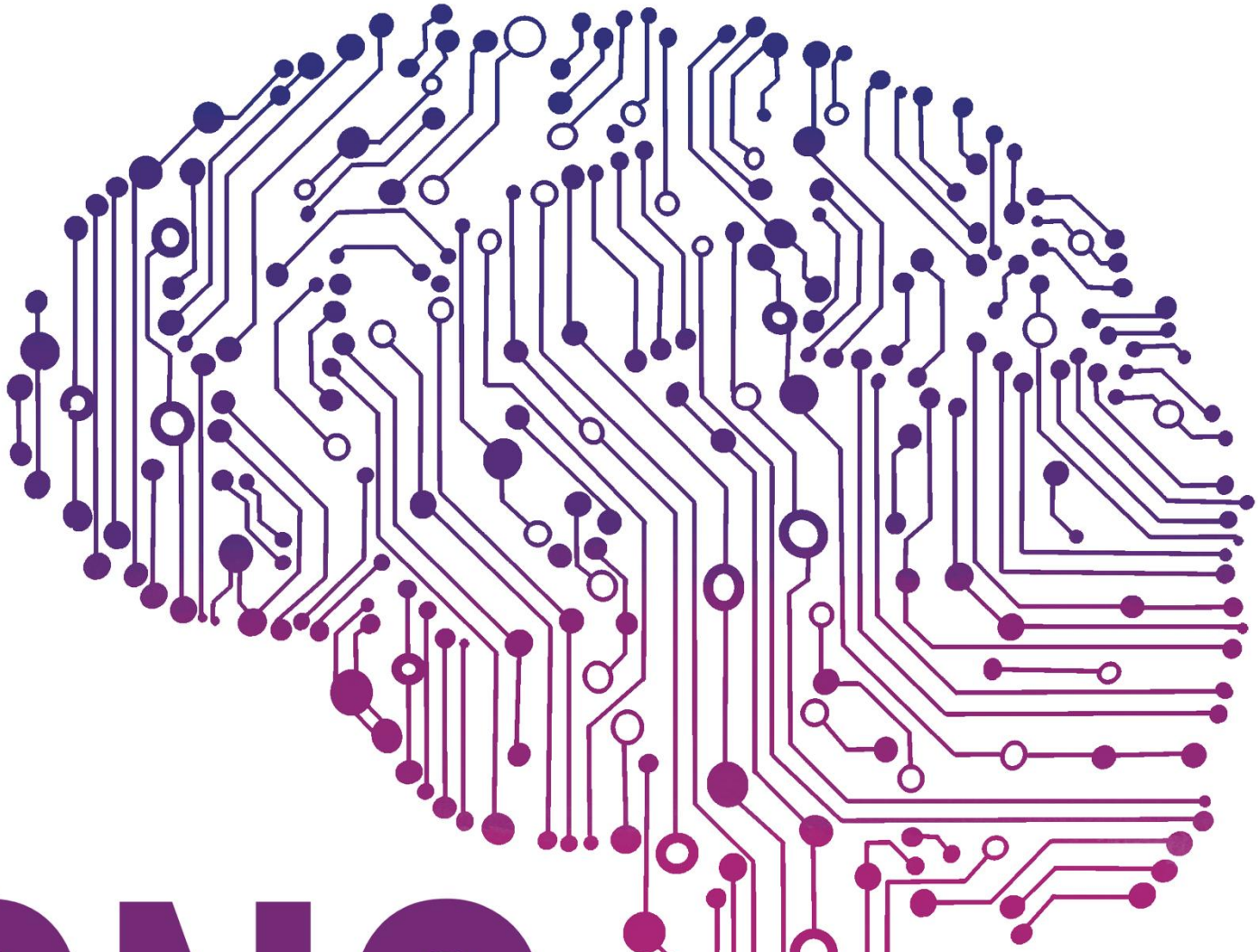

CNS

2

BIOCHEMISTRY

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Sheet 2: Neurotransmitters

References

- **Mark's Basic Medical Biochemistry, 4th ed, pp. 908- 918 or 5th edition**
- **http://what-whenhow.com/neuroscience/neurotransmitters-theneuron-part-1/**

What is a Neurotransmitter?

A neurotransmitter is a chemical substance that is:

- Synthesized in a neuron.
- Released at a synapse e.g., neuromuscular junction where we have at least one nerve terminus, following depolarization of the nerve terminal.
  - They usually depend on the influx of calcium ions to be released into this neurosynaptic junction/cleft.
- Then, they bind to receptors on the postsynaptic or muscle cell and/or presynaptic terminal to elicit a specific response after binding.

Characteristics of a Neurotransmitter

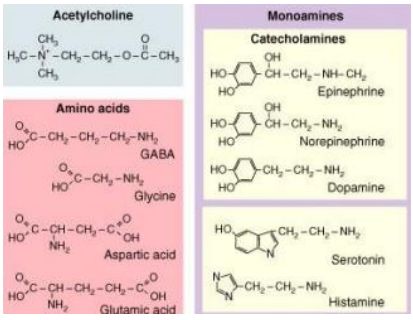
A neurotransmitter is a chemical substance that:

- Is synthesized and stored in a presynaptic neuron (the enzymes needed for its synthesis must be present in the neuron) and released after certain stimuli.
- Is released at a synapse following depolarization of the nerve terminal (usually dependent on influx of calcium ions).
- Binds to receptors on the postsynaptic cell and/or presynaptic terminal.
- Elicits rapid-onset and rapidly reversible responses in the target cell. They elicit different types of responses and durations (slow, fast, long-acting, short-acting…).
- Is removed or inactivated from the synaptic cleft, thus the response is stopped.

Types and Structure of Neurotransmitters

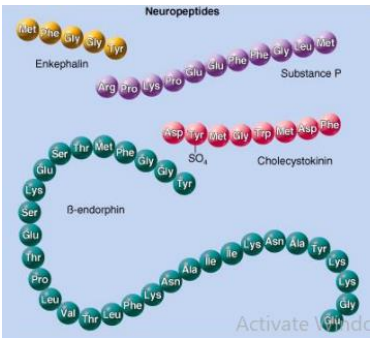
There are different types of neurotransmitters and they are classified into three types:

1. **Small-molecules**
   - Amines (acetylcholine, epinephrine, dopamine, histamine, serotonin, norepinephrine, etc.).
   - Amino acids (glutamate, aspartate, glycine).
   - The following figures display some of the mentioned NT. Notice the amino group in all of them.



**GABA is derived from glutamate. Histamine is derived from histidine by a decarboxylation reaction.* (will be discussed later)

2. **Neuropeptides**: neurotransmitters that have multiple amino acids in their structures but haven't reached the length where they can be considered proteins. So, they are shorter than proteins but larger than small molecules.
   - Examples include B-endorphin, cholecystokinin, substance P, and enkephalin.
3. **Gases:** For example, nitric oxide (NO).



Each neuron may contain any of the following:

- One or more small-molecule neurotransmitters.
- One or more neuropeptide neurotransmitters.
- Both types of neurotransmitters.

➢ It depends on the function of the neuron, action or response needed by these neurons, stimuli they respond to, and the receptor found on their membrane.
➢ The differential release of the various neurotransmitters is the result of the neuron altering its frequency and pattern of firing.

Distribution of Neurotransmitters

- Each neuron synthesizes only those neurotransmitters that it needs/uses for transmission of action potential through a synapse or to another cell.
- The neuronal tracts are often identified by the type of neurotransmitter they release. For example, a dopaminergic tract synthesizes and releases the neurotransmitter dopamine. A cholinergic tract synthesizes and releases the neurotransmitter acetylcholine, and so on.
- More than one transmitter (usually a small-molecule transmitter and a neuroactive peptide) coexist in many mature neurons (e.g., most spinal motor neurons contain acetylcholine and calcitonin gene-related peptide).

The Nature of the Response

The responses induced by the release and binding of these neurotransmitters:

- Can be excitatory or inhibitory depending on different factors.
- Does not depend on the chemical nature of the transmitter.
- Depends on the type of receptor the transmitter binds to and activates in the postsynaptic cell or neuron and the ion species that becomes more permeable and goes through the membrane changing its polarity.

Neuropeptides: Introduction

- More than 50 neuropeptides have been described.
- They usually mediate slow, ongoing brain functions. Here are some of the processes they mediate:
  - Homeostasis
  - Sleep
  - Appetite
  - Thirst
  - Temperature
  - Behavior
  - Pain perception
  - Memory

Neuropeptides: Neurohormones or Neurotransmitters?

- Neurohormone: A messenger that is released by neurons into the hemolymph (blood circulation or lymph circulation) and exerts its effects on distant peripheral targets, such as thyroid stimulating hormone (TSH) and growth hormone (GH).
- Neurotransmitter: a messenger released from a neuron at an anatomically specialized junction, which diffuses across a narrow cleft to affect one or sometimes two postsynaptic neurons, a muscle cell, or another effector cell inducing a certain effect.

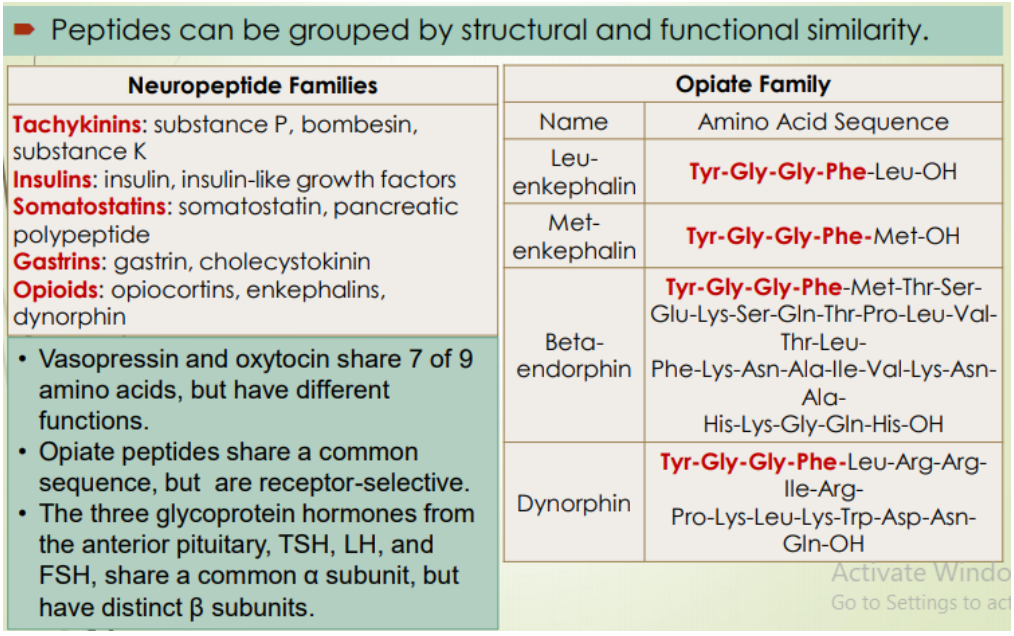
So, the difference between them is more like endocrine and paracrine effects, neurotransmitters act locally while neurohormones act distally.

So, let's classify these neuropeptides:

(please read the following figure carefully)

**Enkephalins* are local/internal pain killers (analgesics) peptides made of five amino acids (pentapeptides).*

Notice that opiates share a part of their structure (amino acids in red).



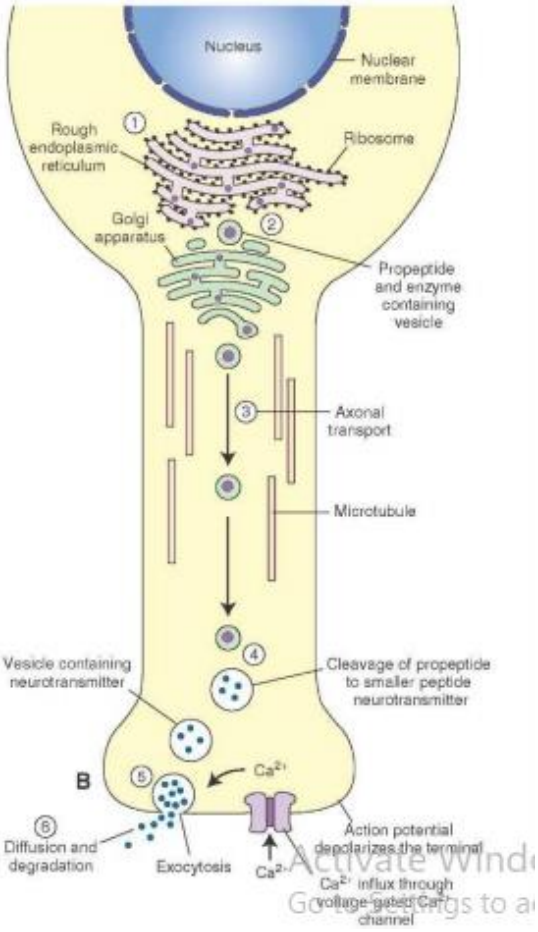
Peptides can be grouped by structural and functional similarity.

Neuropeptide Families
Tachykinins: substance P, bombesin, substance K **Insulins**: insulin, insulin-like growth factors **Somatostatins**: somatostatin, pancreatic polypeptide **Gastrins**: gastrin, cholecystokinin **Opioids**: opiocortins, enkephalins, dynorphin

- Vasopressin and oxytocin share 7 of 9 amino acids, but have different functions.
- Opiate peptides share a common sequence, but are receptor-selective.
- The three glycoprotein hormones from the anterior pituitary, TSH, LH, and FSH, share a common α subunit, but have distinct β subunits.

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

Stages of action of neuropeptides:



1. Their synthesis (ER and Golgi apparatus) is similar to protein synthesis; synthesized by ribosomes and then modified in the ER and further modified in Golgi apparatus.
2. Packaging into large-dense core vesicles (with modifying enzymes), so while they are moving, they are going to be modified as well.
3. Transported into the cytoskeletal elements (fast-axonal transport).
    - During the transport, proteases cleave the precursor neuropeptide into the final mature form. So, they are going to keep getting modified and getting mature during their transport along the neuroaxon.
    - Summary: Started from very close to the nucleus in the ER -> Towards Golgi -> Vesicles on the cytoskeletal elements -> Towards the periphery of the axon where they arrive in their mature and final form).
4. Release by fusion of these vesicles with the presynaptic membrane and exocytosis of these neurotransmitters into the cleft/synapse.
    - They are released slowly and gradually over time in response to general increase in the level of intracellular calcium. So, they depend on the concentration of calcium ions in their release. Ca^{+2} facilitates the fusion of these vesicles with the presynaptic membrane and the release of the NT.
5. Neurotransmitters bind to the post-synaptic receptors producing an action (prolonged).
6. Termination of this action by diffusion (dilution) into local areas and degradation. So, there is no reuptake of the NT back into the presynaptic neuron).

The action of the neurotransmitter is terminated through:

1. Reuptake into the presynaptic terminal.
2. Uptake into glial cells.
3. Diffusion away from the synapse.
4. Enzymatic inactivation:

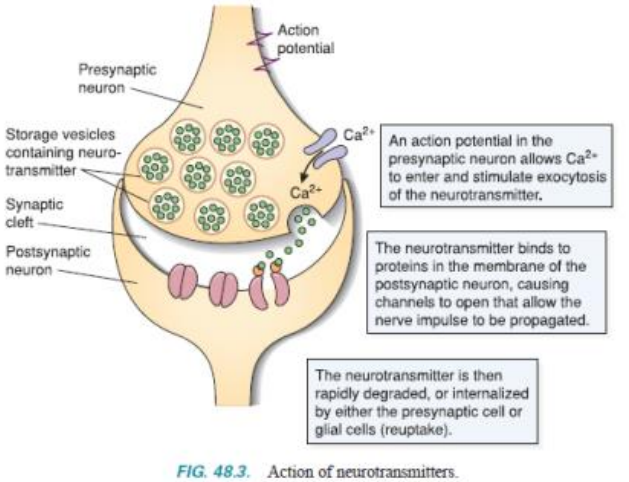
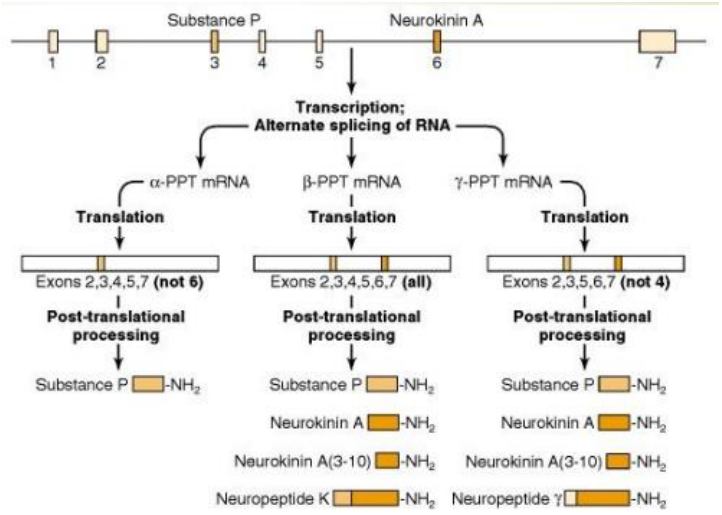


FIG. 48.3. Action of neurotransmitters.

- May occur in the postsynaptic terminal, the presynaptic terminal, or an adjacent astrocyte, microglia cell, or in endothelial cells in the brain capillaries.
- Remember that there is a difference between the mechanisms of action termination for neurotransmitters and neuropeptides. Here, we are talking about neurotransmitters generally, thus the option of reuptake into the presynaptic terminal is possible. However, for neuropeptides, there is no reuptake.

Diversity

Since neuropeptides are made of amino acids, they are surely encoded by genes and synthesized just like proteins but in smaller molecules relative to proteins. So, here are some mechanisms that cause diversity in the neurotransmitters:



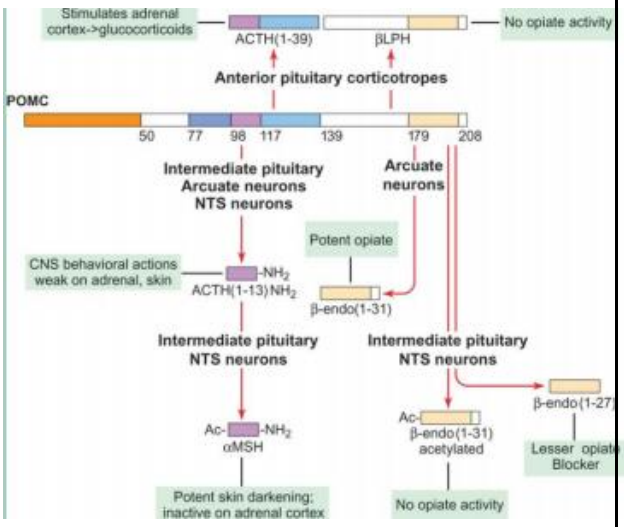
1. Alternative Splicing:

- Alternative splicing is a mechanism in which different types of neuropeptides can be formed. Alternative splicing of mRNA leads to translation of distinct precursors, and subsequent processing leads to unique mature peptides.
- For example, substance P mRNA, normally includes mRNA encoding substance K too. So, substance P and substance K are generated from the same mRNA by alternative splicing of this mRNA forming two different molecules.

2. Post-Translational Modifications:

- Another way to create a diverse group of neuropeptides is by post-translational modifications, like proteolytic, differential, and sequential processing.
- Neuropeptides are produced from a longer precursor protein by:
  - Proteolytic processing by cleaving the longer precursor protein into different parts and different types of neuropeptides.
  - Vesicular packaging of different proteases that recognize different cleavage sequences, thus cleaving different peptides.
  - Hiding a proteolytic site by posttranslational modifications (example: addition of a carbohydrate side chain or glycosylation).
  - Tissue-specificity of these proteases results in the presence of different proteases in different tissues, resulting in different neuropeptides.

- Refer to the figure on the right for the following example: Processing of the pro-opiomelanocortin (POMC) precursor proceeds in an ordered, stepwise fashion. Some of the reactions are tissue specific. This processing results in the formation of adrenocorticotropic hormone (ACTH), corticotropin-like intermediate lobe peptide (CLIP), joining peptide (JP), lipotropin (LPH), melanocyte-stimulating hormone (MSH), and prohormone convertase (PC).



In conclusion, diversity of neuropeptides can result at two levels:

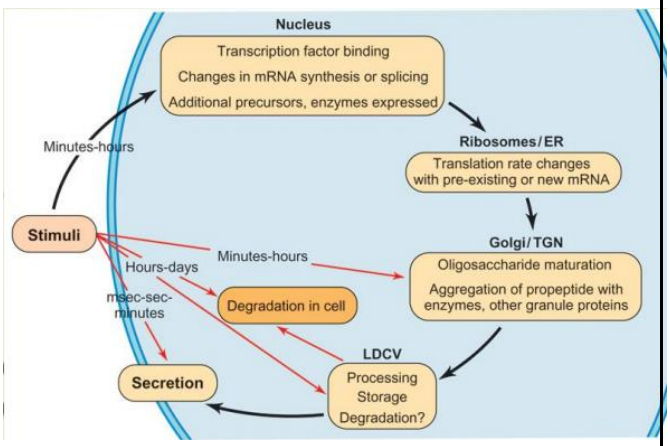
1. Alternative splicing→ at the level of mRNA.
2. Post-translational modifications → after protein synthesis.

Levels of Regulation of Neuropeptide Expression

There are many levels of regulation of neuropeptide expression and formation. This leads to a diverse group of neuropeptides and regulation of their synthesis.

The synthesis of neuropeptides is associated with certain stimuli:

1. Once the <u>stimulus</u> is present or close to the cell, it is going to transmit its message through receptors and secondary messengers until it reaches the nucleus.
2. The regulation of the expression of neuropeptides in the <u>nucleus</u> occurs at the gene level. There will be:
   - Activation or inhibition of transcription factors and their binding to DNA.
   - Activation or inhibition of the expression of certain proteins.
   - Changes in mRNA synthesis or splicing.
   - Additional precursors and/or enzymes expressed.
   - This process, from the stimulus to inducing an action in the nucleus, takes minutes to hours. The time depends on the stimulus, receptor, and other factors.
3. At the level of the <u>ribosomes/ER</u>:



    - Once the mRNA is transcribed, there will be activation of translation. The rate of translation depends on the amount of mRNA present whether pre-existing or newly synthesized.
    - Addition of some sugars in the ER.
4. At the level of the Golgi Apparatus/Trans-Golgi Network (TGN):
    - Addition of some sugars and oligosaccharide maturation.
    - Aggregation of pro-peptide with enzymes and other granule proteins and their cleavage.
5. At the level of Large-Dense Core Vesicles (LDCV):
    - Further maturation, cleavage, and processing.
6. Secretion.

- The stimuli may also have an effect on secretion and degradation in the cell when needed.

These are all different points at which regulation of neuropeptide expression may occur. They occur over a wide duration of time and may vary in their effect on the level of expression. This will contribute to the intricate organization and interplay between the different types of neuropeptides and their availability to induce different actions and responses.

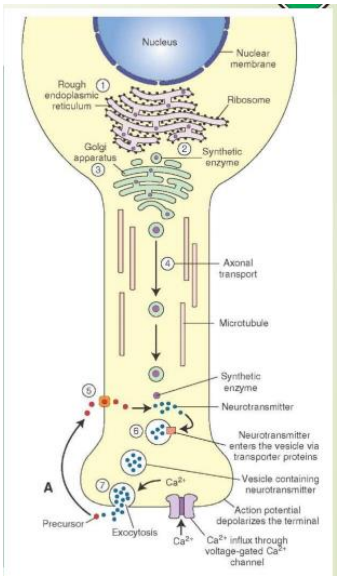
Small-Molecule Neurotransmitters

Types

They are nitrogen containing molecules, so they will be either:

- Amino acids or their derivatives.
- Intermediates of glycolysis and the Krebs Cycle (TCA cycle).

Stages of Action



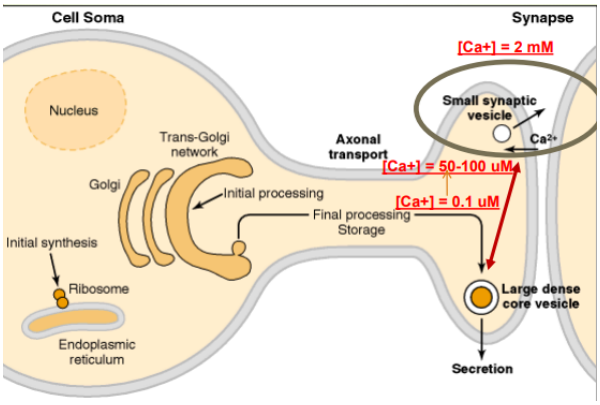
1. The neurotransmitters are synthesized by enzymes (which need to be synthesized themselves).
    - The enzymes (which are proteins) are synthesized by the ribosomes of the RER in the cell body and then modified through the ER-Golgi apparatus.
    - They will then be packaged into large-dense core vesicles.
2. These vesicles transport the enzymes along the cytoskeleton of the axon into the terminus. There are slow and fast forms of axonal transport.

3. At the pre-synaptic terminal, the synthesis of the small molecule neurotransmitters occurs.
4. Once made, the neurotransmitters are packaged into synaptic vesicles which can fuse with the pre-synaptic membrane to release their contents into the synaptic cleft.
    - The neurotransmitters are released in brief pulses each time an action potential triggers the influx of calcium.
5. The action of neurotransmitters is short. Termination of their function is through diffusion, reuptake (by the pre-synaptic neuron: step 5 in the figure), or inactivation (by enzymes).

The professor said in the lecture that the neurotransmitters can be re-uptaken by the pre-synaptic neurons for the synthesis of other molecules again in the terminus. Here is further clarification on this point from the resource linked:

"The released transmitter enters back into the terminal by an uptake mechanism and is recycled for subsequent release. Some neurotransmitters (e.g., Ach) are degraded in the synaptic cleft, and one or more of their degradation products are taken back into the terminal and reused to synthesize the neuro-transmitter in the terminal."

Role of Calcium

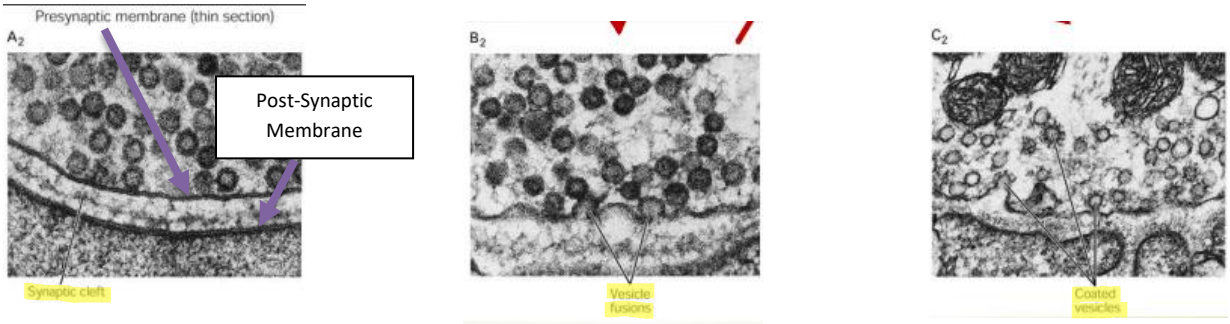


- The concentration of Ca+ in the pre-synaptic terminus (0.1 uM) is much lower than the concentration in the synapse itself (2 mM).
- Vesicles are located further away from the presynaptic membrane and away from the area of Ca influx. As we've discussed, the vesicles are moving along the axon to the pre-synaptic terminal.
- Due to propagation of the action potential along the axon towards the terminus, there will be an influx of calcium inside the cell. The concentration of calcium will increase up to a thousand-fold more (50-100 uM).
    - The calcium influx can be from external or internal sources.
- This influx assists in the fusion and exocytosis of the neurotransmitters from the pre-synaptic membrane.

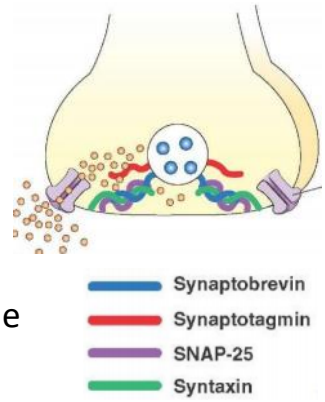
Recycling of the Vesicular Membrane

- The fused vesicular membrane is retrieved and recycled within a minute by a complex process called endocytic budding.

- After the vesicle releases its contents, it's going to bud back into the inside of a pre-synaptic neuron. Therefore, the vesicle does **not** leave the neuron.
- Several proteins, including **clathrin**, form a basket-like lattice on the remnants of the fused vesicle giving the appearance of a coated pit which is then pinched off from the presynaptic membrane back to the presynaptic terminus by another protein called **dynamin**.



Vesicular Fusion Proteins and Exocytosis



- Other proteins involved in vesicular fusion and exocytosis are the **SNARE proteins**. They are present on the pre-synaptic (target) and vesicular membranes. They form complexes in close apposition of the vesicular and pre-synaptic membranes.
- **Synaptotagmin** interacts with the calcium ions that influxed as a result of depolarization. This interaction facilitates the fusion of the vesicular membrane with the pre-synaptic membrane.

Comparing Between Neuropeptides and Small Molecule Neurotransmitters

They differ in their:

- Onset and duration of action.
- Synthesis, transport, and packaging (and the sites of synthesis and modification).
  - The neuropeptides were synthesised in the ER and Golgi apparatus, packaged, and then matured during the vesicular transport.
  - The small molecules neurotransmitters were synthesized in the axon terminus, while the enzymes that synthesize them were packaged and transported through vesicles.
- Concentration needed for a specific action or for binding to receptors. This is due to differences in size.
- Concentration of Ca+ needed for release.
- Their fate and how their action is terminated.
  - Recall that small molecule neurotransmitters can be reuptaken into the pre-synaptic terminal while the neuropeptides can't.

Synthesis of Small Molecule Neurotransmitters

- Most are synthesied from amino acids, intermediates of glycolysis and the TCA cycle, and O_2 in the cytoplasm of the presynaptic terminal.
- The rate of synthesis is generally regulated to correspond to the rate of firing of the neuron.

We will now further discuss some small molecule neurotransmitters.

Tyrosine-Derived Neurotransmitters

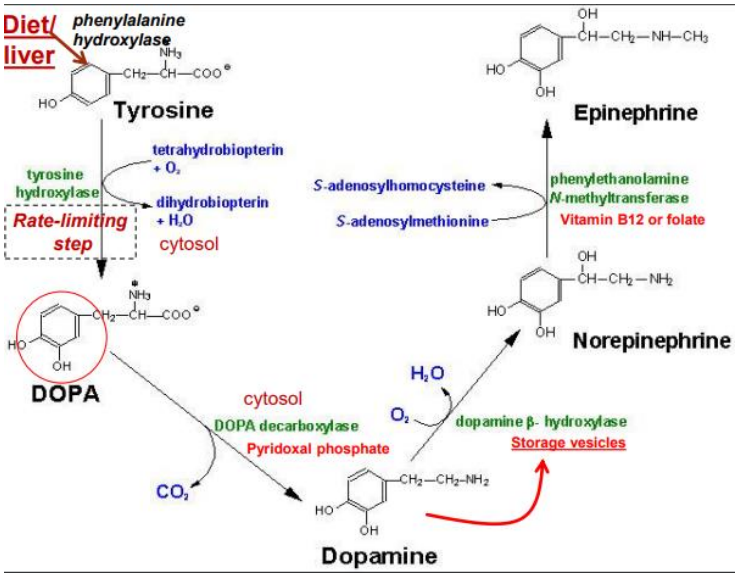
This group includes dopamine, epinephrine and norepinephrine. They're all classified as catecholamines as they contain a catechol ring (circled in red in the figure below). A catechol ring is a benzene ring with two hydroxyl (OH) groups on adjacent positions on the ring.

Synthesis (please refer to the figure below)

1. Synthesis begins with **Tyrosine.**
   - *Process:* Tyrosine can be obtained from the diet or synthesized from phenylalanine in the liver in a hydroxylation reaction catalyzed by the enzyme phenylalanine hydroxylase.
   - *Classification:* Since it can be synthesized in the body tyrosine is a nonessential amino acid.
   - *Structure:* It has a phenol group in the R chain (a benzene ring with a single hydroxyl group).
2. Tyrosine is converted into **DOPA**.
   - *Reaction:* Tyrosine is hydroxylated (because a second OH group is needed) through the enzyme tyrosine hydroxylase.
   - *Requirements:* The enzyme needs the cofactor tetrahydrobiopterin (BH4) for the reaction. It is then oxidized into dihydrobiopterin.
   - *Structure of DOPA:* It still has the amino and carboxyl group of tyrosine in the backbone and a catechol ring.
   - *Note:* This is the rate-limiting step, and it occurs in the cytosol to produce DOPA. Dopa concentration is reduced in patients with Parkinson's disease.
3. DOPA is converted into **Dopamine**, the first neurotransmitter.
   - *Reaction:* The carboxyl group needs to be removed in a decarboxylation reaction catalyzed by the enzyme DOPA decarboxylase. A CO_2 molecule is released.
   - *Requirements:* The coenzyme pyridoxal phosphate (vitamin B6) (mentioned later: it is used for transamination and decarboxylation reactions)

- *Fate of Dopamine:* It can move on to storage vesicles if it is going to be used as dopamine or it can be used to synthesize other catecholamines.

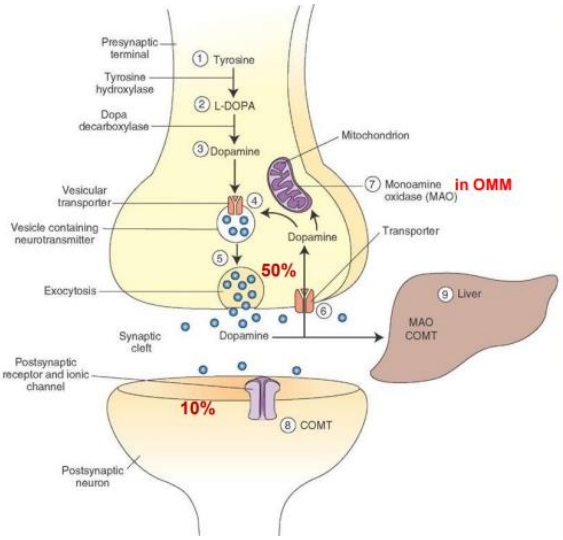
4. Dopamine is converted into **Norepinephrine (NE).**
   - *Reaction:* Dopamine is hydroxylated by dopamine β-hydroxylase to produce NE.
   - *Structure of NE:* It differs from dopamine through the addition of a hydroxyl group.
5. Norepinephrine can be converted into **Epinephrine (E)**.
   - *Reaction:* A methyl group is added onto NE by the enzyme phenyl ethanolamine N-methyltransferase (PMNT) to produce E as the last product in this series of reactions.
   - *Requirements:* SAM (S-adenosylmethionine) to transfer the methyl group and vitamin B12 or folate (as they are involved in the metabolism of methionine to produce SAM).



Now let's look into some details on how they are synthesized and used in axon terminals:

Pathway of dopamine if not converted into other catecholamines:

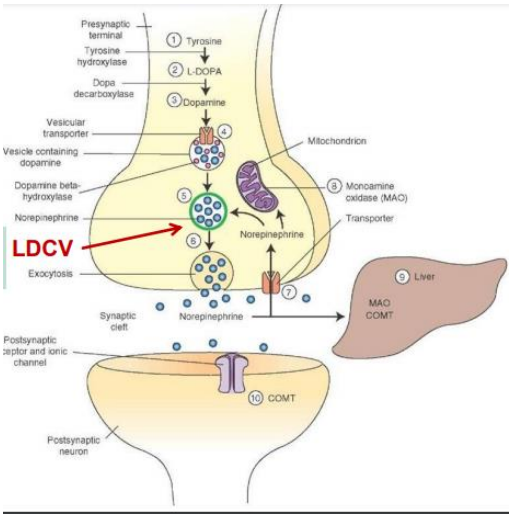
1. After being synthesized from L-dopa (and tyrosine before that), dopamine is packaged into vesicles that can fuse with the membrane to release dopamine into the cleft.
2. In the cleft, the dopamine is going to bind to receptors on the post-synaptic neurons to produce a certain response.



3. Then dopamine needs to have its action terminated. There are multiple options:
    a. 50% - Re-entry of dopamine by a transporter into the pre-synaptic cell to be re-used.
    b. If not needed, the reuptaken dopamine can be metabolized by MAO in the outer mitochondrial membrane.
    c. Another way is to metabolize/degrade dopamine by MAO (monoamine oxidase) and COMT which are present in the liver.
    d. 10% - Dopamine can be degraded by COMT in the post-synaptic neuron.

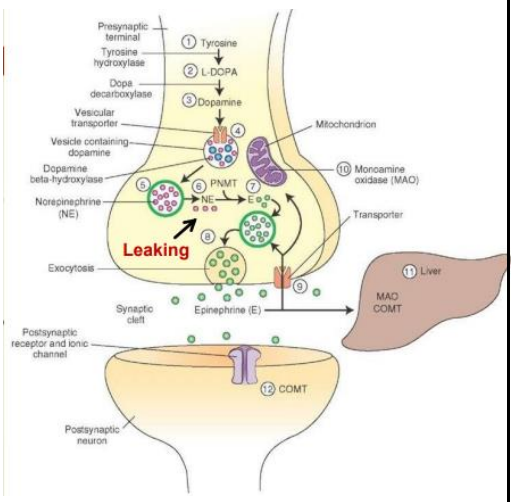
Pathway of Norepinephrine:

1. All the initial steps to reach dopamine are the same as mentioned previously.
2. The vesicles that dopamine is in now have the enzyme dopamine β-hydroxylase which converts dopamine into NE.
3. Once NE is produced, the vesicles become LDCVs that can fuse with the membrane and release NE into the synaptic cleft.
4. Once the function is completed, its concentration needs to be decreased to reduce its activity. This can be done by the same mechanisms that terminated the action of dopamine.



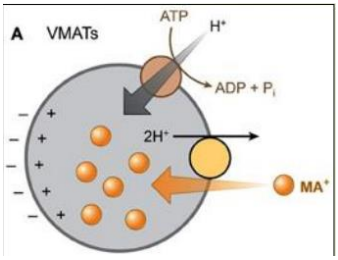
Pathway of Epinephrine:

1. All the initial steps to reach norepinephrine are the same as mentioned previously.
2. The NE leaks out of the vesicles into the cytosol. There, it is converted by the methyl transferase PMNT into epinephrine.
3. This epinephrine re-enters a vesicle that can fuse with the membrane and release its contents into the synaptic cleft.
4. The termination of its action is by the same mechanisms that terminated the action of dopamine.



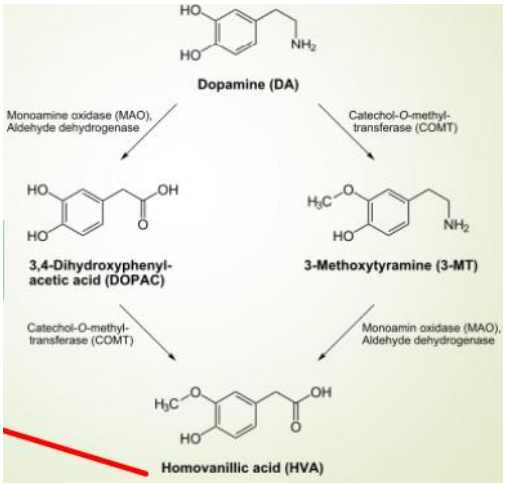
Packaging of Catecholamines Into Vesicles

The catecholamines (dopamine and epinephrine) are transported into vesicles by an ATP-dependent process linked to a proton pump.



1. Protons are pumped into the vesicles by a vesicular ATPase (V-ATPase).
2. Once there is a high concentration of protons inside the vesicles, they can then be exchanged for the entry of positively charged catecholamines via the transporter VMAT2 (vesicle monoamine transporter 2). Note that a transporter is needed as catecholamines are relatively large molecules and cannot diffuse through the membrane.

Catecholamine Degradation – COMT and MAO



- Catecholamine degradation is achieved by a set of two enzymes, COMT (catechol-O-methyl transferase) and MAO. Any one of these can start the degradation process and the other can complete it (So MAO works first then COMT, or vice versa).
- The end product of dopamine degradation is homovanillic acid (HVA) via both enzymes, but the intermediate differs based on which enzyme was used first.
- COMT is a methyl transferase so the inactivation process is dependent on SAM (a methyl carrier). And SAM production is dependent on vitamin B12 and folate.
- HVA is reduced in Parkinson's patients as they do not produce enough dopamine due to a problem in their dopaminergic neurons.

Regulation of catecholamine synthesis:

- Recall that the enzyme **tyrosine hydroxylase** catalyzes the committed, rate-limiting first step of this process, in which hydroxylation of tyrosine into L-DOPA takes place.

Short term regulation:

1. Tyrosine hydroxylase enzyme is inhibited by the presence of free cytosolic catecholamines; so, the end product's presence will inhibit the enzyme responsible for its synthesis (feedback inhibition).
    - How? Catecholamines, when abundant, will compete with cofactor BH4 and interfere with its binding to the enzyme. Thus, the enzyme will not be able to perform its function and catalysis within the speed and activation required.
2. On the other hand, this enzyme can be activated by depolarization.
    - Depolarization of the neurons and the changes in the ion concentration are going to activate a group of enzymes, like PKA, Ca^{2+} Calmodulin (CAM kinases), and PKC. These kinases (PKA, CAM kinases, PKC) will phosphorylate the enzyme

tyrosine hydroxylase, activating it by tightening the binding of the enzyme to BH4.

- The professor's explanation: When there is depolarization, there is no action potential taking place yet → So, there is no catecholamines (they had been deactivated or degraded and their action is halted)→ Thus, we need to activate the synthesis of these neurotransmitters. In conclusion, depolarization induced the synthesis of catecholamines.
- The book's explanation: By tightening the binding of the enzyme to BH4, it makes the enzyme less sensitive to end-product inhibition.

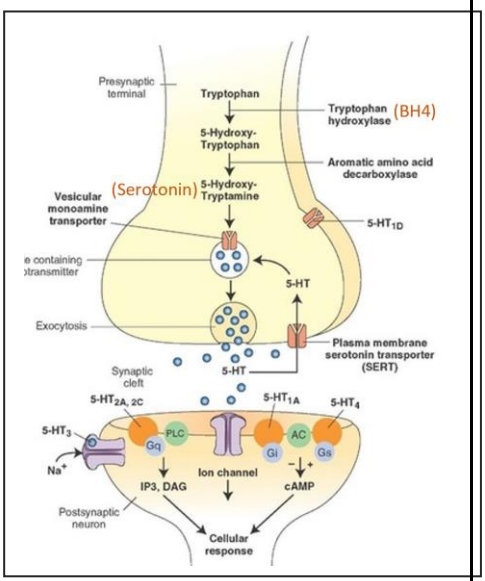
Long term regulation:

- It involves gene regulation and increasing the production or the translation of the tyrosine hydroxylase and dopamine β-hyroxylase enzymes needed for this pathway (i.e., increase in the amounts of these enzymes).
- When sympathetic neuronal activity is increased for a prolonged period, alterations (increase) in the enzyme amounts will happen.

Tryptophan-Derived Neurotransmitters: Serotonin and Melatonin

- Serotonin and melatonin are derived from the amino acid tryptophan.
- Serotonin is the hormone of happiness. It does not cross the BBB, so it has to be synthesized in the nervous system.

Synthesis of Serotonin



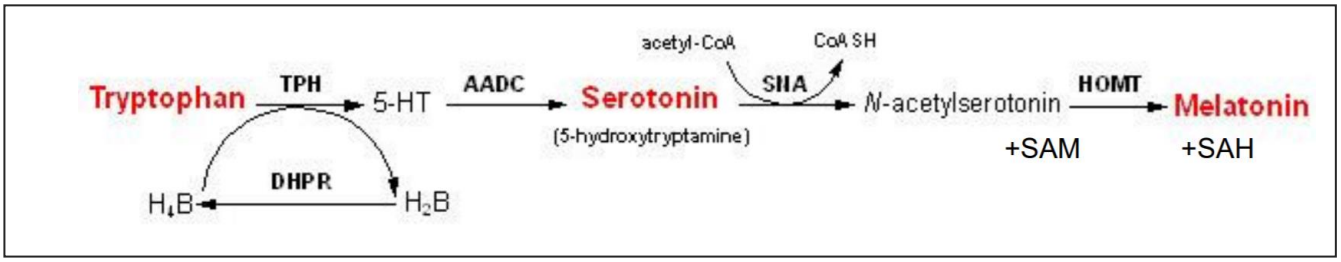
1. The precursor, Tryptophan, will be hydroxylated into 5-Hydroxy-Tryptophan by **Tryptophan hydroxylase**, which needs cofactor **BH4**.
2. 5-Hydroxy-Tryptophan is then decarboxylated into 5-Hydroxy-Tryptamine (also called serotonin) by **Aromatic amino acid decarboxylase**. Now, it's not an amino acid anymore.
3. Serotonin is then packaged/ transferred into vesicles by the **vesicular monoamine transporter**.
4. The vesicles fuse with the presynaptic membrane and release their serotonin content into the synaptic cleft.
5. This serotonin then binds to the postsynaptic membrane receptor, which is a G-protein coupled receptor. Thus, it will activate different types of second messengers, such a IP3/DAG and cAMP, resulting in a set of cellular responses.

Degradation of Serotonin (once its action is finished)

- It can be re-uptaken by the plasma membrane **serotonin transporter (SERT),** after which it is recycled into vesicles to be reused again.
- Or, if we don't need it, it will be metabolized by **monoamine oxidase enzyme (MAO)** into 5-hydroxyindoleacetic acid, which is excreted into urine.
- Antidepressants (like Prozac®), called selective serotonin re-uptake inhibitors (SSRIs), inhibit the reuptake process, thus resulting in prolonged serotonin presence in the synaptic cleft.

Melatonin

- Melatonin is a molecule derived from serotonin.
- Serotonin synthesized in the pineal gland serves as a precursor for the synthesis of melatonin, because it is very similar in structure to melatonin.
- Methylation of serotonin by a methyl transferase (the source of methyl group is from SAM) produces melatonin. (refer to the picture below)
- It is a neurohormone involved in regulating:
    - Sleep patterns
    - Seasonal and circadian (daily) rhythms
    - Dark-light cycle

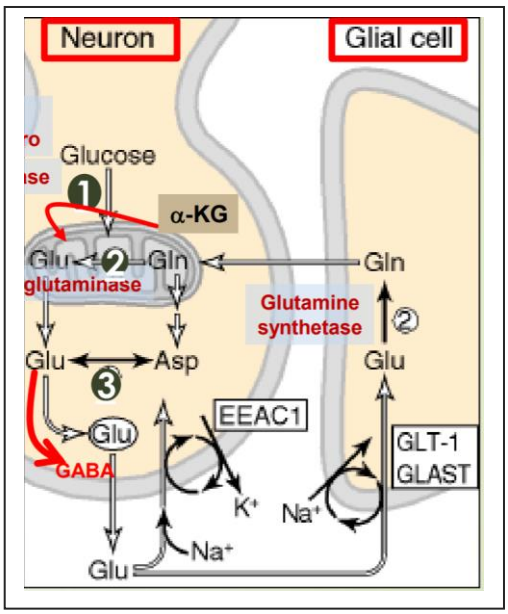


Glutamate and aspartate

- They are nonessential amino acids, which means they can be synthesized in our cells.
- They can act in the nervous system as neurotransmitters. They are excitatory neurotransmitters.
- They do not cross the BBB, thus must be synthesized in neurons de novo from glucose rather than taken up from the blood in order to be used as neurotransmitters.
    - The main synthetic compartments in which they are synthesized are neurons and glial cells.

Now, we will focus on glutamate:

Synthesis of glutamate

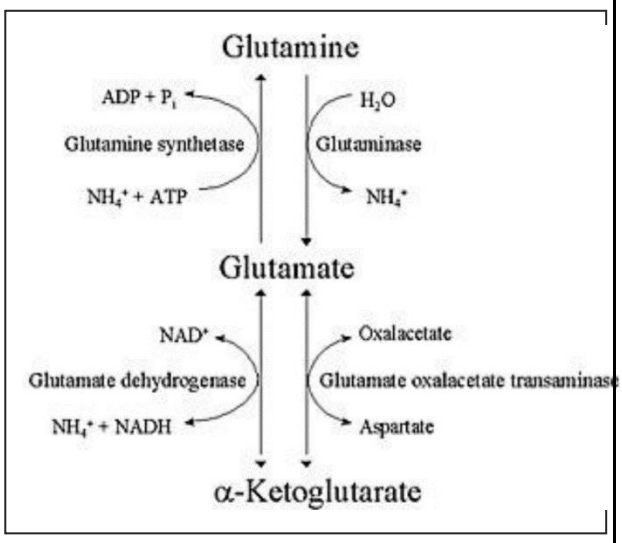


1. There are different sources of glutamate:
   - Glucose→ enters Glycolysis→ Krebs cycle→ Dehydrogenation of α- ketoglutarate (which is a product of Krebs cycle) produces glutamate.
   - Deamination of Glutamine (Gln amino acid) by **glutaminase** produces glutamate. Recall that the difference in structure between glutamate and glutamine is that the latter has an amino group attached to the amide functional group in its R-chain.
   - Transamination of Aspartate amino acid by **aminotransferases** produces glutamate.
2. Once produced, glutamate is stored in vesicles, and its release is Ca2+ dependent.

- Once its action is done, it's removed by high-affinity uptake systems in both nerve terminals and glial cells.
  - On the neuronal membrane, there is excitatory amino acid carrier-1 (EAAC1) that can reuptake it back to stop its action.
  - On the cell membrane of the glial cell, there is glutamate transporter-1 (GLT-1) and glutamate-aspartate transporter (GLAST) that can uptake the glutamate into the glial cell and remove it from the synaptic cleft.

Sources of glutamate (further explanation)

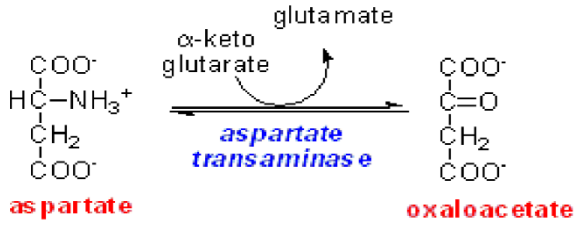
1. Glutamate can be produced from α-Ketoglutarate, a Krebs cycle intermediate, by **glutamate dehydrogenase** enzyme.
   - Note that this enzyme can catalyze the reaction both ways, but the cofactor needed for the forward reaction is different than the reverse reaction.
2. Also, **Glutamate oxaloacetate transaminase** can convert α-Ketoglutarate into glutamate by adding an amino group from aspartate, converting aspartate into oxaloacetate.



3. It can result from the deamination of Glutamine (Gln) by **glutaminase**. This reaction can be reversed from glutamate to glutamine by **glutamine synthetase**, which adds this lost amino group back using ATP as a source of energy for this reaction.

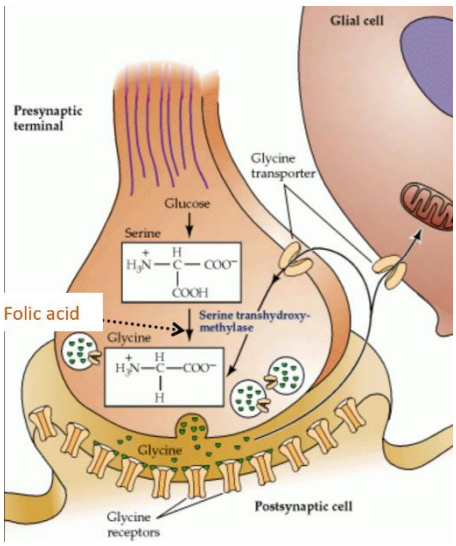
Now, let's discuss aspartate:

- A vesicular uptake mechanism for aspartate has not yet been demonstrated, somewhat weakening the case for considering aspartate to be a neurotransmitter. It is a controversial topic, and some scientists don't consider aspartate as a neurotransmitter.
- The precursor of the synthesis of aspartate is oxaloacetate. Transamination of oxaloacetate by **aspartate transaminase (AST)** (or called aminotransferase) produces aspartate. (refer to the picture below)
    - The amino group comes from glutamate, which becomes α-Ketoglutarate.



Glycine

- It is the major inhibitory neurotransmitter in the spinal cord.



Synthesis

1. It can be synthesized de novo from serine by **serine hydroxy methyltransferase** through 3-phosphoglycerate. (Clarification from book: Serine is synthesized from the intermediate 3-phosphoglycerate in the glycolytic pathway)
    - Serine has a hydroxymethyl group (CH2OH) in its R-chain. So, hence the name, hydroxymethyltransferase or methylase removes this hydroxymethyl group from serine → serine now becomes glycine.
2. Glycine can be packaged in the vesicles.
3. Vesicles fuse with the presynaptic membrane releasing the glycine into the synaptic cleft.

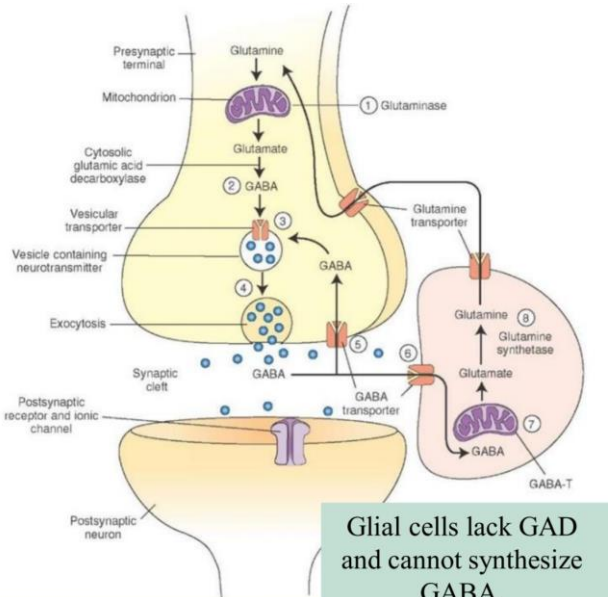
- Removal of glycine is by a high-affinity transporter.

GABA (gamma aminobutyric acid)

- It is derived from the amino acid glutamate.
- It is a major inhibitory neurotransmitter of the CNS.
- GABA is present in high concentrations (millimolar) in many brain regions in comparison to other types of neurotransmitters.
    - These concentrations are about 1,000 times higher than concentrations of the classical monoamine neurotransmitters in the same regions.

There is a closed loop of reactions that happen to GABA called the **GABA shunt.** This process has the dual purpose of producing and conserving the supply of GABA. In other words, it is a series of reactions that recycles GABA in the CNS to conserve glutamate and GABA. It occurs in the following steps:

1. Glutamine is converted into glutamate by **glutaminase**.
2. Glutamate is α-decarboxylated forming GABA by **glutamate decarboxylase (GAD),** which requires pyridoxal phosphate (vitamin B6).
3. GABA is then stored in vesicles until released.
4. GABA will then bind to the post-synaptic membrane receptors inducing a certain action in the postsynaptic neuron.
5. It is then either taken up into the presynaptic terminal and repackaged (so we preserve it) to be released back when needed OR it goes through the GABA Shunt again where it is taken up by the **GABA transporter** into the glial cells.
    - Inside the glial cells, GABA is converted back to glutamate.
    - Glutamate can be converted into glutamine, which is then transported by **glutamine transporter** out of the glial cells into the neighboring nerve terminals to synthesize glutamate (which can be used to synthesize GABA).



Acetylcholine (AC)

- It is the major neurotransmitter at the neuromuscular junction (NMJ).

Synthesis

1. **Choline acetyltransferase** attaches a choline group to an acetyl group, releasing CoA and producing Acetylcholine in the cytoplasm.
    - The choline group is derived from membrane phospholipids and phosphatidylcholine (lecithin) from the diet.
    - The acetyl group (acetylcoenzyme-A) is derived principally from glucose metabolism. First, glucose oxidation forms pyruvate. Then, decarboxylation of pyruvate forms acetyl-CoA via the **pyruvate dehydrogenase** reaction.

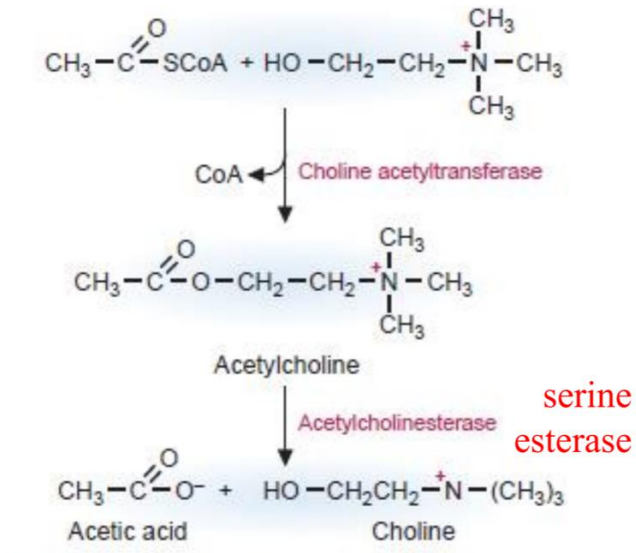
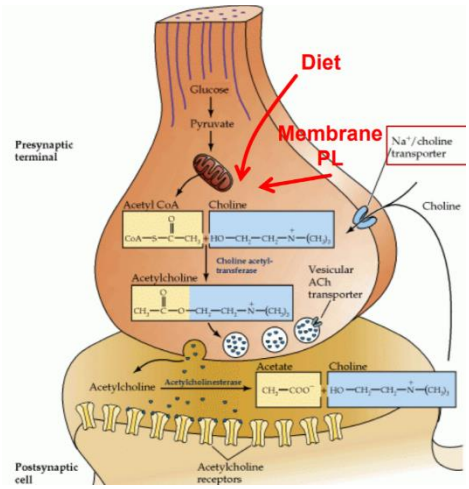


FIG. 48.9 Acetylcholine synthesis and degradation

2. It is then transported into and stored in vesicles which can fuse with the presynaptic membrane.

Degradation

1. **Acetylcholinesterase** enzyme degrades/hydrolyzes AC into its constituents, choline and acetic acid in the NMJ.
2. Choline can be re-uptaken by the **Na+/choline transporter** into the neuron to be reused for the synthesis of acetylcholine again.

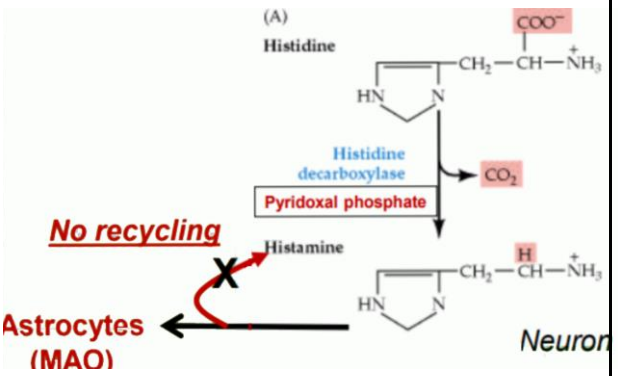


- The sarin gas (nerve gas) inhibits the acetylcholinesterase enzyme. Thus, accumulation of acetylcholine causes constant activation of the nerve-muscle synapses, leading to varying degrees of paralysis.

Histamine

- It is an amine made from the amino acid histidine.
- It does not penetrate the blood-brain barrier and, hence, must be synthesized in the brain or neurons themselves.

Synthesis



1. It is produced by the decarboxylation of histidine by **histidine decarboxylase** (which needs pyridoxal phosphate as a coenzyme) to remove the carboxyl group.
2. Newly synthesized neuronal histamine is stored in the nerve terminal vesicles.
3. Once released from neurons, histamine is thought to activate both postsynaptic and presynaptic receptors (it acts on both).

Histamine Inactivation

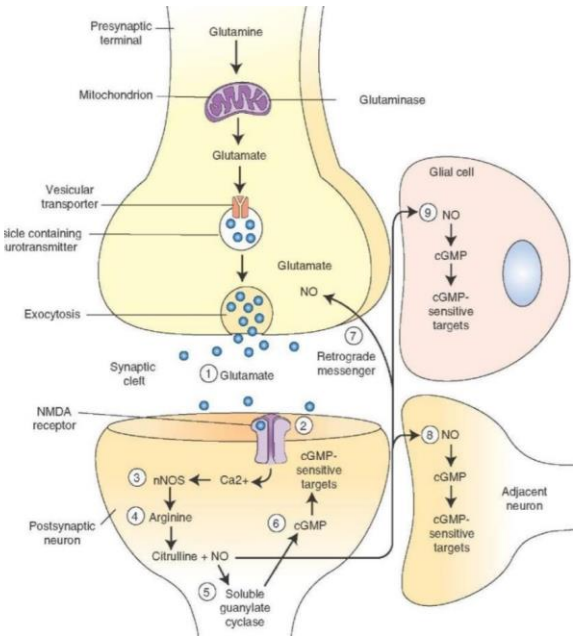
1. **Histamine methyltransferase** and then oxidation by **MAO-B** (in the brain, specifically astrocytes).
2. **Diamine oxidase** (histaminase) (in the peripheral tissues).

- Notice that there is no recycling of histamine back into the presynaptic terminal.

NO synthesis by NO synthase

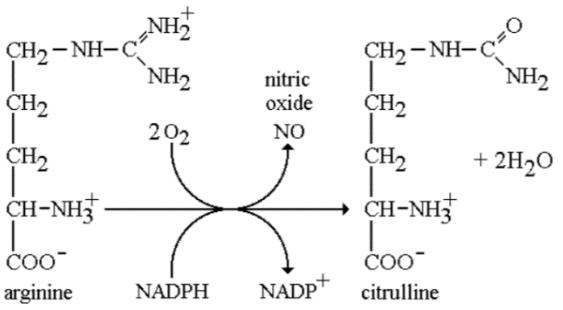
- NO (nitric oxide) is a gaseous molecule that is synthesized by **NO synthase**.
- Half-life: 2-4 seconds.
- NO is inhibited by hemoglobin and other heme proteins which bind it tightly.

A



Synthesis (refer to figure A)

1. Inside the presynaptic neuron, glutamine → glutamate → vesicles → Glutamate is released.
2. Glutamate acts on NMDA (N-methyl-D-aspartate) receptors located on the post-synaptic neuron.
   - These receptors can auto-transport glutamate into the neuron.
3. As a result, Ca2+ enters the postsynaptic neuron activating NO synthase (NOS).
4. NOS converts arginine, the precursor, to citrulline, producing NO as a side-product. (refer to figure B)



5. NO stimulates/activates guanylate cyclase forming cGMP, which results in a physiological response.
6. NO can diffuse out of the neuron because it's a small gaseous molecule:
    - To the presynaptic terminal (acting as a retrograde messenger), prolonging the effect.
    - To adjacent neurons, activating guanylate cyclase to produce cGMP.
    - To glial cells stimulating guanylate cyclase.

NO synthase has three different isoforms. All three isoforms require BH2 (dihydrobiopterin) as a cofactor and nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme.

1. Isoform I (nNOS or cNOS):
    - Present in **Neurons** and **epithelial cells**.
    - Activated by the influx of extracellular calcium.
2. Isoform II (iNOS):
    - Present in **Macrophages** and **smooth muscle cells**.
    - Since it is present in macrophages, it is induced by cytokines (inflammatory mediators).
3. Isoform III (eNOS):
    - Present in **Endothelial cells** lining blood vessels.
    - Activated by the influx of extracellular calcium.

Is NO a neurotransmitter? Yes, but it has some differences from the traditional neurotransmitters.

1. It is not stored in vesicles because it's a small gaseous molecule that can diffuse easily.
2. It is not released by calcium-dependent exocytosis (it diffuses).
3. Its inactivation is passive. There is no active process that terminates its action, as in no molecule can bind it or degrade it. Thus, it decays spontaneously.
4. It does not interact with receptors on target cells since it diffuses through the membranes.
    - Its sphere of action depends on the extent to which it diffuses, and its action is not confined to the conventional presynaptic-postsynaptic direction.
5. It acts as a retrograde messenger and regulates the function of axon terminals presynaptic to the neuron in which it is synthesized.