Biochemistry of Neurotransmitters

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Central Nervous System

References

- Mark's Basic Medical Biochemistry, 4th ed, pp. 908-918 or 5th edition
- http://what-when-how.com/neuroscience/neurotransmitters-the-neuron-part-1/

What is a neurotransmitter?

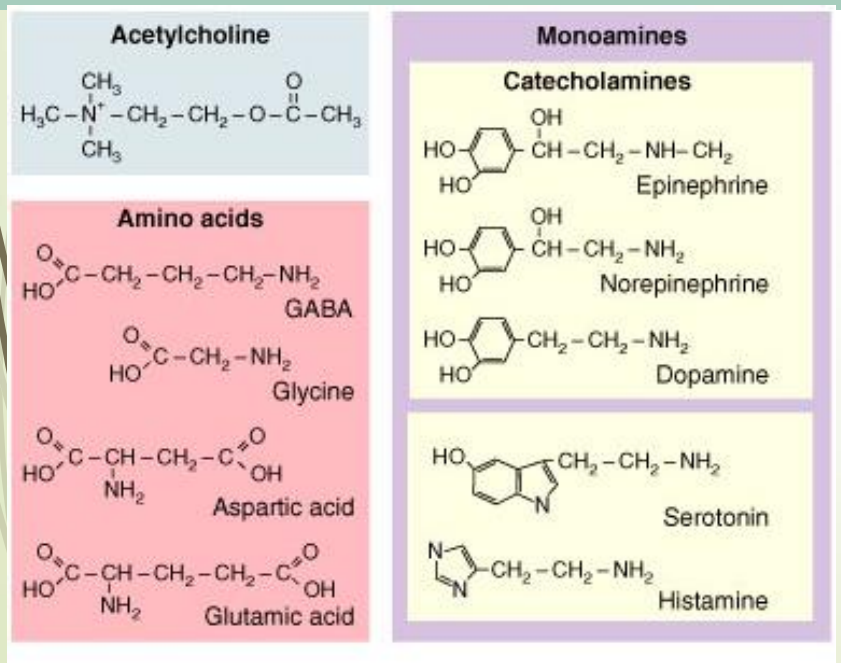
- A neurotransmitter is a chemical substance that is:
  - synthesized in a neuron,
  - Is released at a synapse following depolarization of the nerve terminal (usually dependent on influx of calcium ions),
  - binds to receptors on the postsynaptic cell and/or presynaptic terminal
  - to elicit a specific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

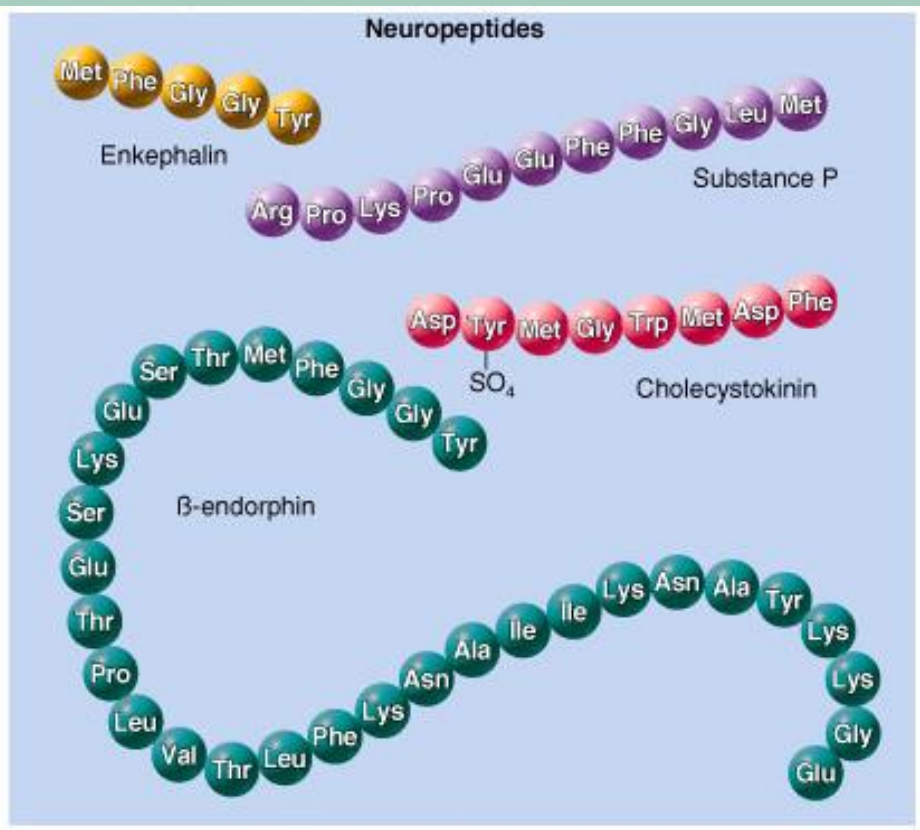
Characteristics of a neurotransmitter

- A chemical substance that:
  - Is synthesized and stored in a presynaptic neuron (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)
  - binds to receptors on the postsynaptic cell and/or presynaptic terminal
  - elicits rapid-onset and rapidly reversible responses in the target cell
  - Is removed or inactivated from the synaptic cleft.

Types and structure of neurotransmitters

- Small-molecule
  - Amines (acetylcholine, epinepherine, dopamine, histmaine, serotonin, norepinephrine, etc.)
  - Amino acids (glutamate, aspartate, glycine)
- Neuropeptides
- Gases (nitric oxide)





Neuron may contain

- (1) more than one small-molecule neurotransmitter
- (2) more than one neuropeptide neurotransmitter,
- or (3) both types of neurotransmitters.
- 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 uses for transmission through a synapse or to another cell.
- The neuronal tracts are often identified by their neurotransmitter; e.g, a dopaminergic tract synthesizes and releases the neurotransmitter dopamine.
- *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

- Excitatory or inhibitory
- Does not depend on the chemical nature of the transmitter.
- Depends on the type of receptor being activated and the ion species that becomes more permeable.

Neuropeptides

Introduction

- Usually mediate slow, ongoing brain functions
- More than 50 neuropeptides have been described
  - Behavior
  - Pain perception
  - Memory
  - Appetite
  - Thirst
  - Temperature
  - Homeostasis
  - Sleep

Neuropeptides: neurohormones or neurotransmitters?

- Neurohormones: a messenger that is released by neurons into the haemolymph and exert its effects on distant peripheral targets, e.g (TSH, 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.

Classification of neuropeptides

- Peptides can be grouped by structural and functional similarity.

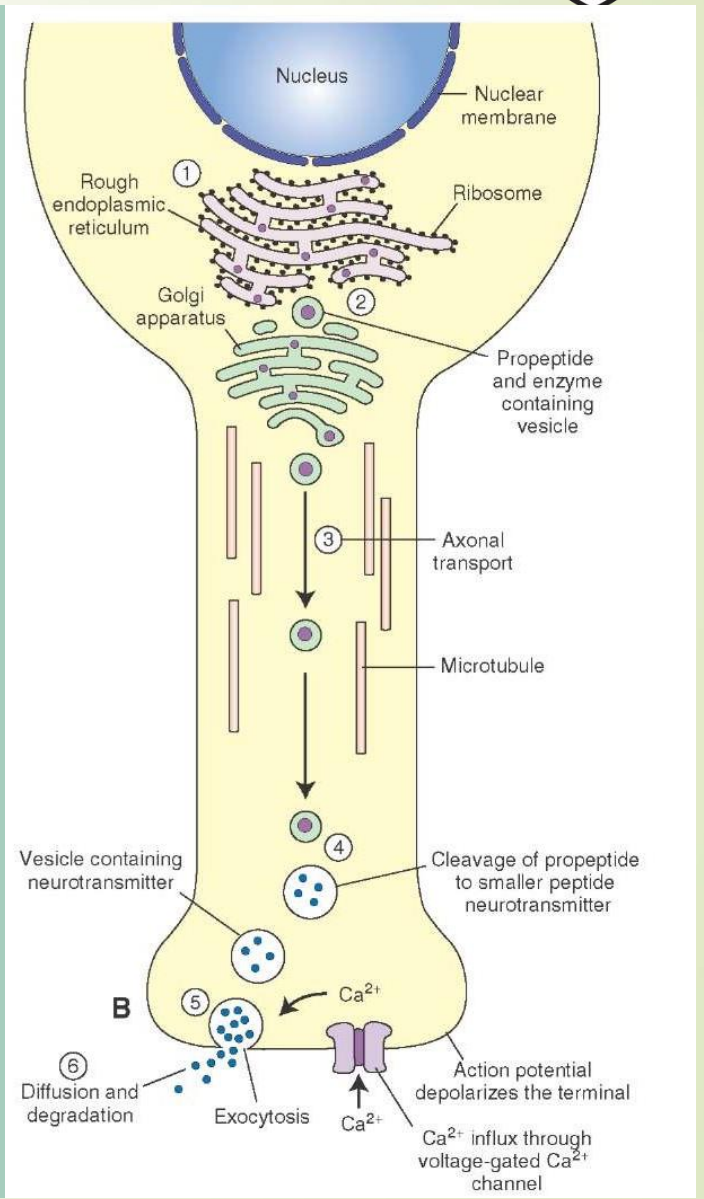
Neuropeptide Families
Tachykinins: substance P, bombesin, substance K **Insulins**: insulin, insulin-like growth factors **Somatostatins**: somatostatin, pancreatic polypeptide **Gastrins**: gastrin, cholecystokinin **Opioids**: opiocortins, enkephalins, dynorphin

- 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

- Synthesis (ER and Golgi apparatus)
- Packaging into large-dense core vesicles (with modifying enzymes)
- Transport (fast-axonal transport)
  - During the transport, proteases cleave the precursor neuropeptide into the final mature form.
- Release
  - They are released gradually over time in response to general increases in the level of intracellular calcium.
- Action (prolonged)
- Termination by diffusion and degradation (no reuptake)



The action of the neurotransmitter is terminated through:

- Reuptake into the presynaptic terminal
- Uptake into glial cells
- Diffusion away from the synapse
- Enzymatic inactivation.
  - May occur in the postsynaptic terminal, the presynaptic terminal, or an adjacent astrocyte microglia cell or in endothelial cells in the brain capillaries.

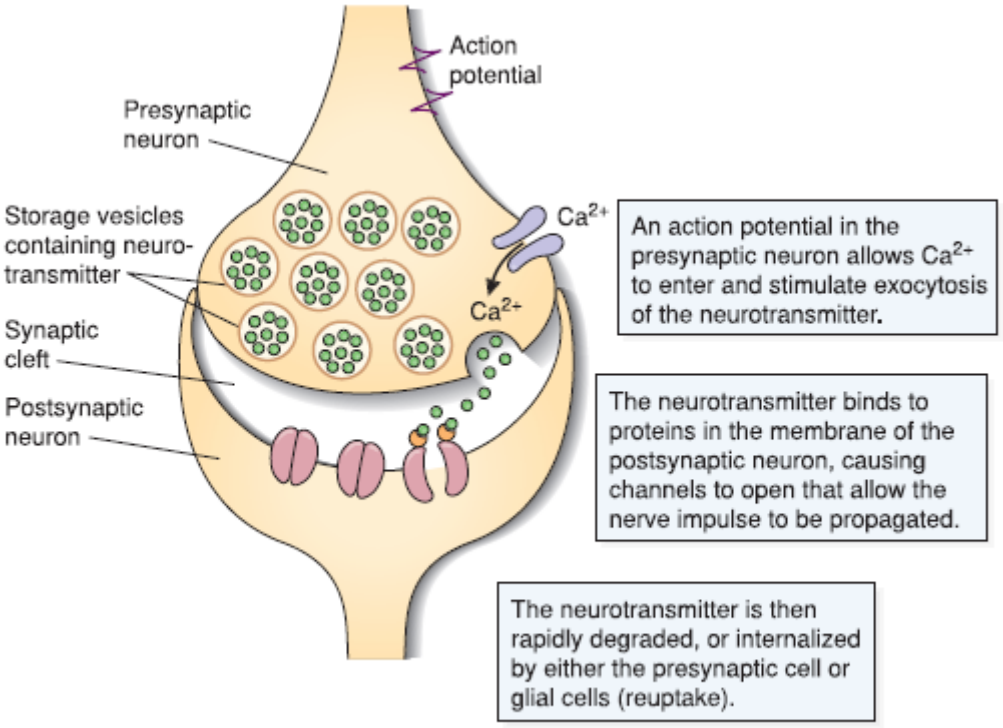
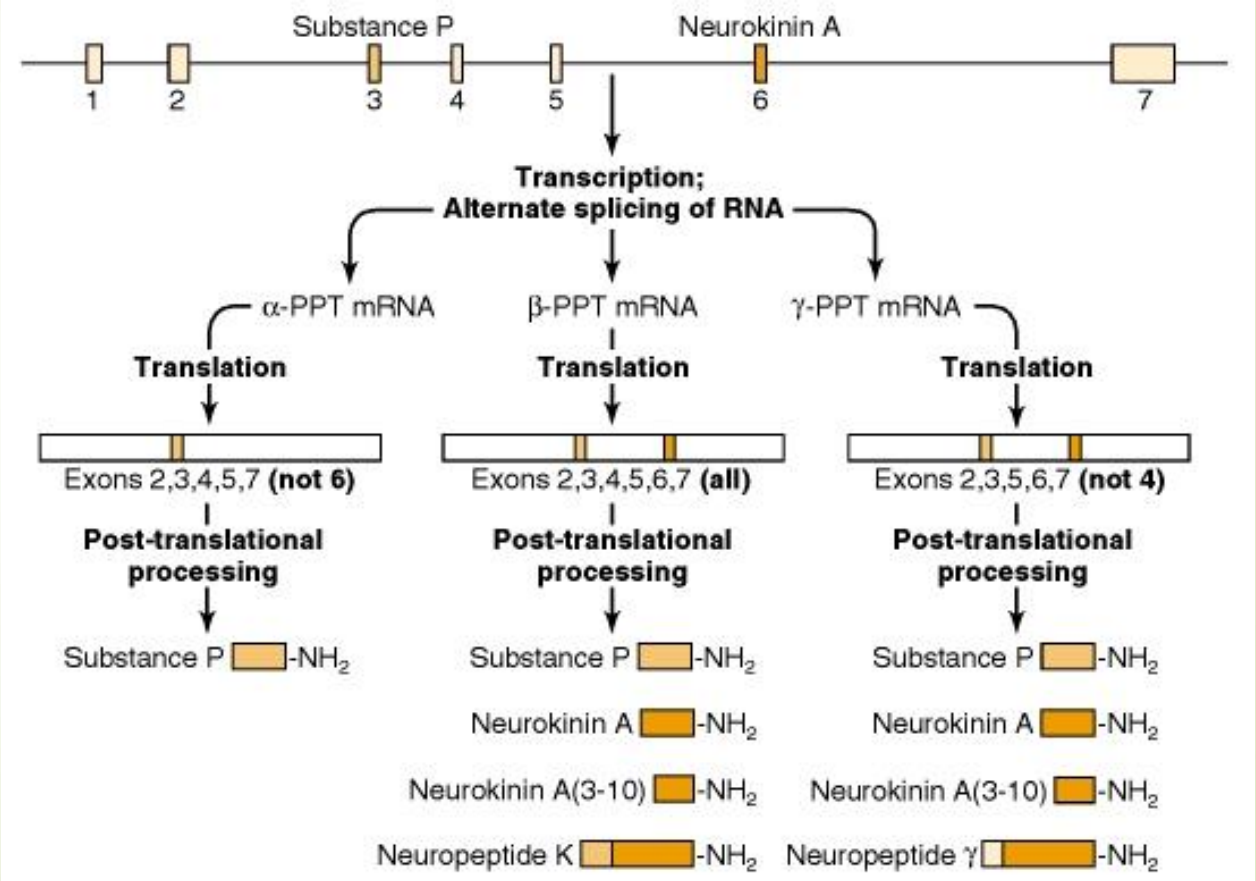


FIG. 48.3. Action of neurotransmitters.

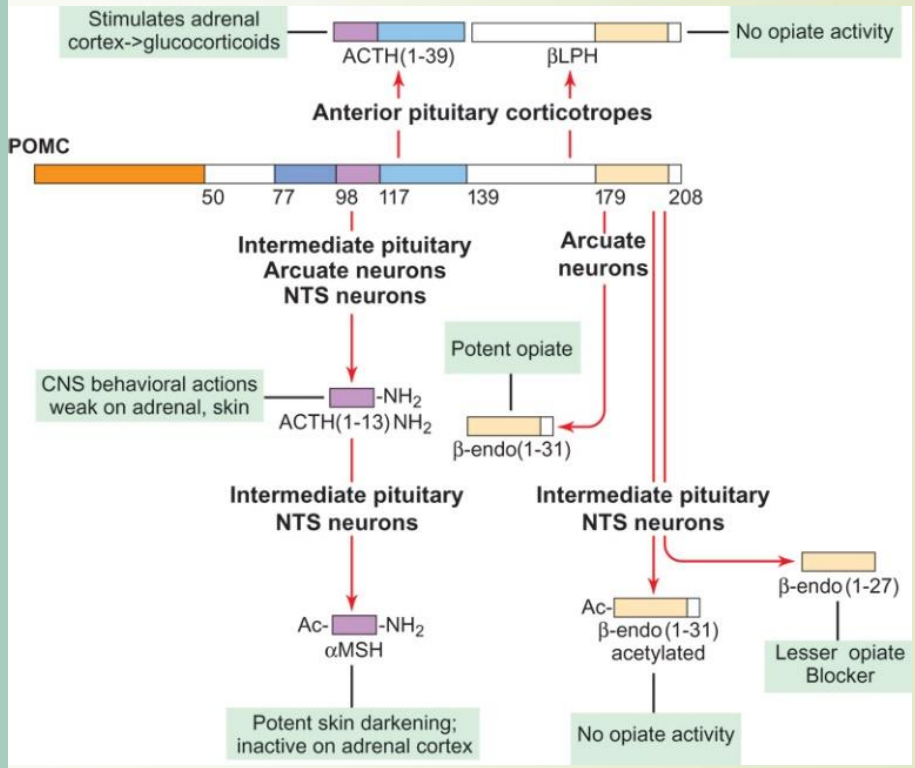
Diversity: alternative splicing

- Alternative splicing of mRNA 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.



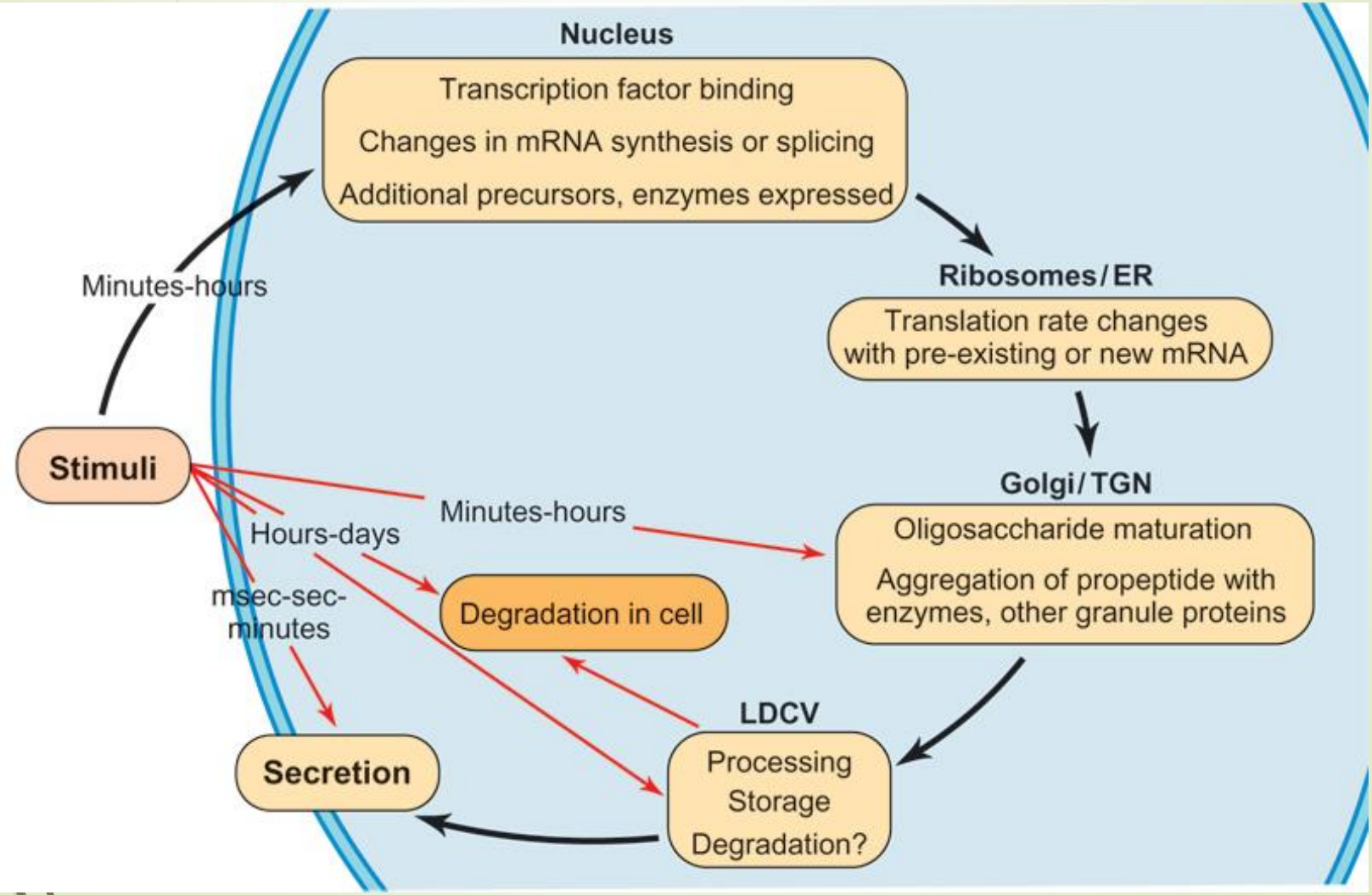
Diversity: proteolytic, differential, sequential processing

- Neuropeptides are produced from a longer precursor protein by
  - Proteolytic processing.
  - Vesicular packaging of different proteases that recognize different cleavage sequences
  - Hiding a proteolytic site by post-translational modifications (example: addition of a carbohydrate side chain).
  - Tissue-specific



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.

The levels of regulation of neuropeptide expression



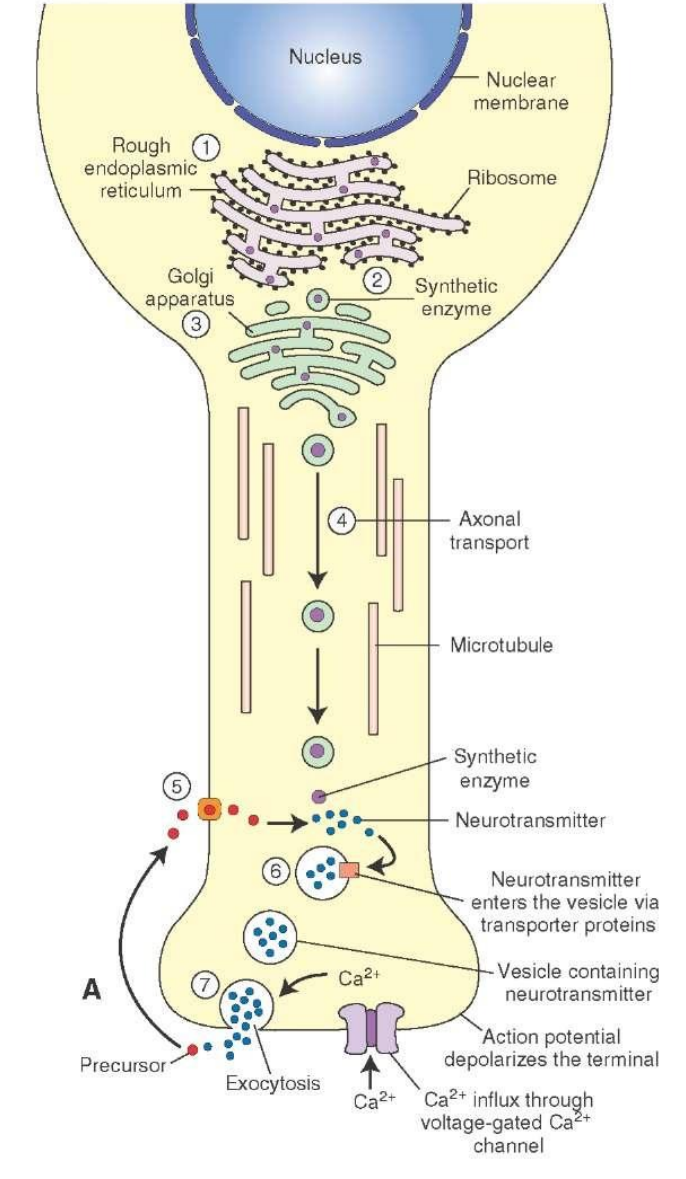
Small-molecule neurotransmitters

Types of small-molecule neurotransmitters

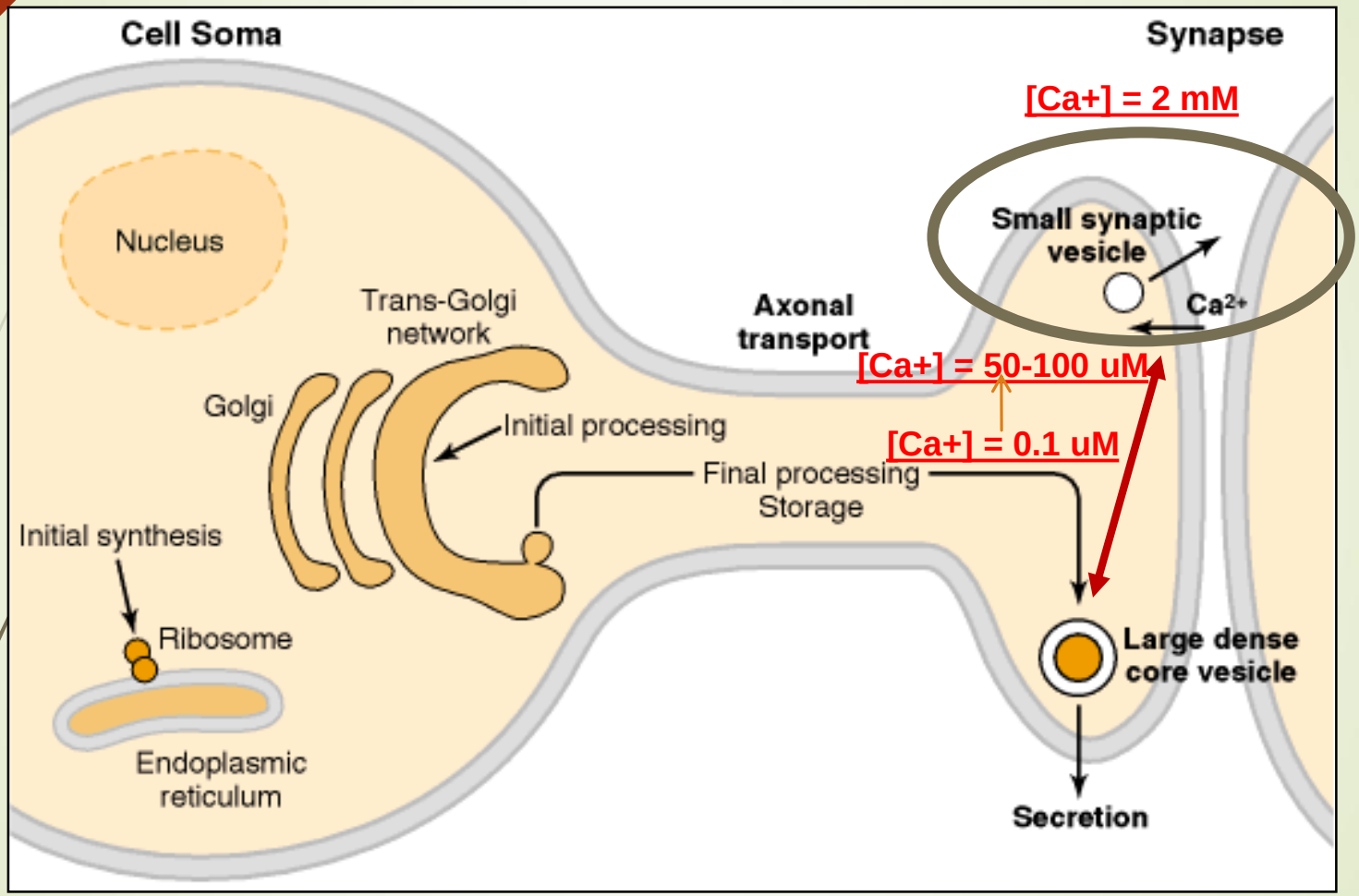
- Nitrogen-containing molecules
  - amino acids and their derivatives
  - intermediates of glycolysis and the Krebs cycle (TCA cycle)

Stages of action

- Synthesis of enzymes
  - RER in the cell body
  - ER-Golgi apparatus (packaging into large-dense core vesicles)
- Transport of enzymes (slow and fast-axonal transport)
- Synthesis in pre-synaptic terminal
- Packaging in synaptic vesicles
- Release
  - They are released in brief pulses each time an action potential triggers the influx of calcium
- Action (short)
- Termination by diffusion, re-uptake, or inactivation

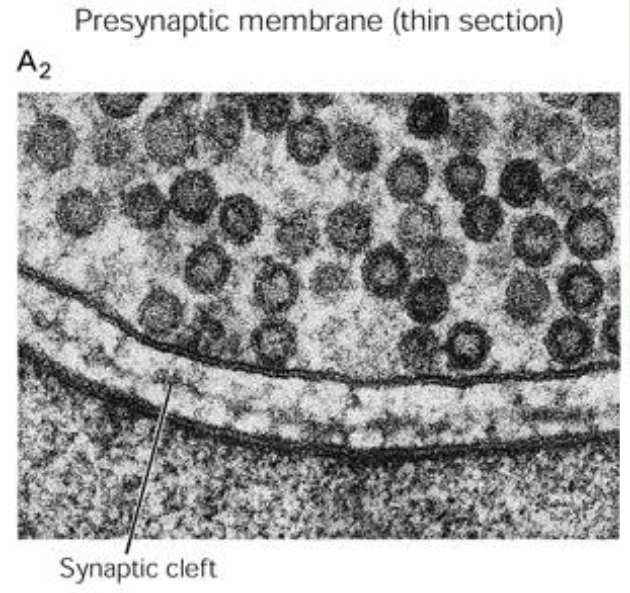


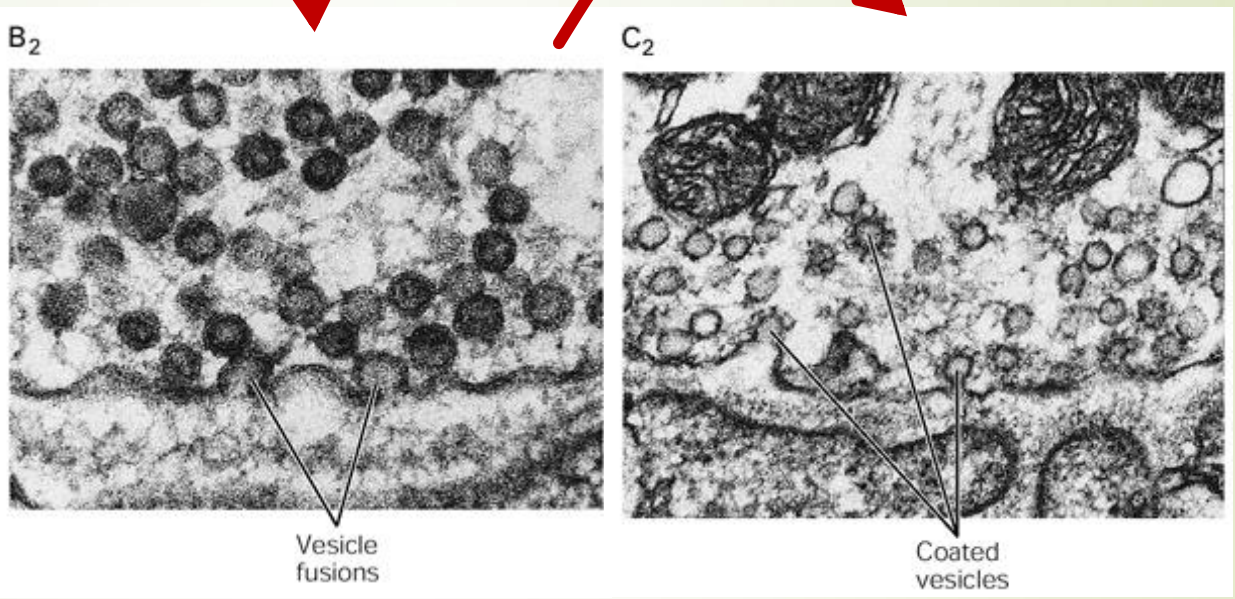
Role of calcium



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

Vesicular Membrane Recycle

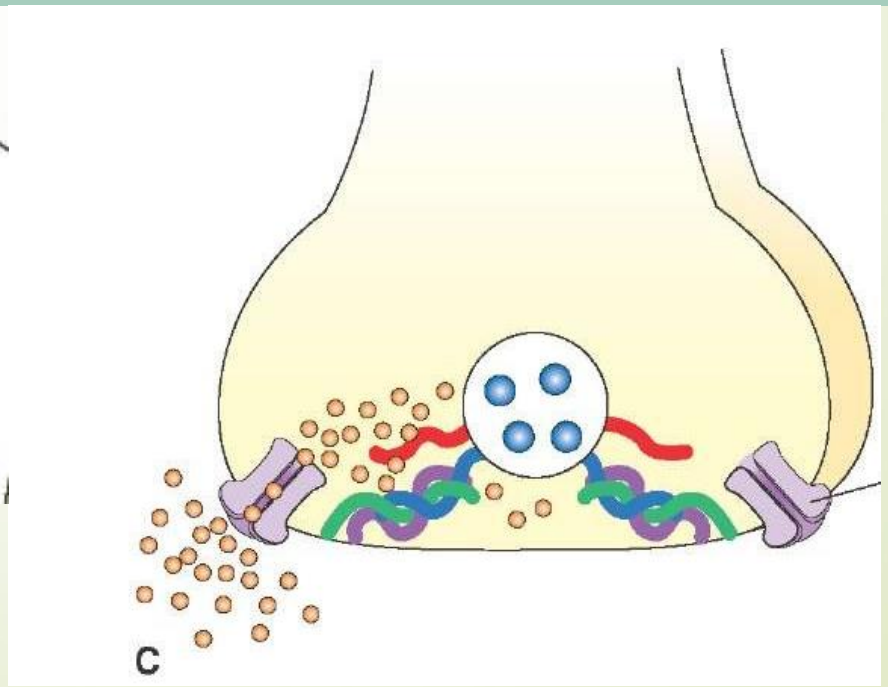




- The fused vesicular membrane is retrieved and recycled within a minute by a complex process called endocytic budding.
- Several proteins, including clathrin, form a basket-like lattice on the remnants of the fused vesicle giving the appearance of a coated pit which is then pinched off from the presynaptic membrane by another protein called dynamin

Vesicular Fusion Proteins and exocytosis

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.



Differences between neuropeptides and small molecule neurotransmitters

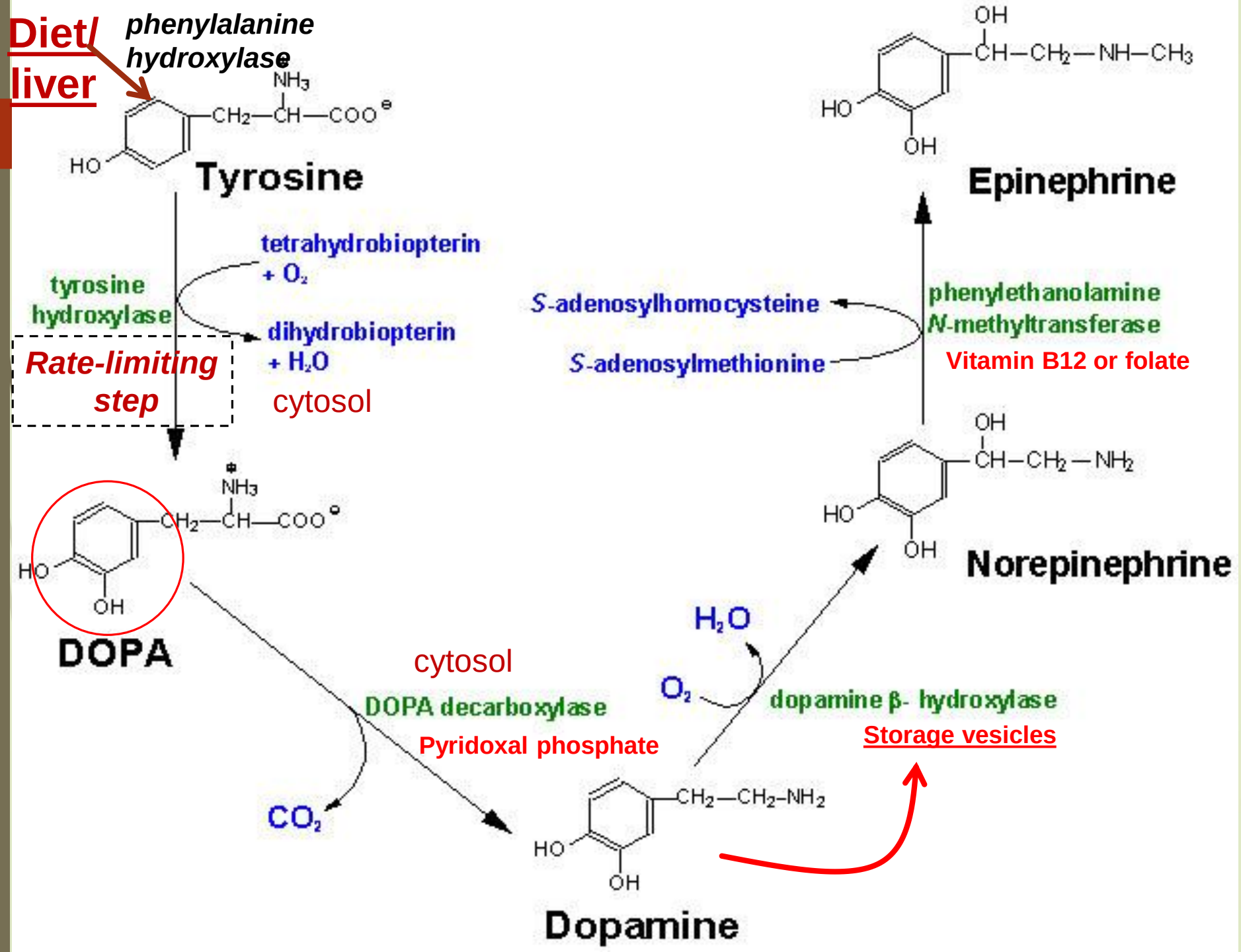
- Onset and duration of action
- Synthesis, transport, and packaging
- Concentration for action and receptor binding
- Concentration of [Ca+] for release
- Site of synthesis, modification
- Fate

Synthesis of neurotransmitters

- Most are synthesized from amino acids, intermediates of glycolysis and the TCA cycle, and O2 in the cytoplasm of the presynaptic terminal.
- The rate of synthesis is generally regulated to correspond to the rate of firing of the neuron.

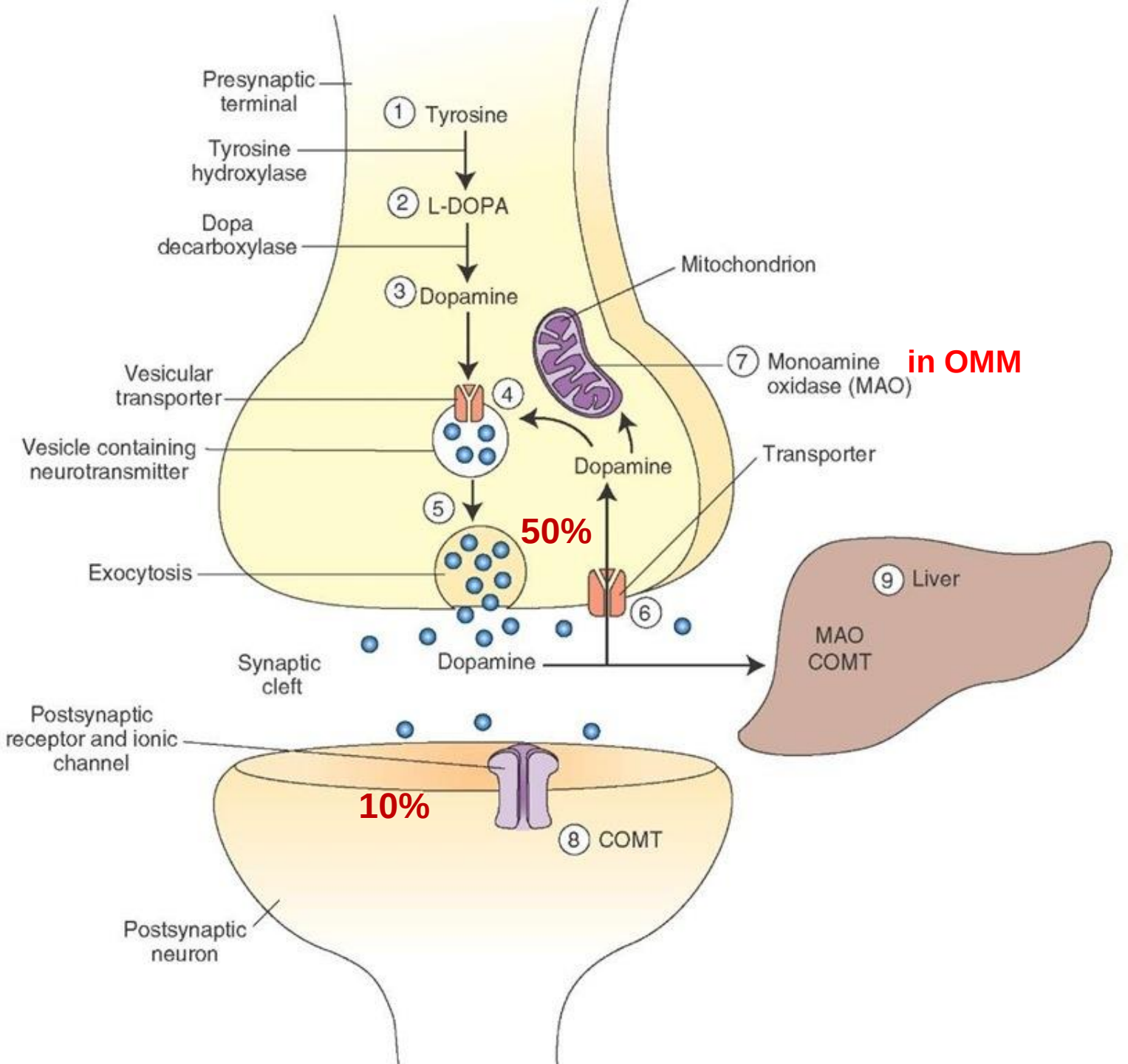
Tyrosine-Derived Neurotransmitters

Dopamine, norepinephrine, and epinephrine

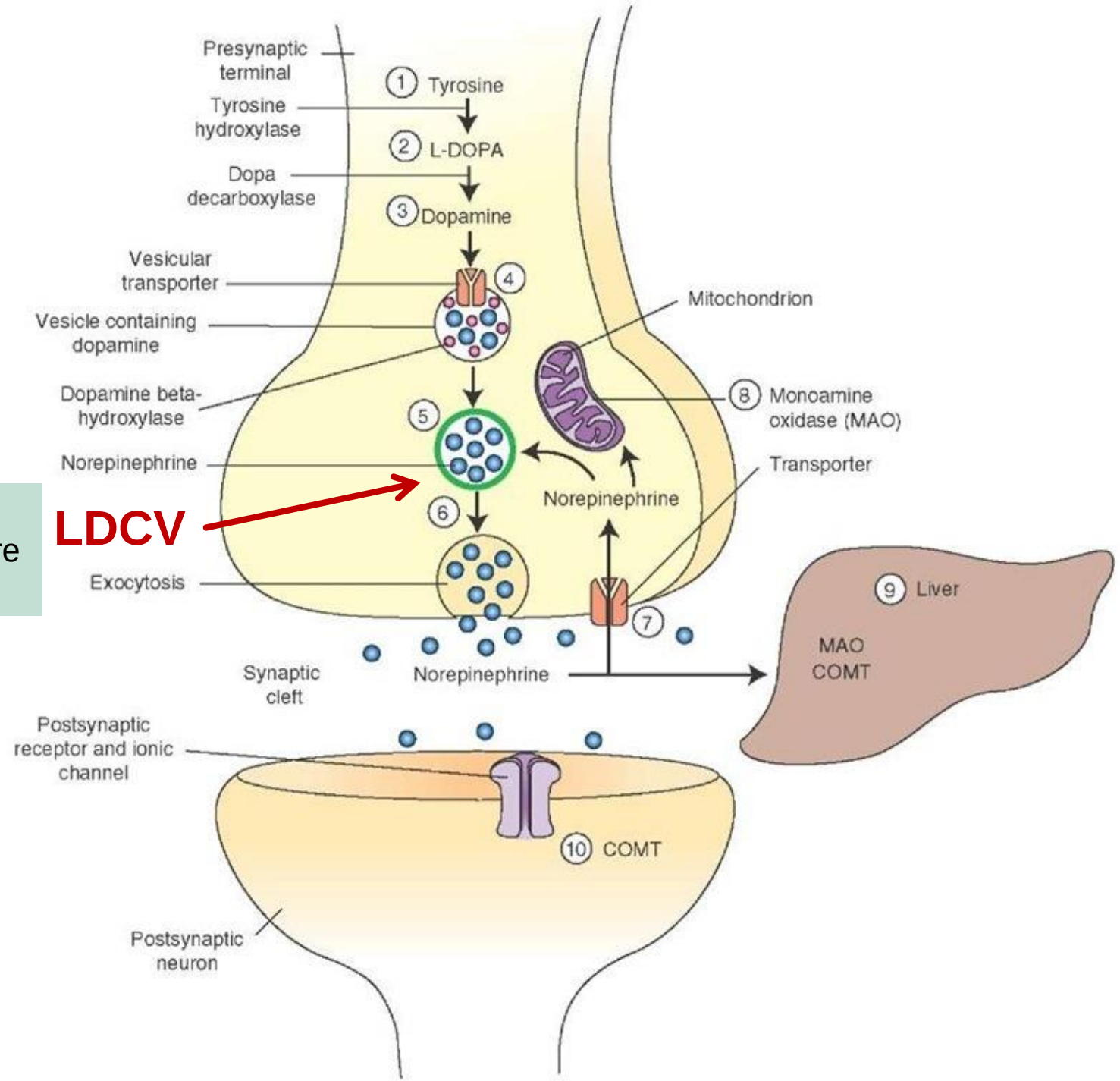

Diet/ liver
phenylalanine hydroxylase
Tyrosine
tyrosine hydroxylase
tetrahydrobiopterin + O2
dihydrobiopterin + H2O
Rate-limiting step
cytosol
DOPA
cytosol
DOPA decarboxylase
Pyridoxal phosphate
CO2
H2O
O2
dopamine β- hydroxylase
Storage vesicles
Dopamine
S-adenosylhomocysteine
S-adenosylmethionine
phenylethanolamine N-methyltransferase
Vitamin B12 or folate
Norepinephrine
Epinephrine

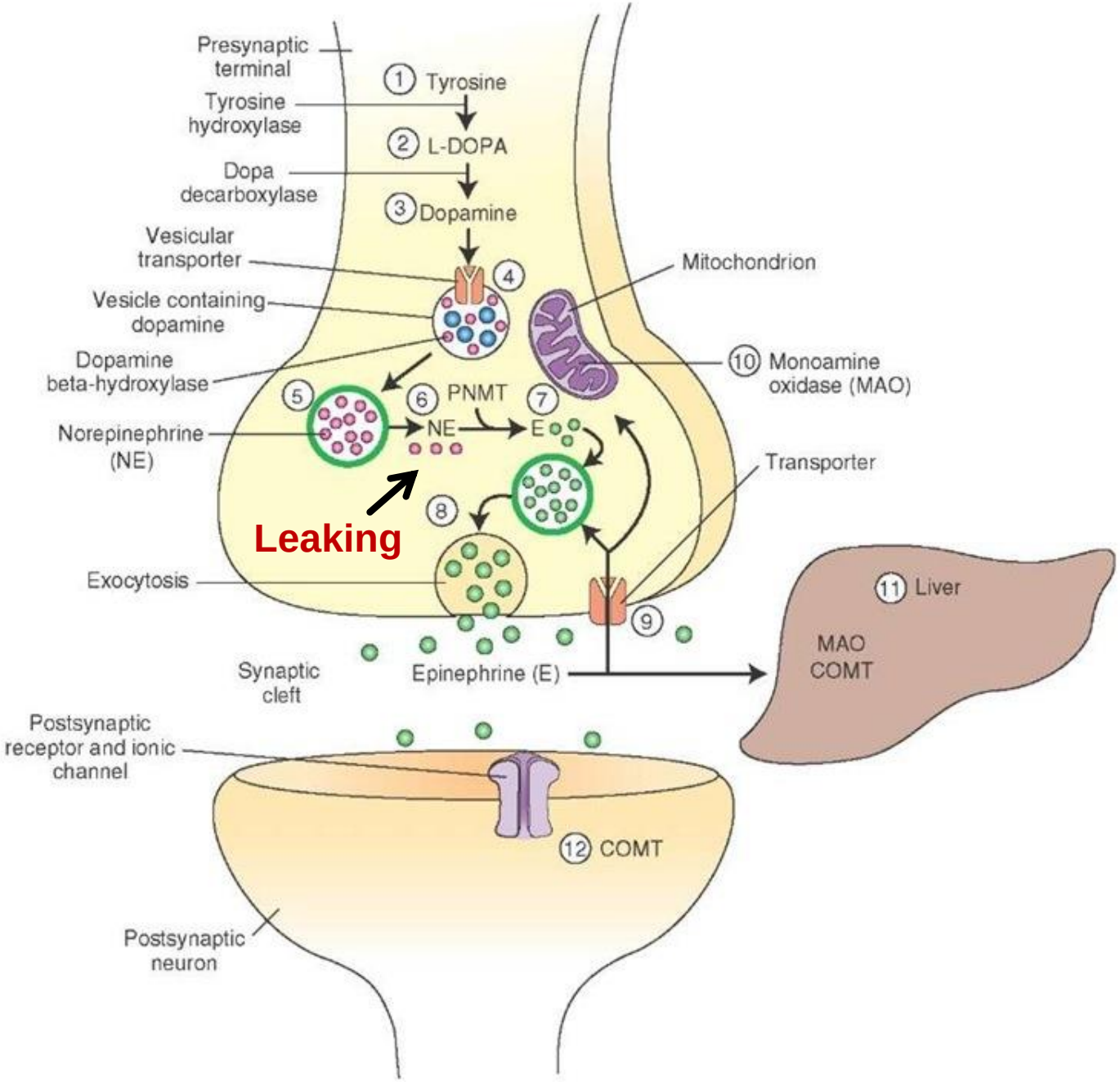
Notes

- Role of cofactors
  - S-adenosylmethionine (methyl transfer)
  - Pyrodoxal phosphate (vitamin B6): transamination, decarboxylation
  - Tetrahydrobiopterin (BH4)


Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Mitochondrion
7 Monoamine oxidase (MAO)
in OMM
Vesicular transporter
4
Vesicle containing neurotransmitter
Dopamine
Transporter
5
50%
Exocytosis
9 Liver
MAO
COMT
6
Synaptic cleft
Dopamine
Postsynaptic receptor and ionic channel
10%
8 COMT
Postsynaptic neuron

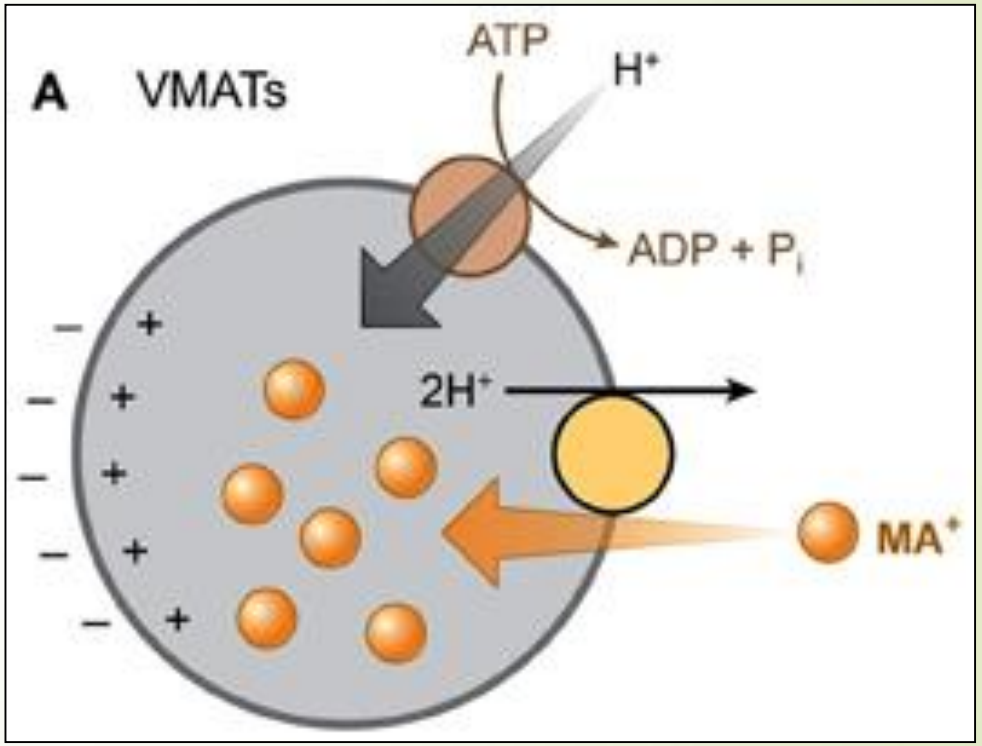
large dense-core vesicles



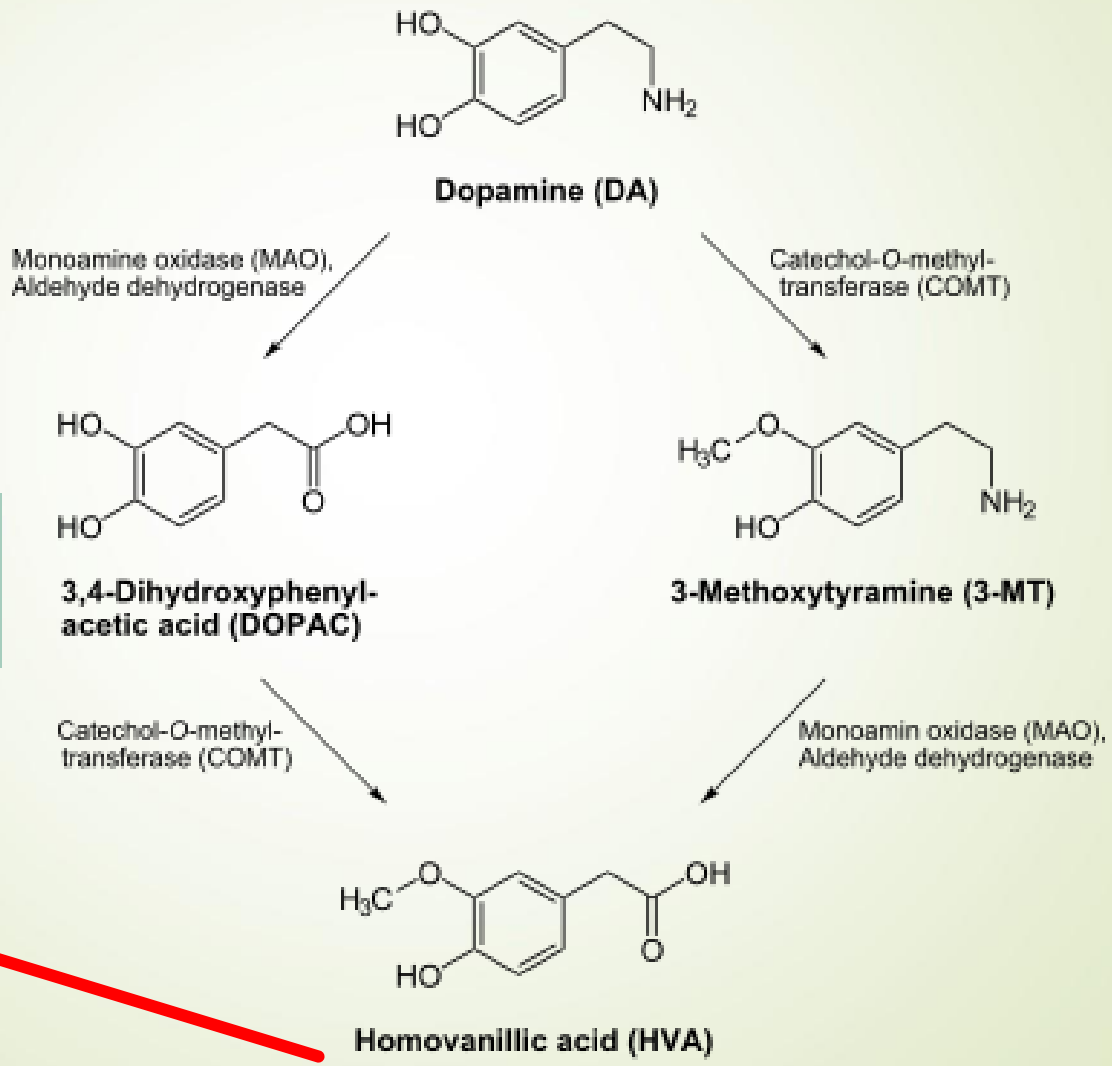

Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Vesicular transporter
4
Mitochondrion
Vesicle containing dopamine
Dopamine beta-hydroxylase
10 Monoamine oxidase (MAO)
5
6 PNMT
7
Norepinephrine (NE)
NE
E
Transporter
Leaking
8
Exocytosis
11 Liver
MAO
COMT
9
Synaptic cleft
Epinephrine (E)
Postsynaptic receptor and ionic channel
12 COMT
Postsynaptic neuron

Packaging of catecholamines into vesicles

- The catecholamines (dopamine an epinepherine) are transported into vesicles by an ATP-dependent process linked to a proton pump.
- Protons are pumped into the vesicles by a vesicular ATPase (V-ATPase).
- The protons then exchange for the positively charged catecholamine via the transporter VMAT2 (vesicle monoamine transporter 2).



Catecholamine Degradation- COMT and MAO



Inactivation is dependent on SAM, vitamin B12 and folate

Parkinson's disease

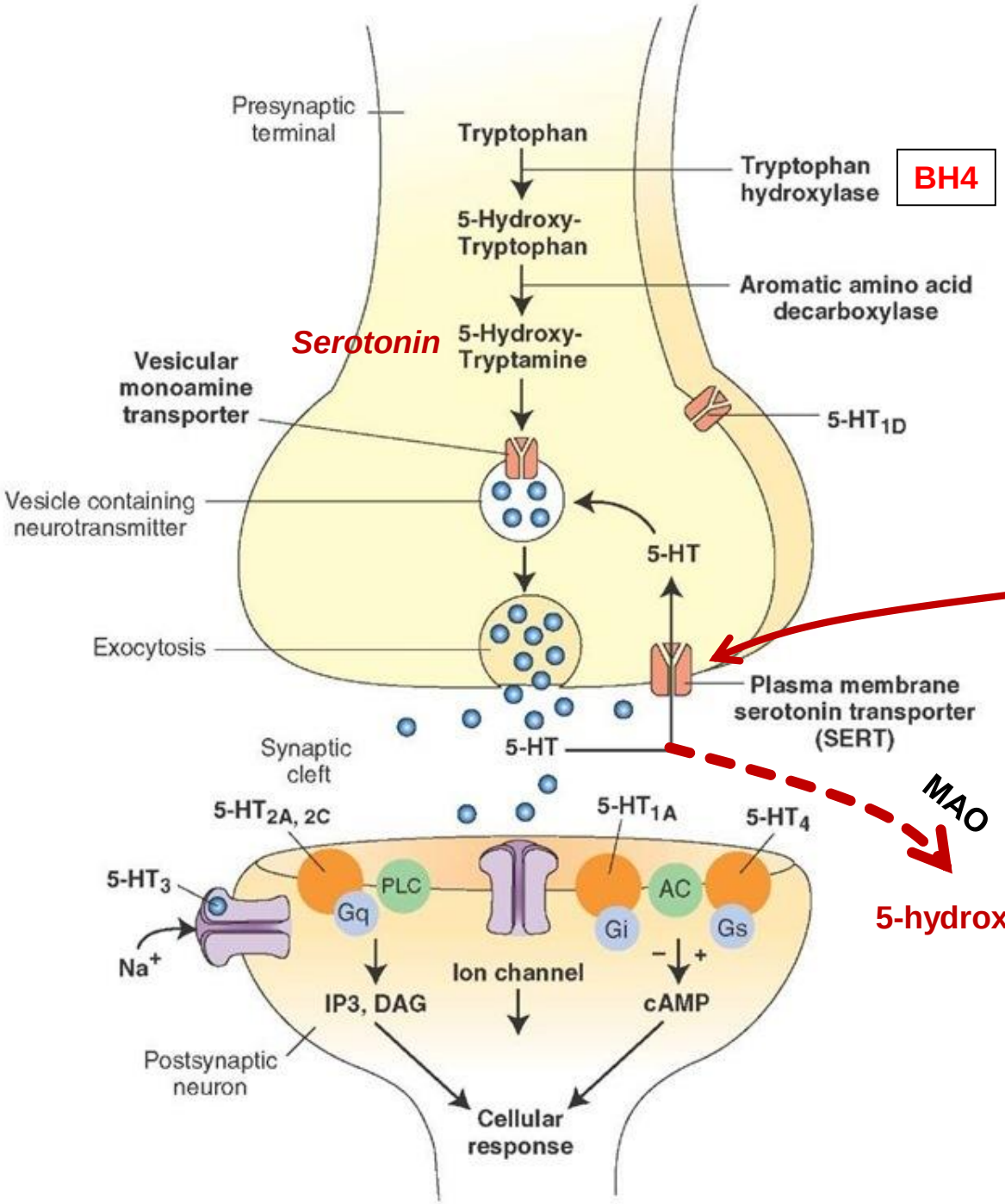
Regulation of catecholamine synthesis

- Tyrosine hydroxylase
  - Short term
    - Inhibition by free cytosolic catecholamines
      - Catecholamines compete with BH4 binding to enzyme
    - Activation by depolarization
      - Tight binding of the enzyme to BH4 following phosphorylation by PKA, CAM kinases, PKC
  - Long-term (increases in the amounts of tyrosine hydroxylase and dopamine β-hyroxylase)
- Alterations (increase) in the enzyme amounts when sympathetic neuronal activity is increased for a prolonged period

Tryptophan-Derived Neurotransmitters

Serotonin and melatonin

Serotonin



- Does not cross BBB

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

5-hydroxyindoleacetic acid

urine

Melatonin

- Serotonin synthesized in the pineal gland serves as a precursor for the synthesis of melatonin, which is a neurohormone involved in regulating:
  - sleep patterns
  - Seasonal and circadian (daily) rhythms
  - Dark-light cycle

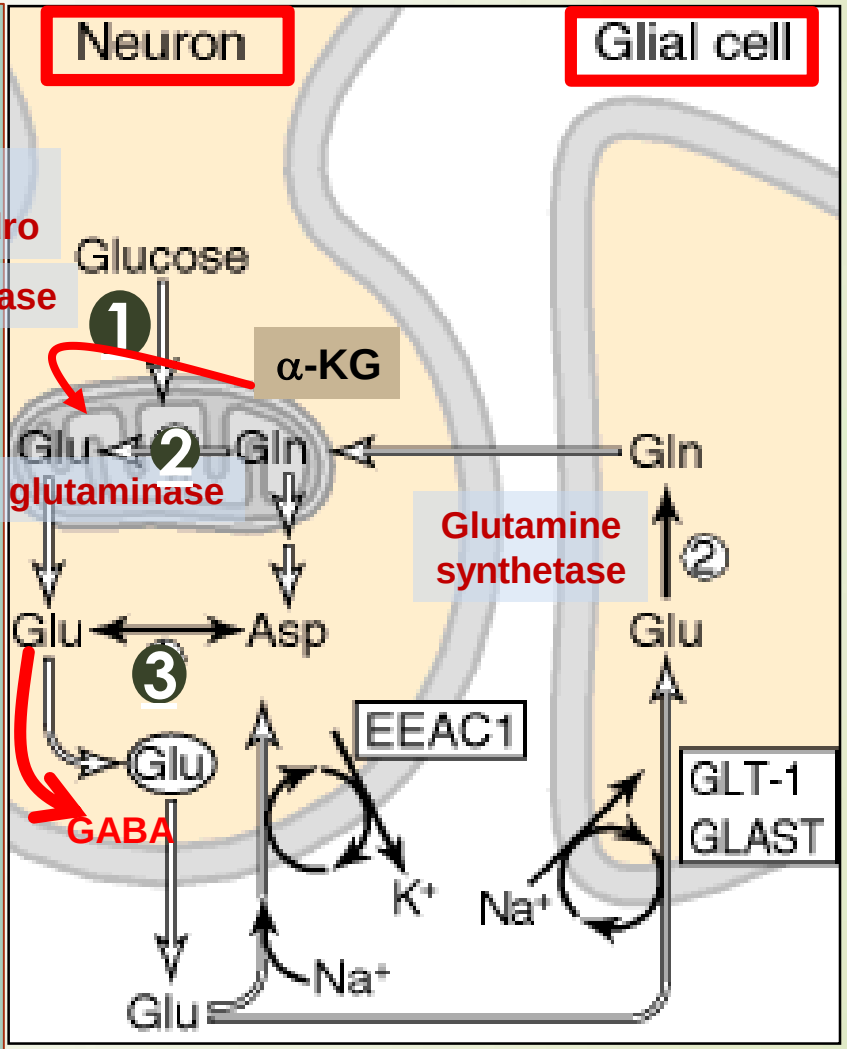
Glutamate and aspartate

Glutamate and aspartate

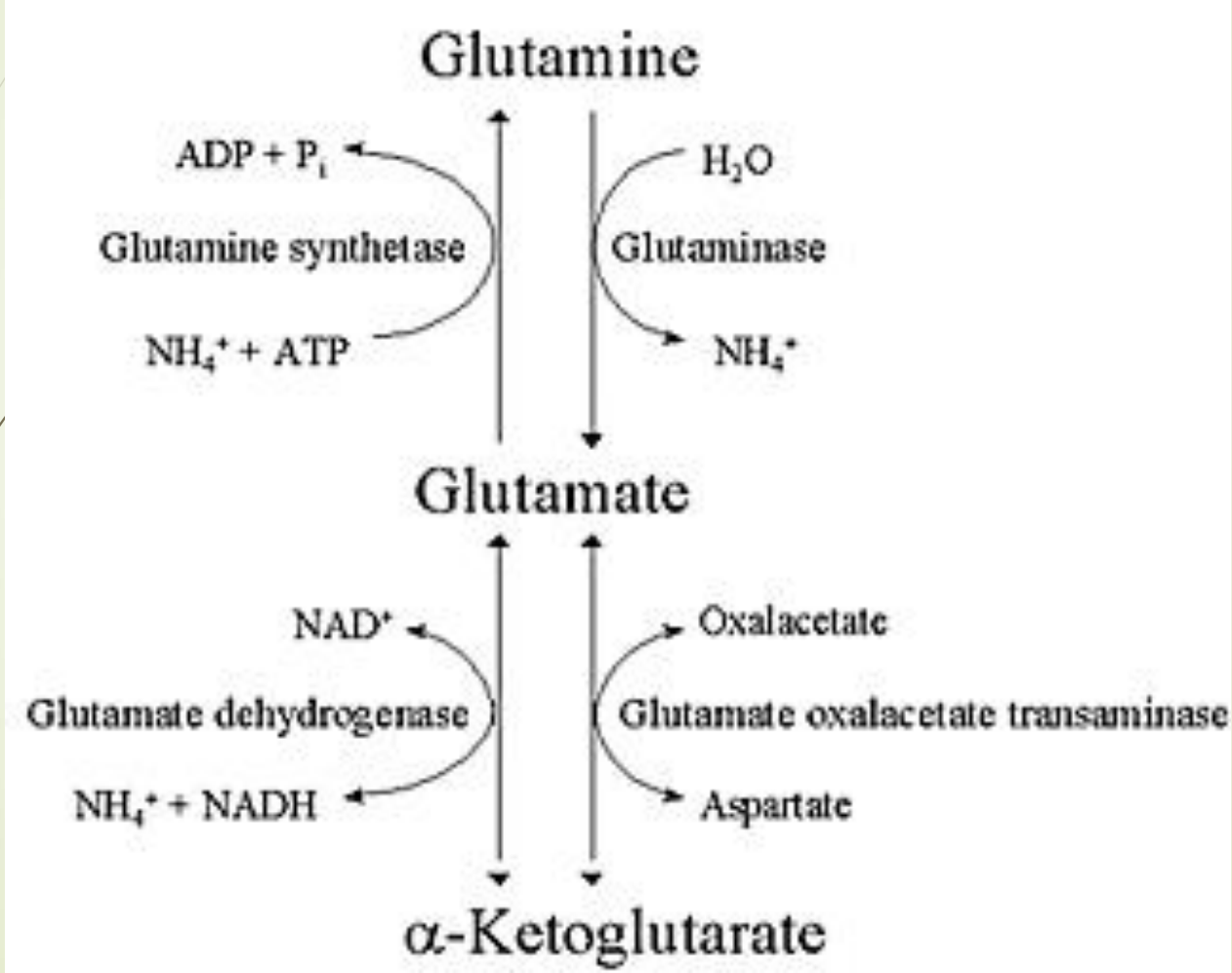
- Nonessential amino acids
- Do not cross BBB
  - must be synthesized in neurons de novo from glucose rather than taken up from the blood
- Main synthetic compartments
  - neurons
  - glial cells
- Both are excitatory neurotransmitters.

Synthesis of glutamate

- Sources:
  1. Glycolysis → Krebs cycle → dehydrogenation of α-ketoglutarate
  2. Glutamine (deamination by glutaminase)
  3. Aspartate (transamination)
- Is stored in vesicles, and its release is Ca2-dependent.
- Removal by high-affinity uptake systems in nerve terminals and glial cells.
  - excitatory amino acid carrier-1 *(EAAC1)*
  - glutamate transporter-1 (GLT-1) and glutamate—aspartate transporter (GLAST)

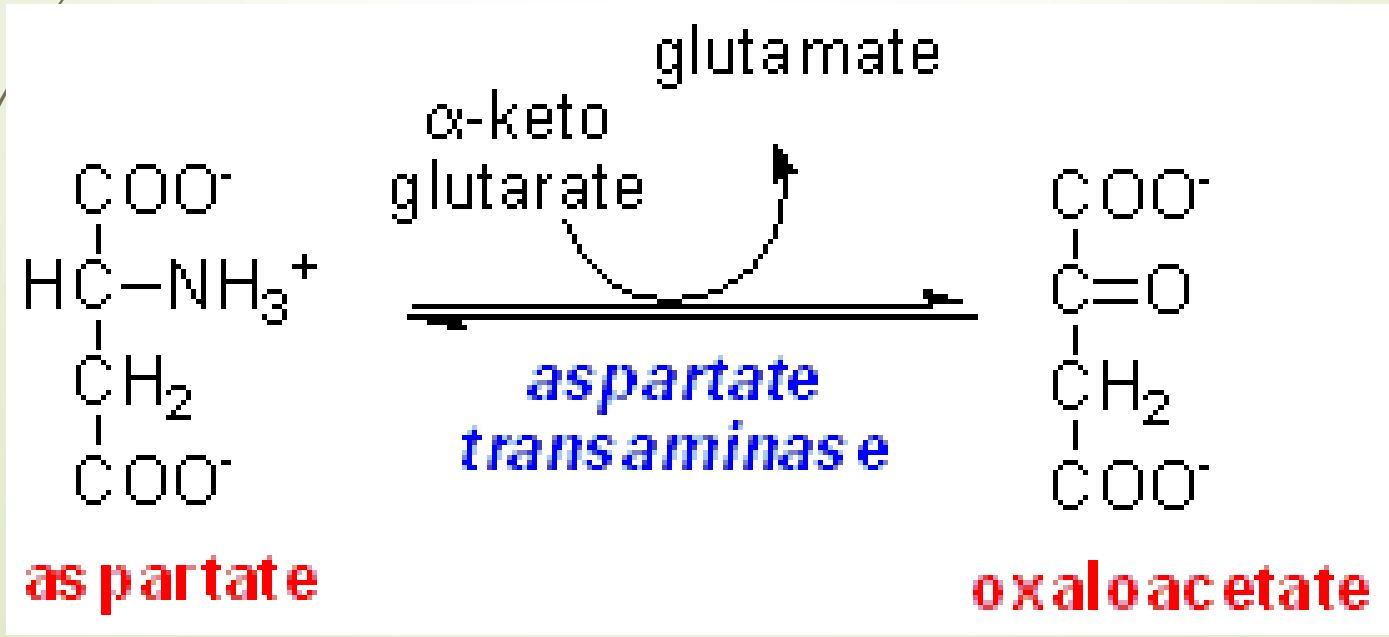


Sources of glutamate *(supplementary)*



Aspartate

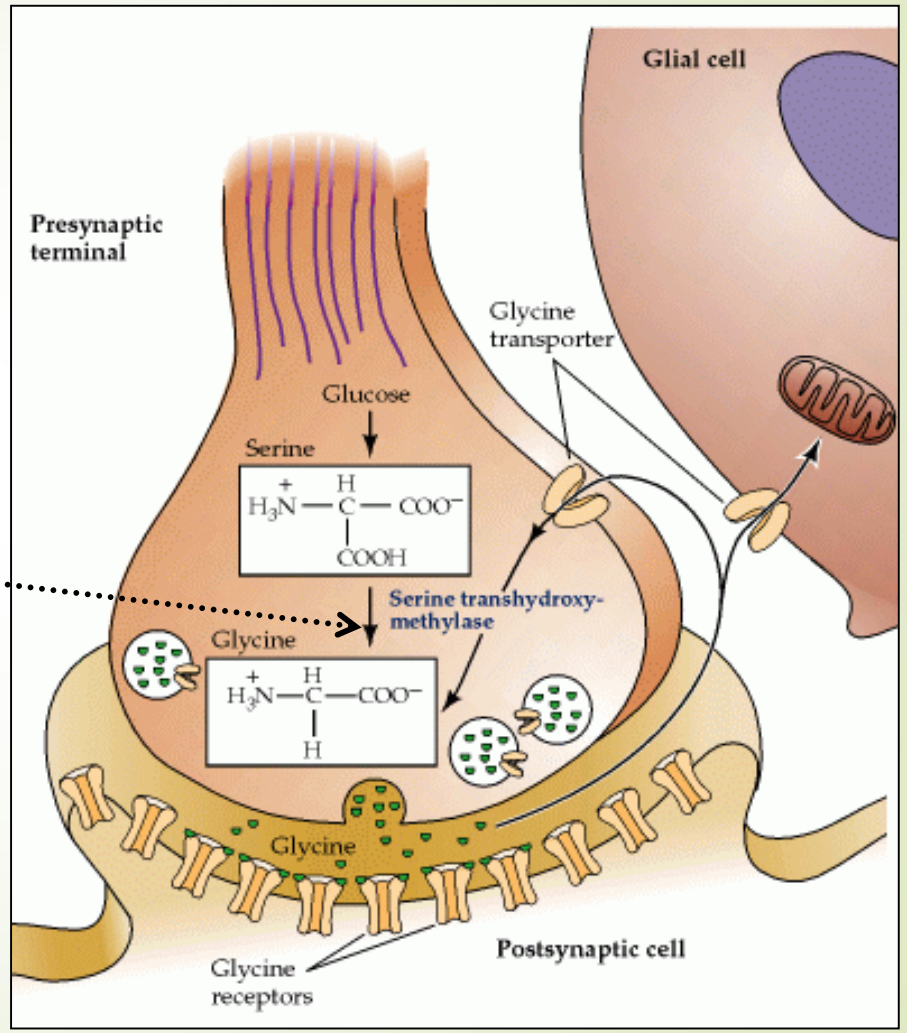
- A vesicular uptake mechanism for aspartate has not yet been demonstrated, somewhat weakening the case for considering aspartate to be a neurotransmitter
- Precursor: oxaloacetate (transamination)



Glycine

- The major inhibitory neurotransmitter in the spinal cord
- Synthesized de novo from serine by serine hydroxymethyltransferase through 3-phosphoglycerate
- Removal: high-affinity transporter

Folic acid



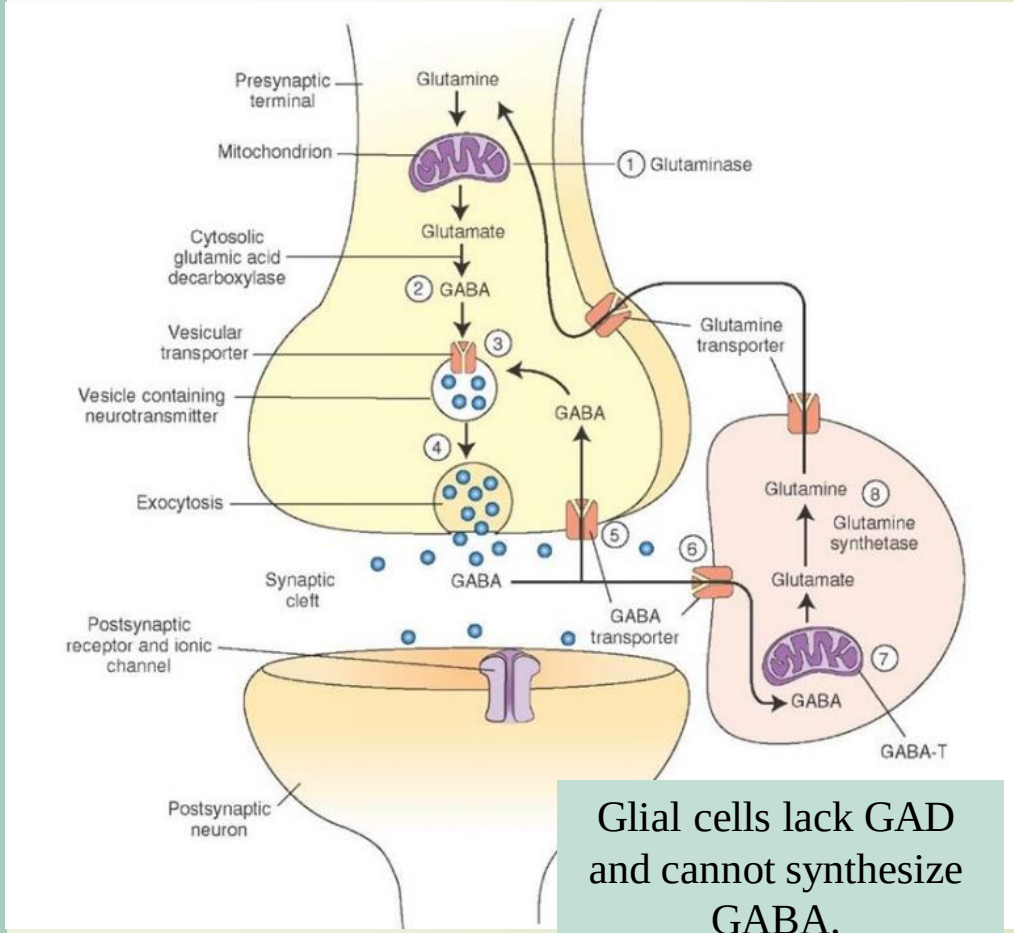
GABA

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

GABA shunt

- Glutamine is converted into glutamate by glutaminase.
- Glutamate is α-decarboxylated forming GABA via glutamate decarboxylase (GAD), which requires pyridoxal phosphate (vitamin B6).
- GABA is the stored in vesicles until released.
- GABA is either taken up into presynaptic terminal and repackaged OR goes into the GABA Shunt where it is taken up into the glia and converted to glutamate.
- Glutamate is converted into glutamine, which is transported into the neighboring nerve terminals to synthesize glutamate.

- GABA shunt is a series of reactions that recycles GABA in CNS to conserves glutamate and GABA



Glial cells lack GAD and cannot synthesize GABA.

Acetylcholine

The acetyl group used for acetylcholine synthesis is derived principally from glucose oxidation to pyruvate and decarboxylation of pyruvate to form acetyl-CoA via the pyruvate dehydrogenase reaction.

- Acetylcholine (AC) is the major neurotransmitter at the NMJ
- Inability to inactivate AC leads to constant activation of the nerve–muscle synapses, leading to varying degrees of paralysis.

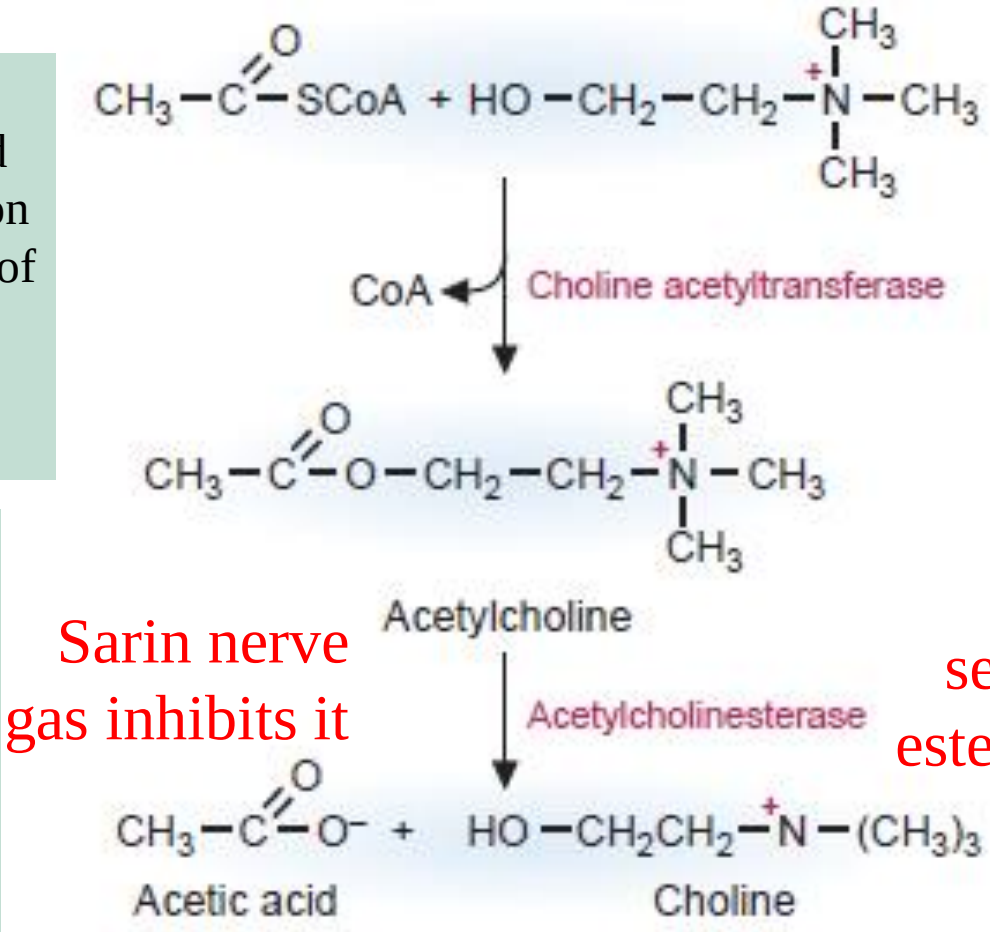


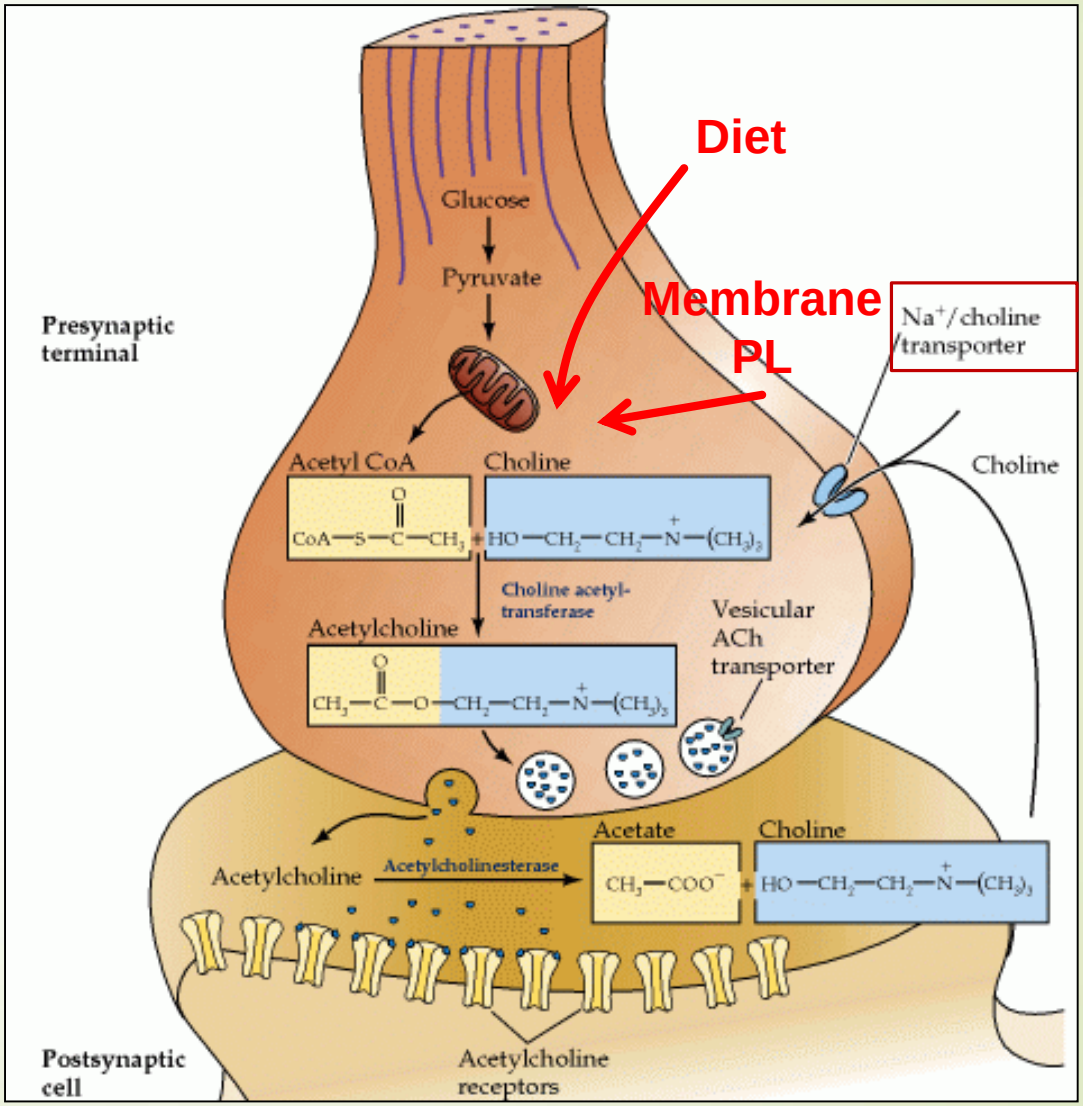
FIG. 48.9. Acetylcholine synthesis and degradation.

Sarin nerve gas inhibits it

serine esterase

Synthesis of acetylcholine

- Choline + acetylcoenzyme-A by choline acetyltransferase in cytoplasm
- Transported into and stored in vesicles.
- Removal: hydrolysis by acetylcholinesterase

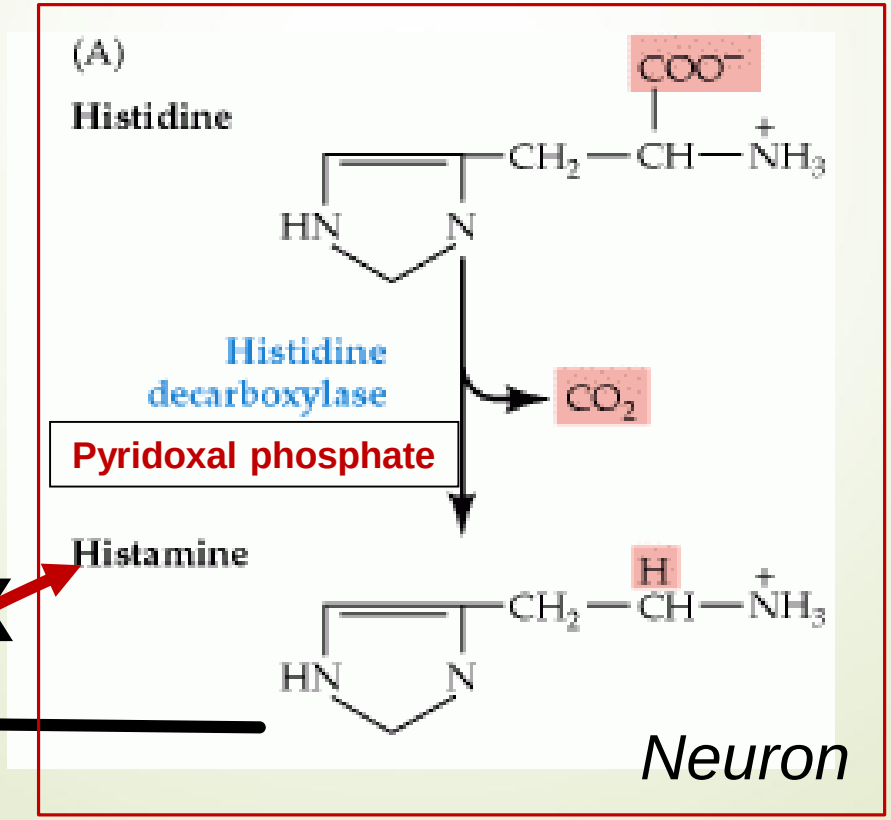


Histamine

- It does not penetrate the blood-brain barrier and, hence, must be synthesized in the brain.
- Histamine is inactivated by two enzymes—histamine methyltransferase then oxidation by MAO-B (brain) and diamine oxidase (histaminase) (peripheral tissues).

Once it is released from neurons, histamine is thought to activate both postsynaptic and presynaptic receptors.

No recycling into presynaptic terminal



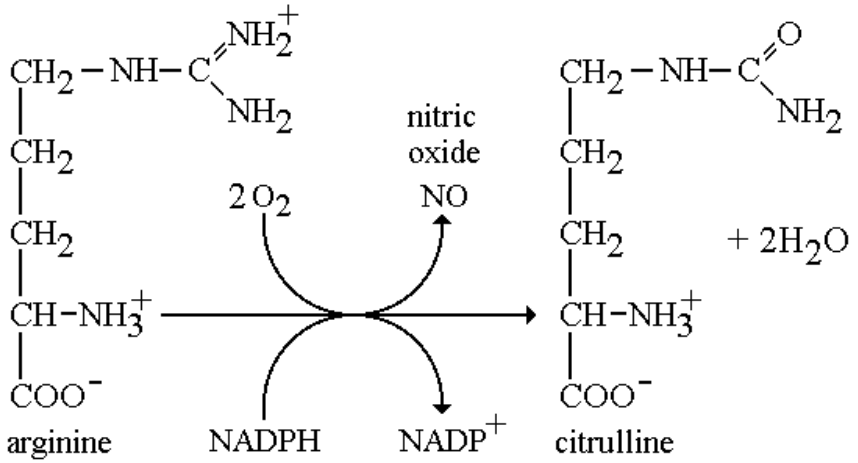
X

Astrocytes (MAO)

Newly synthesized neuronal histamine is stored in the nerve terminal vesicles.

NO synthesis by NO synthase

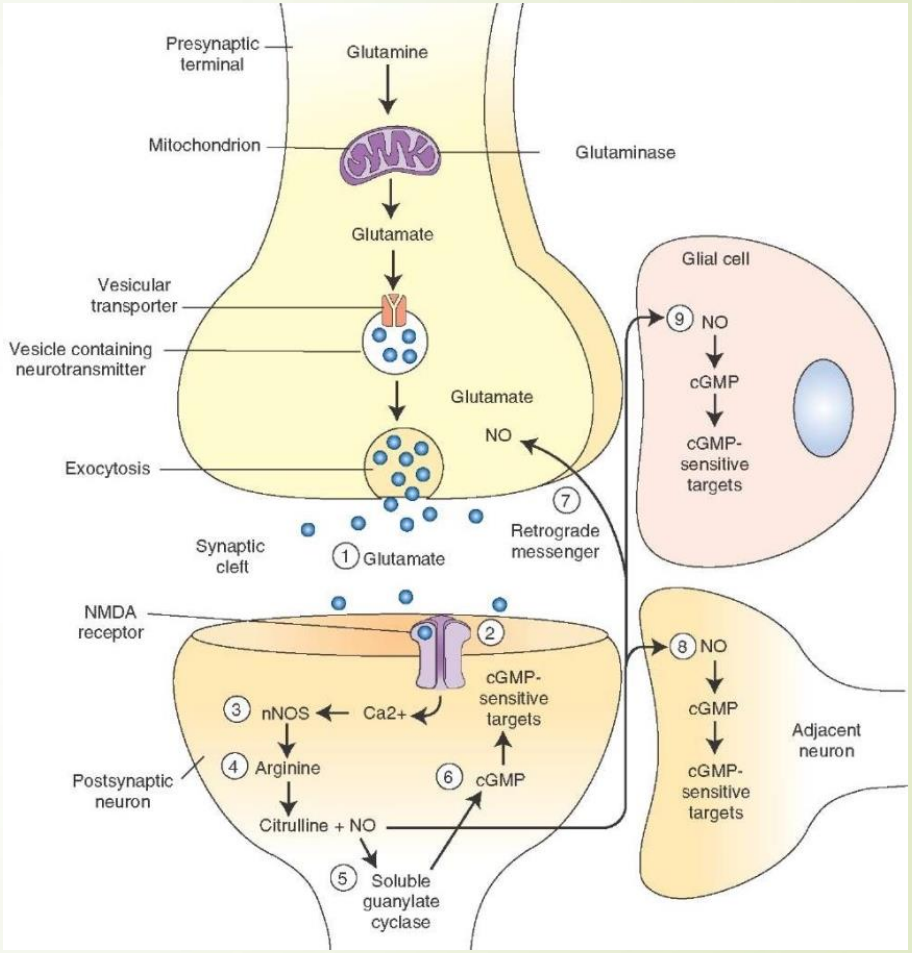
- Isoform I (nNOS or cNOS)
  - Neurons and epithelial cells
  - activated by the influx of extracellular calcium
- isoform II (iNOS)
  - Macrophages and smooth muscle cells
  - induced by cytokines



- and isoform III (eNOS)
  - Endothelial cells lining blood vessels
  - activated by the influx of extracellular calcium
- All three isoforms require BH2 as a cofactor and nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme

Nitric oxide(NO)

- Glutamate is released (1) and acts on NMDA receptors located on the post-synaptic neuron (2)
- Ca2+ enters the postsynaptic neuron activating NOS (3), which forms NO from 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 (*retrograde messenger*) (7) prolonging effect and b) into adjacent neurons (8) and glial cells (9) stimulating guanylate cyclase.



Half-life: 2-4 seconds

NO is inhibited by hemoglobin and other heme proteins which bind it tightly

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)
    - It decays spontaneously
  - 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.