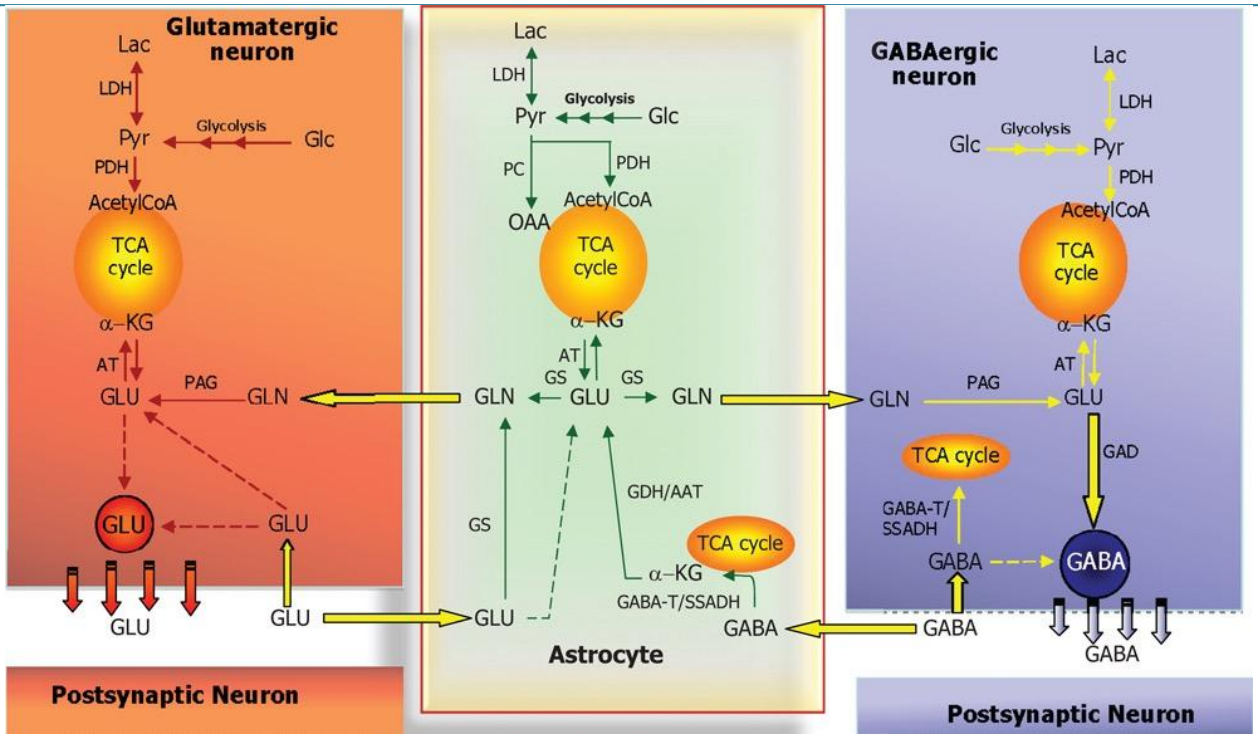
- GABA is an important neurotransmitter that is needed, and thus is present, in high concentrations (millimolar) in many brain regions.
  - These concentrations are about 1,000 times higher than concentrations of the classical monoamine neurotransmitters in the same regions.
- This neurotransmitter is not inactivated as it must be preserved. It is recycled via what is known as a GABA shunt. The GABA shunt is a closed-loop process with the dual purpose of producing and conserving the supply of GABA.

GABA Shunt

The GABA shunt is a way by which GABA is preserved. It is preserved by astrocytic cells or glial cell.

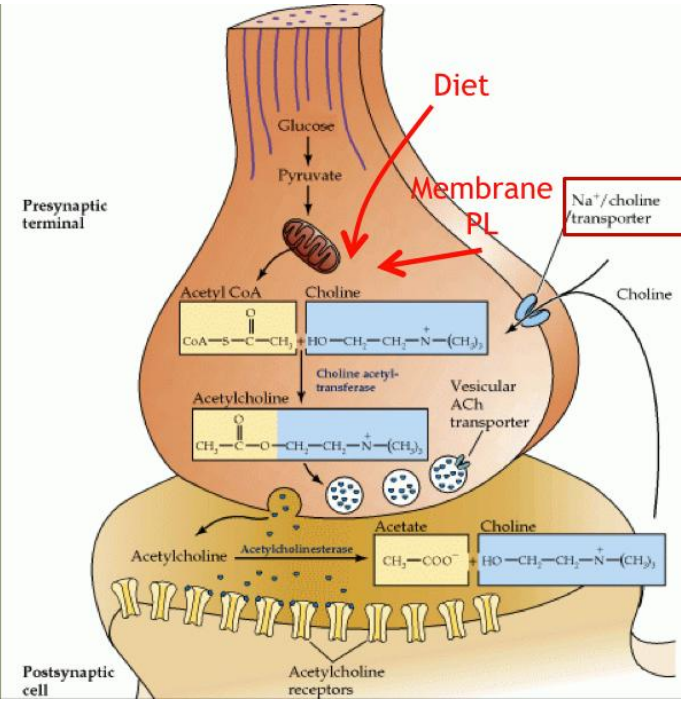
Some neurons have the ability to synthesize GABA from glutamate. Once GABA is released, it is taken up by neighboring cells like glial cells and astrocytes. This GABA can be converted into α-ketoglutarate (an intermediate in the Kreb's cycle). This is followed by transamination and results in the production of glutamate, which is then converted into glutamine. Glutamine can leave the astrocytes and enter the neurons, where it is converted again into glutamate. Glutamate is then converted into GABA and so on.

The GABA shunt in short: glutamate → GABA (GABA is released and does its action, it is then taken up by astrocytic cells and glial cells)



GABA → α-ketoglutarate → glutamate → glutamine (glutamine leaves the cells again into neurons) → glutamate → GABA and cycle continues.

ACETYLCHOLINE:



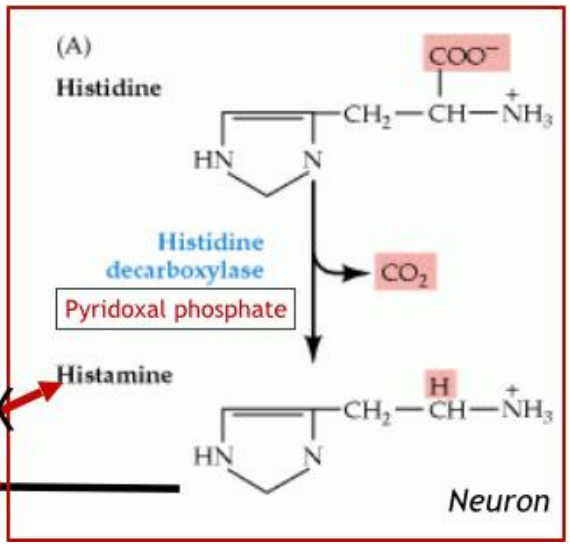
Synthesis

- Choline + acetylcoenzyme-A are conjugated by choline acetyltransferase in the cytoplasm, resulting in the formation of acetylcholine.
- Transported into and stored in vesicles. Release is dependent on calcium influx.
- Removal: It is either taken up by the postsynaptic cell, or it is eliminated by diffusion followed by enzymatic inactivation via hydrolysis by acetylcholinesterase

There are two main sources for choline 1- Diet 2- Plasma membrane.

HISTAMINE:

- it does not penetrate the blood—brain barrier and, hence, must be synthesized.



Inactivation of Histamine

What is different about histamine is the absence of a re-uptake mechanism for it. The main mechanism by which it is inactivated is diffusion followed by inactivation by MAO [it is inactivated b oxidation] and methyltransferase.

In the case of histamine, its MAO is present within astrocytes. However, the MAO

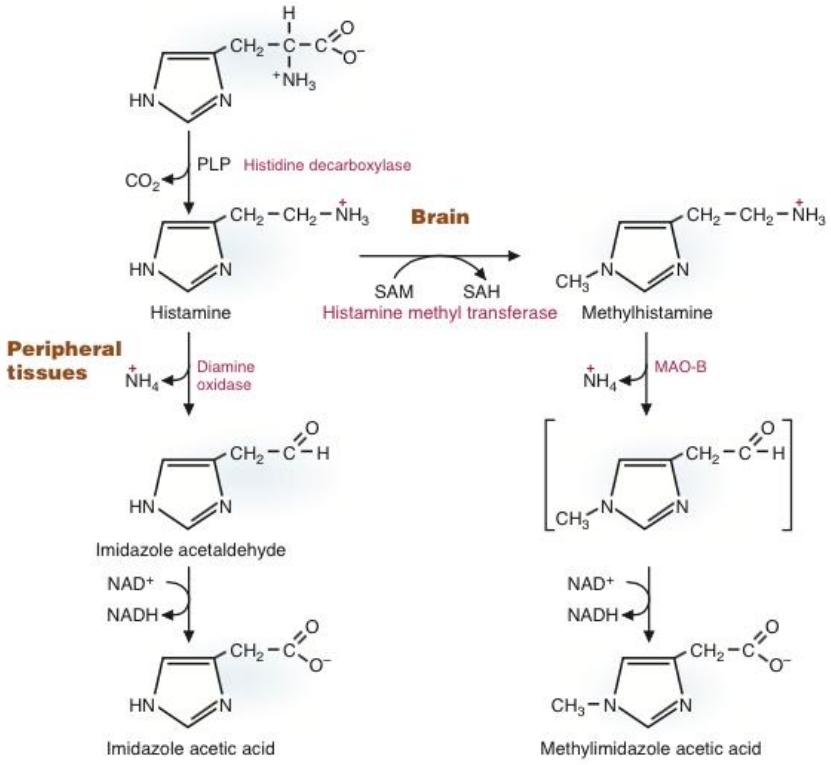
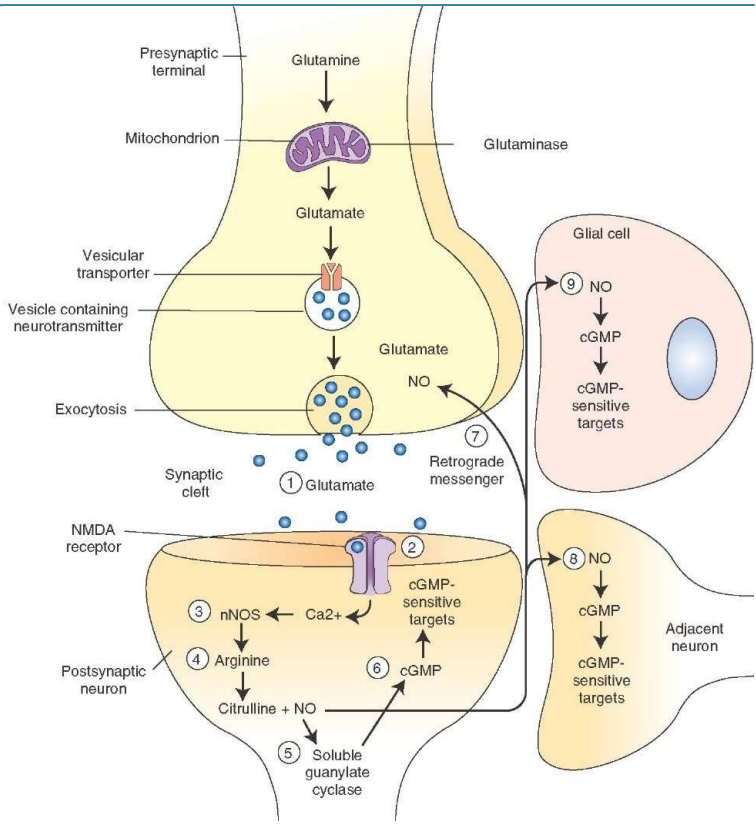


FIG. 48.8. Synthesis and inactivation of histamine; note the different pathways for brain and peripheral tissues. *SAH*, *S*-adenosylhomocysteine; *SAM*, *S*-adenosylmethionine.

of other neurotransmitters is present in the liver.

NITRIC OXIDE (NO):

- is an exception to all other neurotransmitters. It is a gas with a very short half-life.
- It is not synthesized by the presynaptic nerve, but rather by the postsynaptic nerve.
- A signal results in the release of glutamate (1) which acts on NMDA receptors located on the post-synaptic neuron (2)
- Ca2+ enters the postsynaptic neuron and binds with calmodulin activating NOS (3) resulting in formation of NO and citrulline from L-arginine (4).
- NO stimulates guanylate cyclase forming cGMP (5), which results in a physiological response (6)
- NO can diffuse out: a) to the presynaptic terminal via a mechanism knowns as retrograde transport (and is thus known as a *retrograde messenger*) (7) prolonging its effect and b) into adjacent neurons (8) and glial cells (9) stimulating guanylate cyclase. Its action depends on how much it diffuses.
- Inactivation: by binding to other protein as well as cell diffusion.



Half-life: 2-4 seconds
NO is inhibited by hemoglobin and other heme proteins which bind it

The professor said the following:

"Signaling inside these cells activates guanylate cyclase and results in an influx of calcium.This activates NO synthase in the cells resulting in the synthesis of NO, which then diffuses out of the postsynaptic cell. Some of this NO can get back into the presynaptic cell inducing a certain signal. What is important about NO is that it stimulates guanylate cyclase, forming cGMP which binds to certain enzymes and factors eliciting a signal."

According to many references and the set of slides provided by the professor, signaling results in an influx of calcium ions which then bind to calmodulin, activating nNOS. All pieces of information mentioned after the one explained above are true.

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 (it depends on diffusion and there is no active process that terminates its action)
    - It decays spontaneously (it is not enzymatically inactivated)
  - 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.

NO Synthase

There are three important enzymes that are responsible for the synthesis of NO. These are actually just three different isoforms.

- 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
- and isoform III (eNOS)
  - Endothelial cells lining blood vessels
  - activated by the influx of extracellular calcium
  - NO causes vasodilation of these vessels

- All three isoforms require BH4 as a cofactor and nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme