Biochemistry of neurotransmitters

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Neuroscience

Resources

- This lecture
- Mark's Basic Medical Biochemistry, 6th ed, pp. 1027-1037
- http://what-when-how.com/neuroscience/neurotransmitters-the-neuron-part-1/

Definition and characteristics of a neurotransmitter

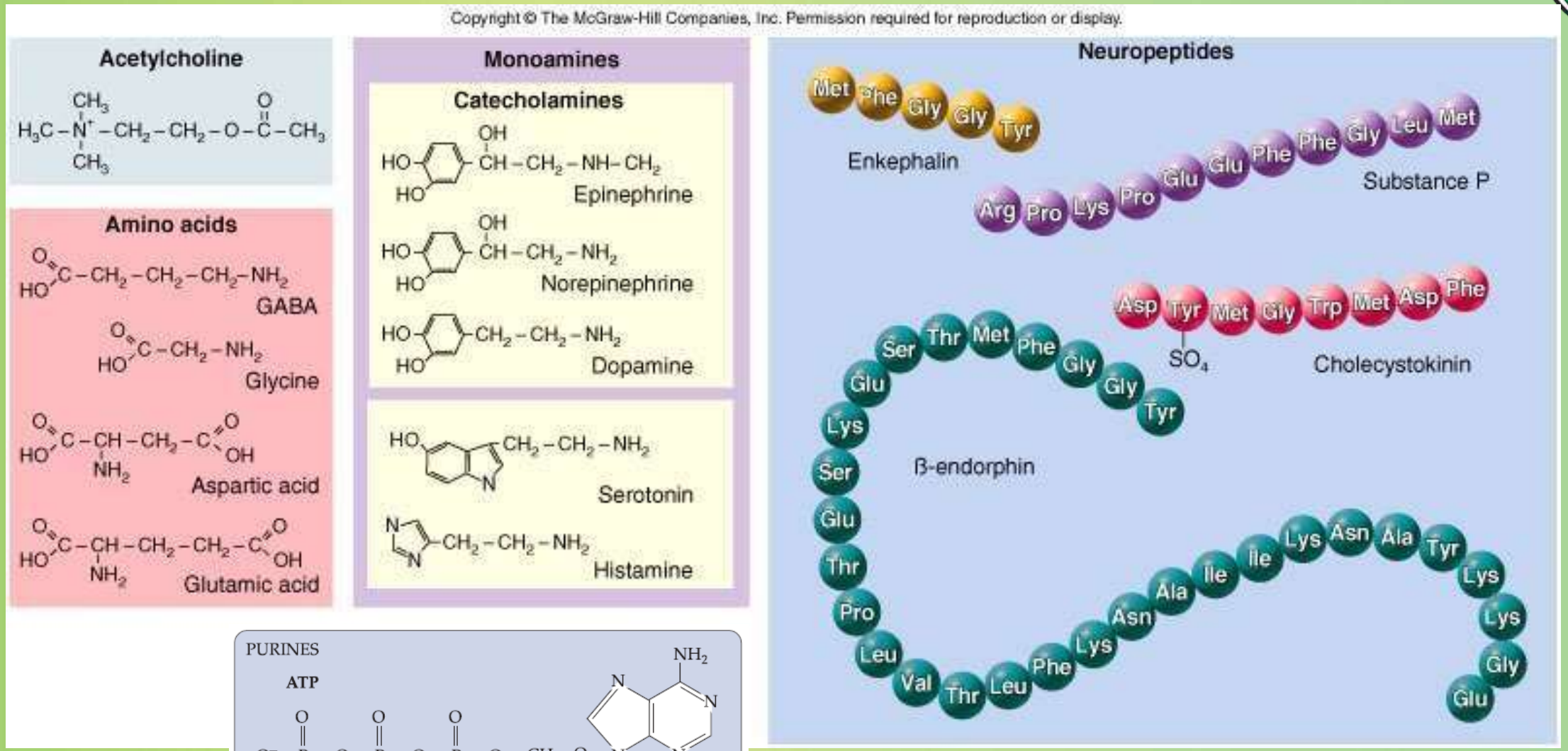
- A chemical substance that:
  - is synthesized and stored in a presynaptic neuron (the enzymes needed for its synthesis must be present in the neuron),
  - is released at a synapse following depolarization of the nerve terminal (usually dependent on an influx of calcium ions),
  - binds to receptors 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 of neurotransmitters

- Small-molecule neurotransmitters
  - Biogenic amines (epinephrine, dopamine, histamine, serotonin)
  - Amino acids (GABA, glutamate, aspartate, glycine)
  - Acetylcholine
  - Purines (ATP)
- Neuropeptides
- Gases (nitric oxide, carbon monoxide)

- Two or more transmitters (usually a small-molecule neurotransmitter and a neuropeptide) can coexist in neurons (e.g., most spinal motor neurons contain acetylcholine and calcitonin gene-related peptide).

Structures of neurotransmitters



Neuropeptides	Small-molecule neurotransmitters
Short-chain peptides (3-60 aa's), Large MW	Endogenous chemicals, Low MW
Slow-acting	Fast-acting
Slow response	Acute response
Prolonged action	Short-term response
Acts on several receptors	Acts on specific a receptor
Can change metabolism	Most do not change metabolism
Alter gene expression	Most do not change gene expression
Synthesized in the ER and Golgi apparatus	Synthesized in the presynaptic nerve terminal (mainly in cytosol)
Synthesized in low concentrations	Synthesized in high concentrations
Found allover the neuron	Found in the axon terminals of presynaptic neurons
Stored in large dense-core vesicles	Stored in small secretory vesicles
released at low cytosolic Ca^{2+} concentrations	released at high cytosolic Ca^{2+} concentrations
Have a different site of actions than their origin	Acts in direct apposition of to the releasing cell
Not re-taken up and not reused	Can be re-taken up and reused
Relatively more potent	Relatively less potent
Terminated when proteolytically degraded or diffused	Terminated by reuptake, uptake by glial cells, diffused, or enzyme degradation

NEUROPEPTIDES

LISTS OF NEUROPEPTIDES

NEUROPEPTIDES | HUGO GENE NOMENCLATURE COMMITTEE

NEUROPEPTIDE GENE FAMILIES

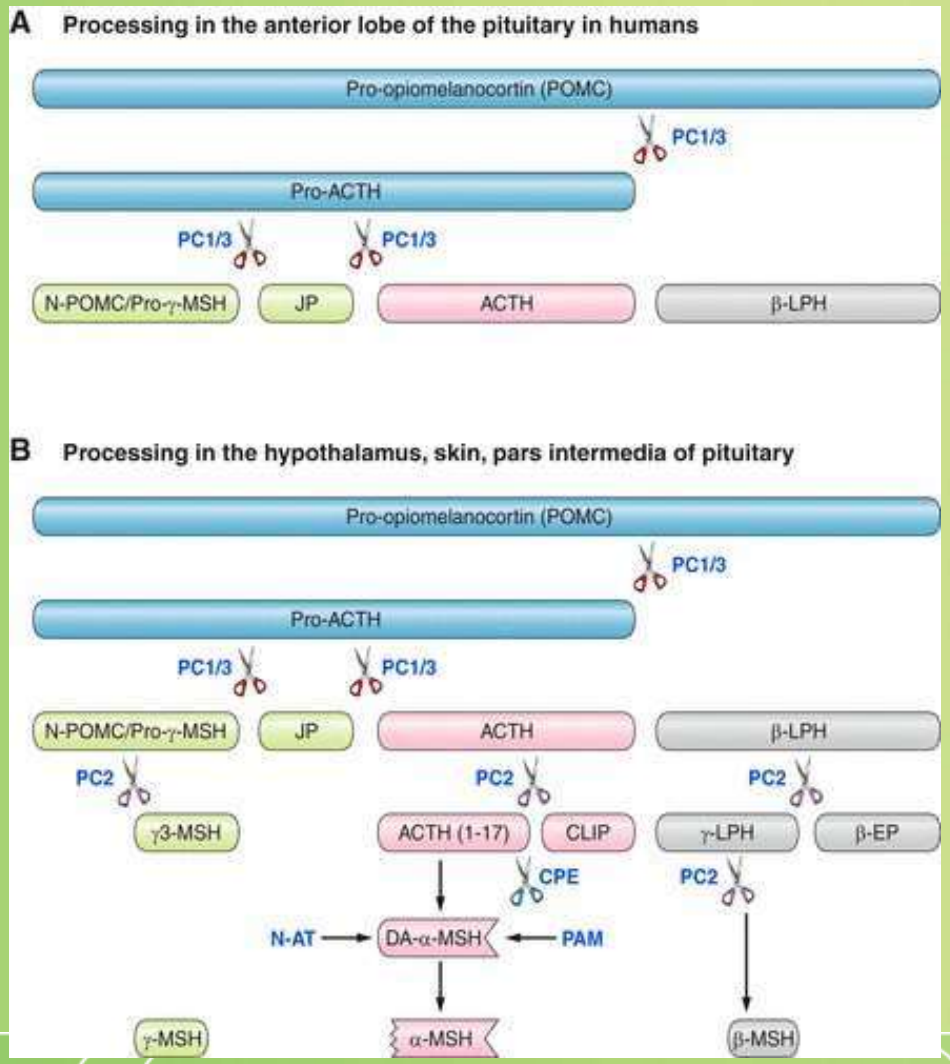
LIST OF NEUROTRANSMITTER INHIBITORS AND NEUROPEPTIDES

Some characteristics of neuropeptides

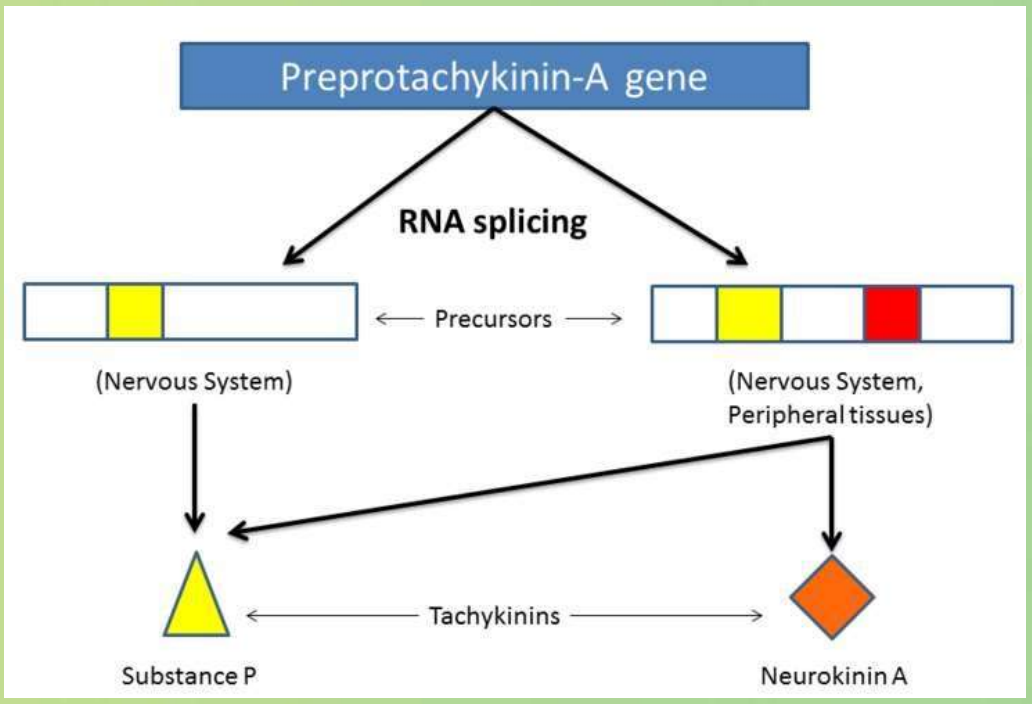
- More than 50 neuropeptides have been described affecting:
  - Behavior, pain perception, memory, appetite, thirst, temperature, homeostasis, and sleep
- They can be considered neurotransmitters or neurohormones.
- They are synthesized just like proteins are.
  - They are subject to alternative splicing and protein processing.
  - They can be tissue-specific.
  - Examples: substance P, neurokinin A and proopiomelanocortin

Just enjoy the colors

Post-translational processing

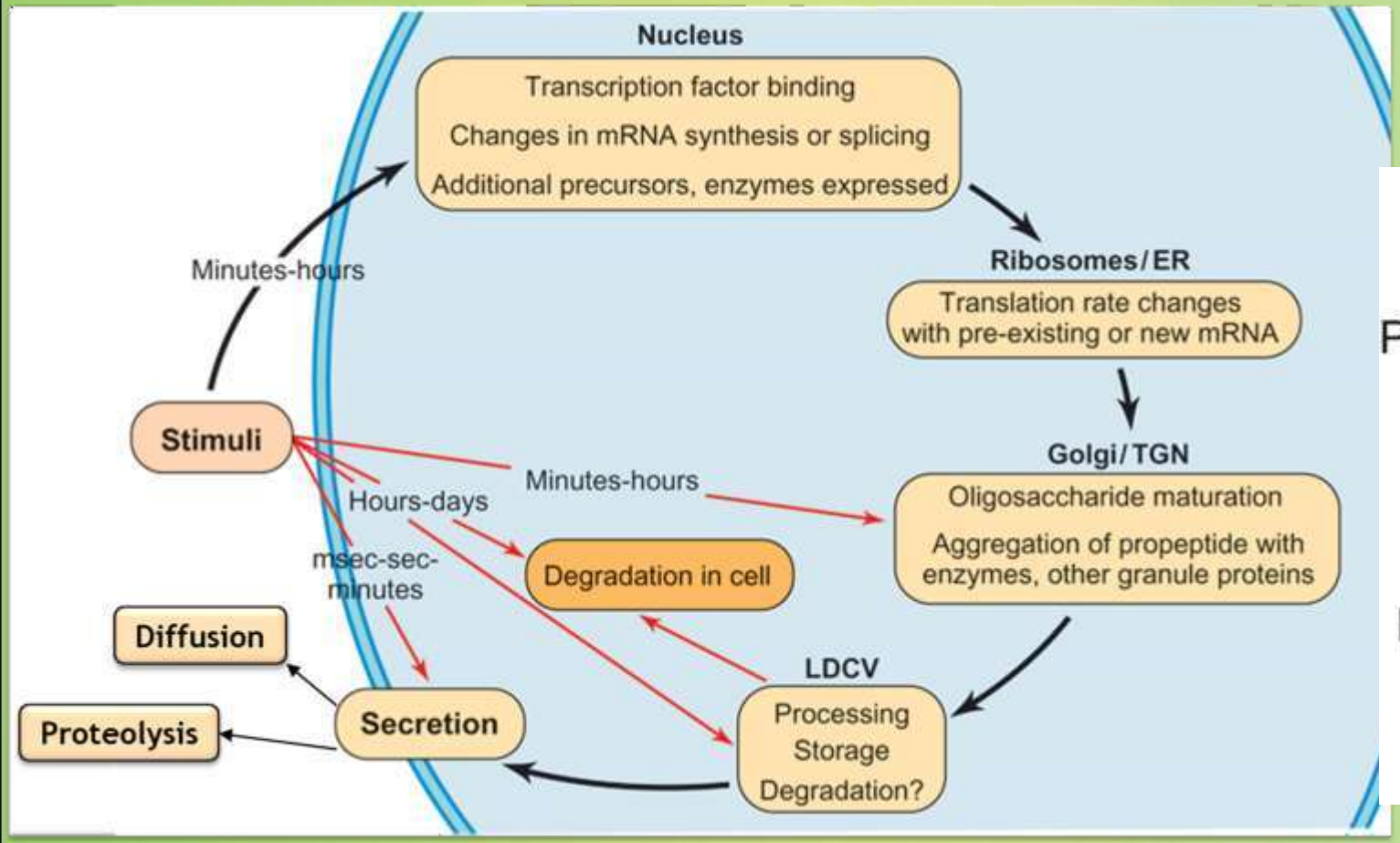


Post-transcriptional processing



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



Transcriptional
- Transcription factors
- Histone modifications

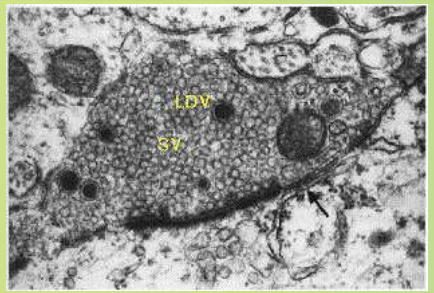
Post-Transcriptional
- ncRNA
- RBPs
- mRNA localization
- RNA decay

RNA transport, processing, and splicing

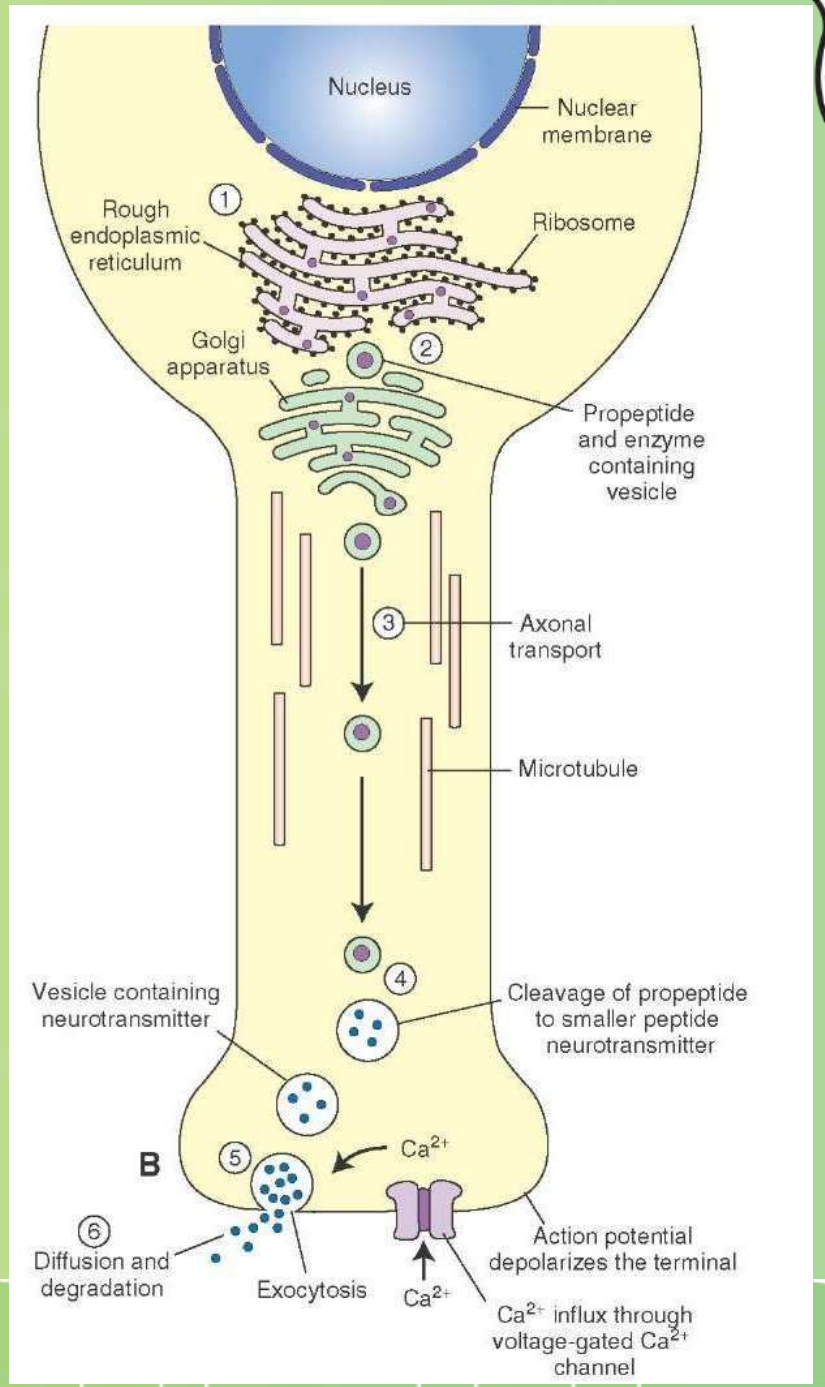
Translational

Post-Translational
- Phosphorylation
- Ubiquitination
- Acetylation
- Glycosylation
- Protein-protein interactions

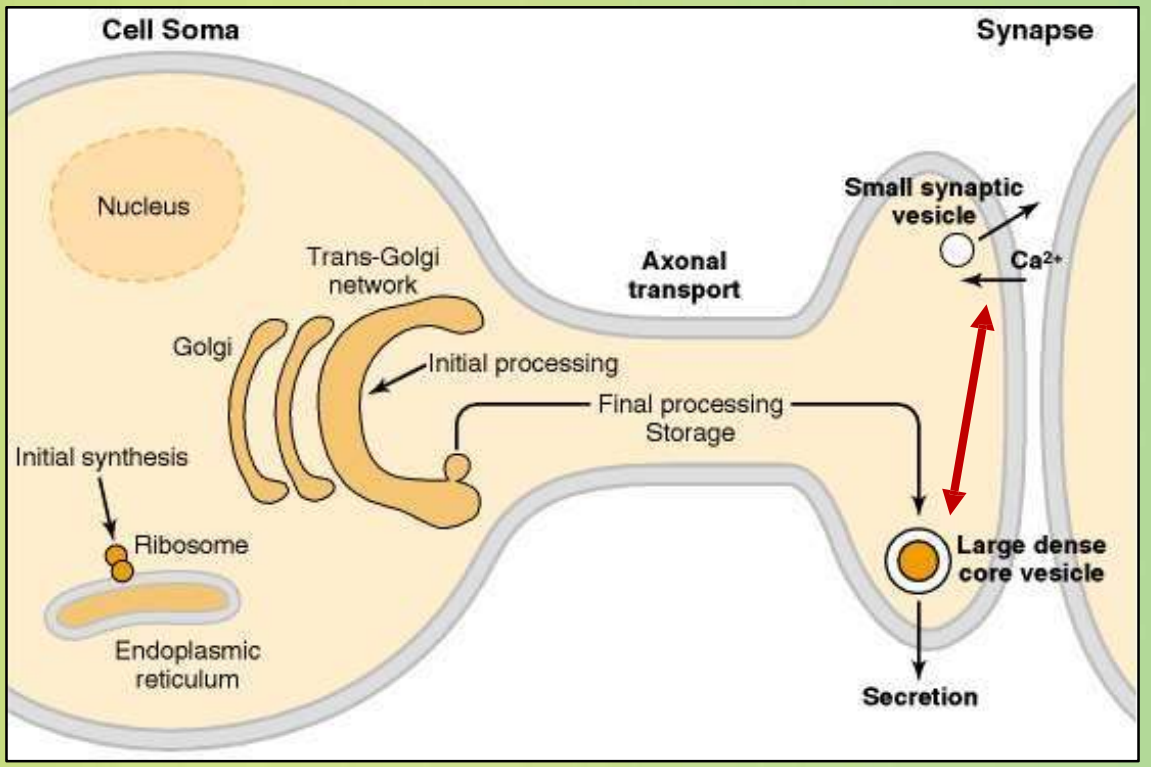
Stages of action



- Synthesized on ER (1) as pre-propeptides then propeptides, and then go into Golgi apparatus (2)
- Packaged into large-dense-core vesicles (with modifying enzymes)
- Transported via (3) fast-axonal transport 
  - During the transport, proteases cleave the precursor neuropeptide into the final mature form (4).
- Released (5)
  - Release is gradual over time in response to general increases in the level of intracellular calcium.
- Action (prolonged)
  - Mainly via GPCR
- Termination by diffusion and degradation (6)



Role of Ca^{2+} ions

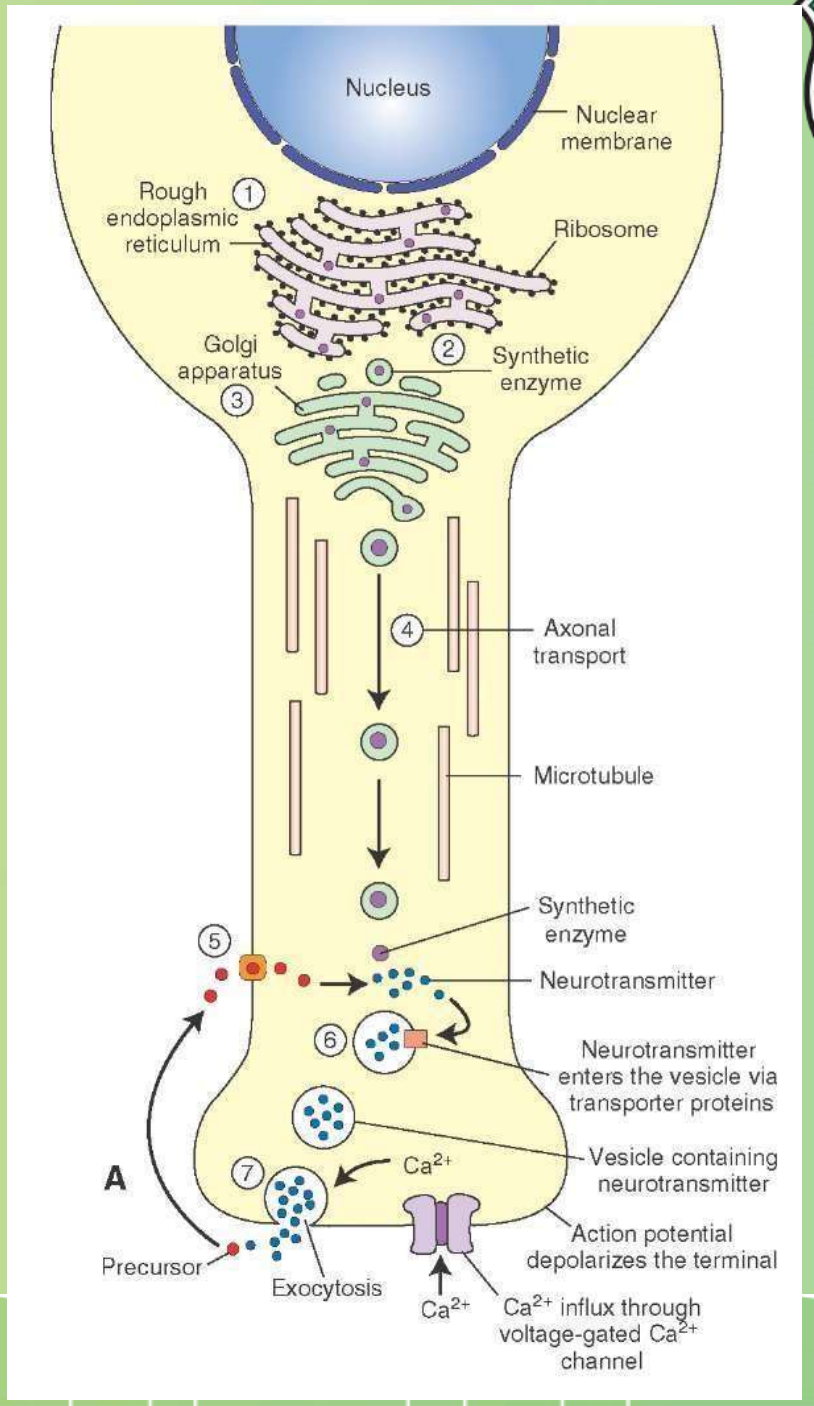


- Vesicles are located further away from the presynaptic membrane and away from the area of Ca^{2+} ions influx
- Ca^{2+} ion influx can be from external of internal sources and at lower concentrations than required for small-molecule neurotransmitters.

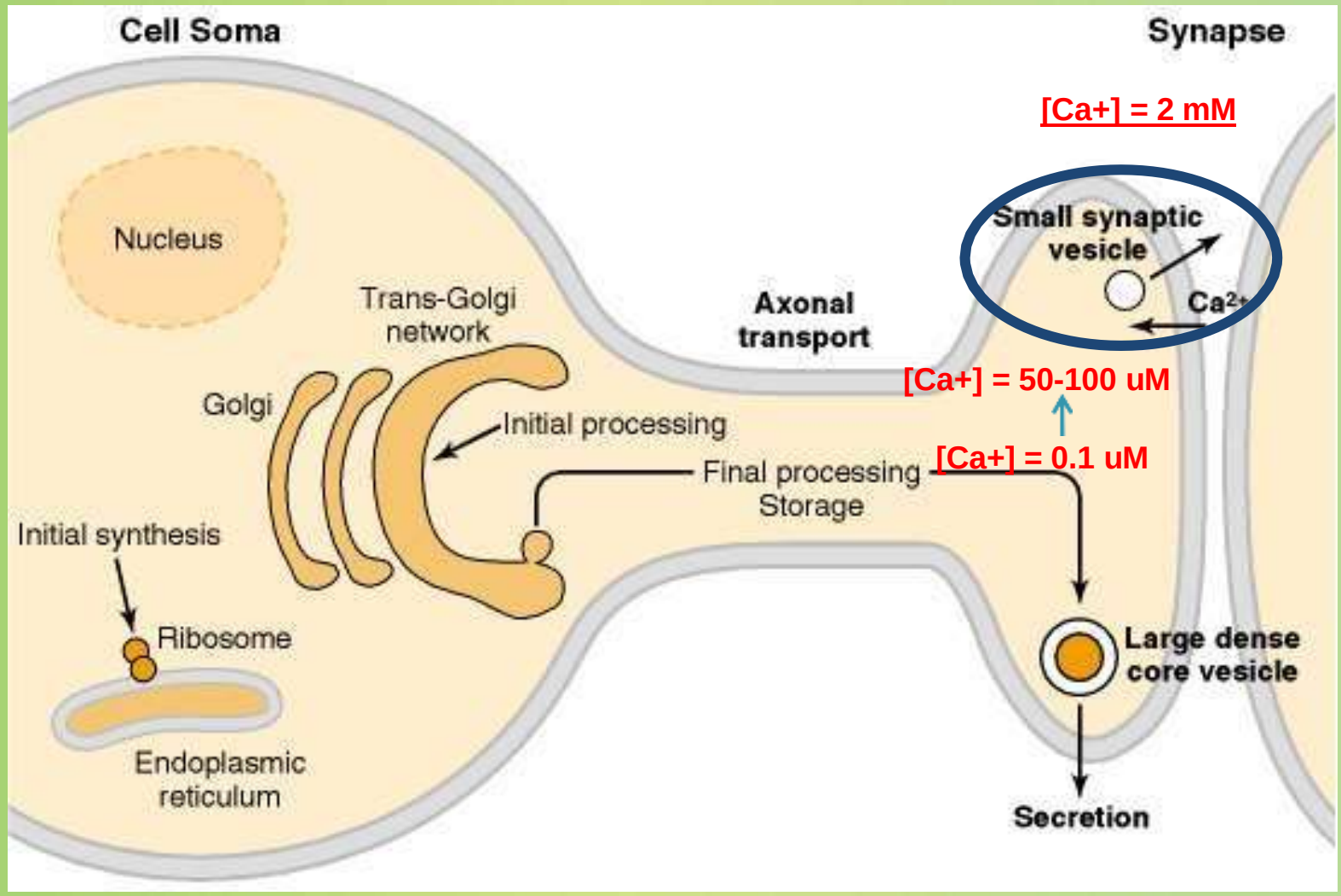
SMALL-MOLECULE NEUROTRANSMITTERS

Stages of synthesis and action

- Synthesis of **the enzymes** in ER (1) and Golgi apparatus (2) where they are modified (3).
- Transport of **soluble** enzymes via slow axonal transport (4) 
- Neurotransmitter precursors are taken up into the cells via transporter proteins located in the plasma membrane of the nerve terminal (5), and the neurotransmitter is synthesized in the presynaptic nerve terminal and then packaged in small synaptic vesicles (6).
- Release is stimulated by brief pulses each time an action potential triggers the influx of calcium (7).
- Action (short)
- Termination by diffusion, re-uptake, glial cell uptake, or inactivation



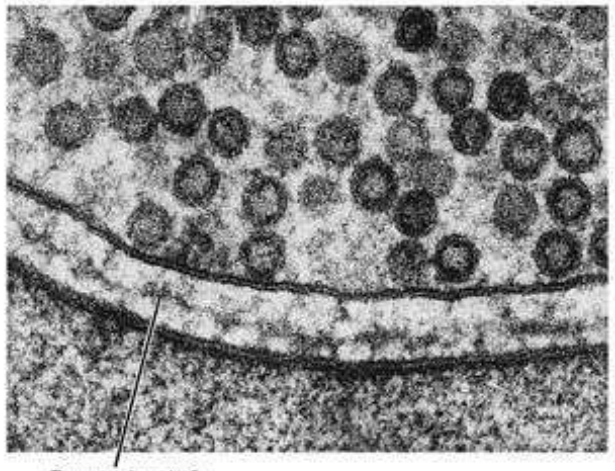
Role of Ca^{2+} ions



Vesicles are located near the presynaptic membrane and the area of Ca^{2+} ions influx.

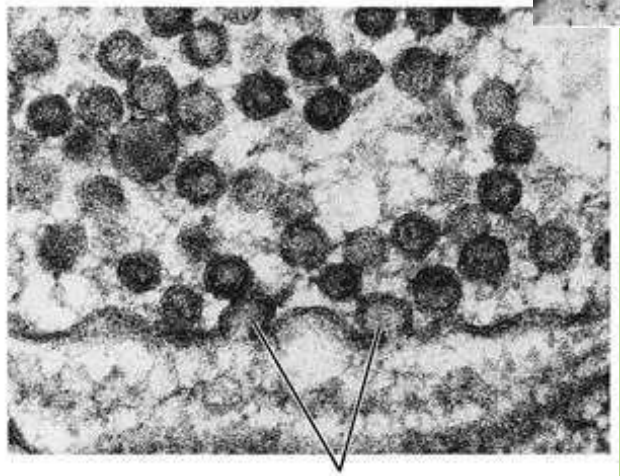
Presynaptic membrane (thin section)

A_2

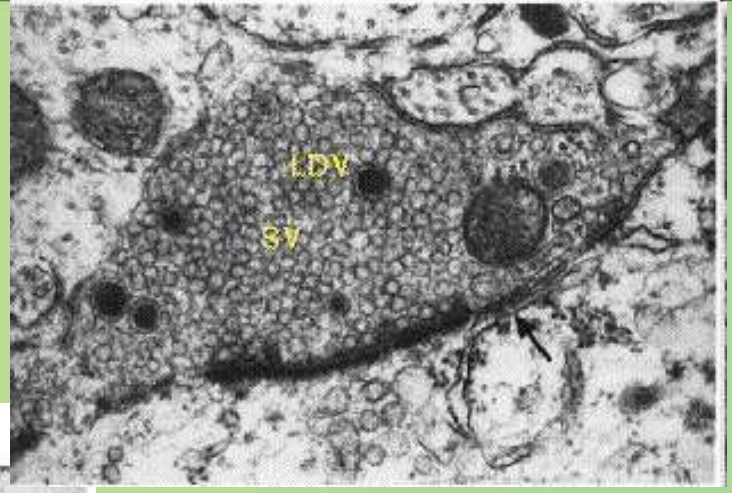


Synaptic cleft

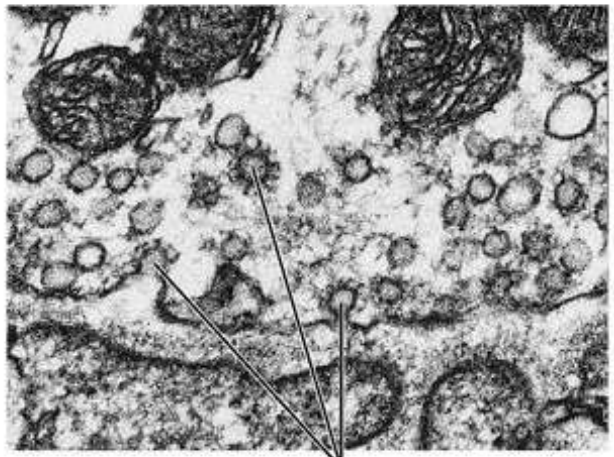
B_2



Vesicle fusions



C_2



Coated vesicles

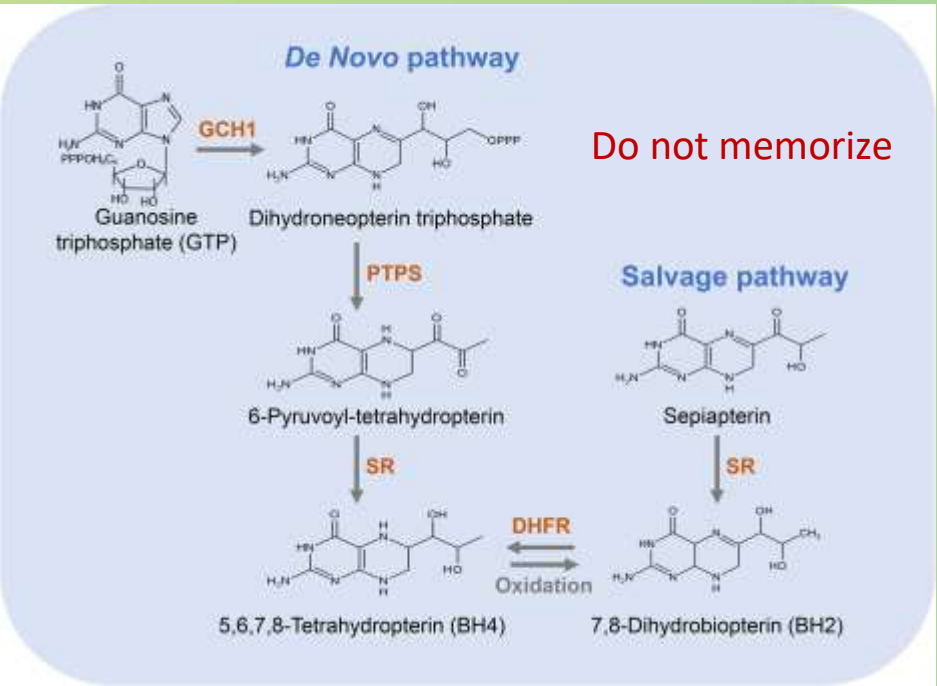
The influx of Ca^{2+} ions allows the vesicles to fuse with the presynaptic membranes.

TYROSINE-DERIVED NEUROTRANSMITTERS

Dopamine, norepinephrine, and epinephrine

Notes

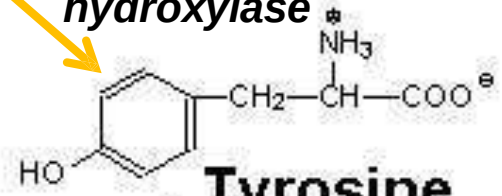
- Role of cofactors
  - S-adenosylmethionine (methyl transfer)
  - Pyrodoxal phosphate (vitamin B6): transamination, decarboxylation
  - Tetrahydrobiopterin (BH4)
  - Vitamin B12
  - Folate



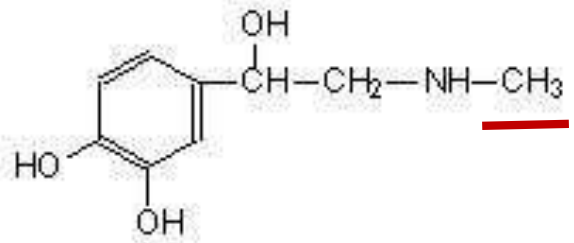
Diet/ liver

phenylalanine hydroxylase

Tyrosine *(hydroxy-phenylalanine) is present in all food products and synthesized from phenylalanine. It enters the neuron by active transport.*



Tyrosine



Epinephrine

tyrosine hydroxylase

tetrahydrobiopterin (BH_4) + O_2

dihydrobiopterin + H_2O (BH_2)

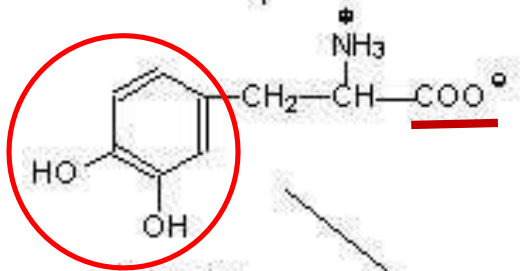
Rate-limiting step

S-adenosylhomocysteine

S-adenosylmethionine

phenylethanolamine *N*-methyltransferase

Vitamin B12 or folate



DOPA

Norepinephrine

cytoplasm

DOPA decarboxylase

Pyridoxal phosphate

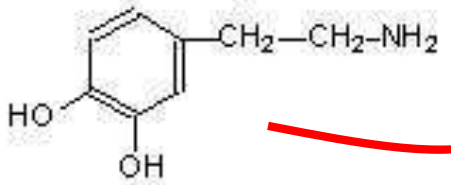
CO_2

H_2O

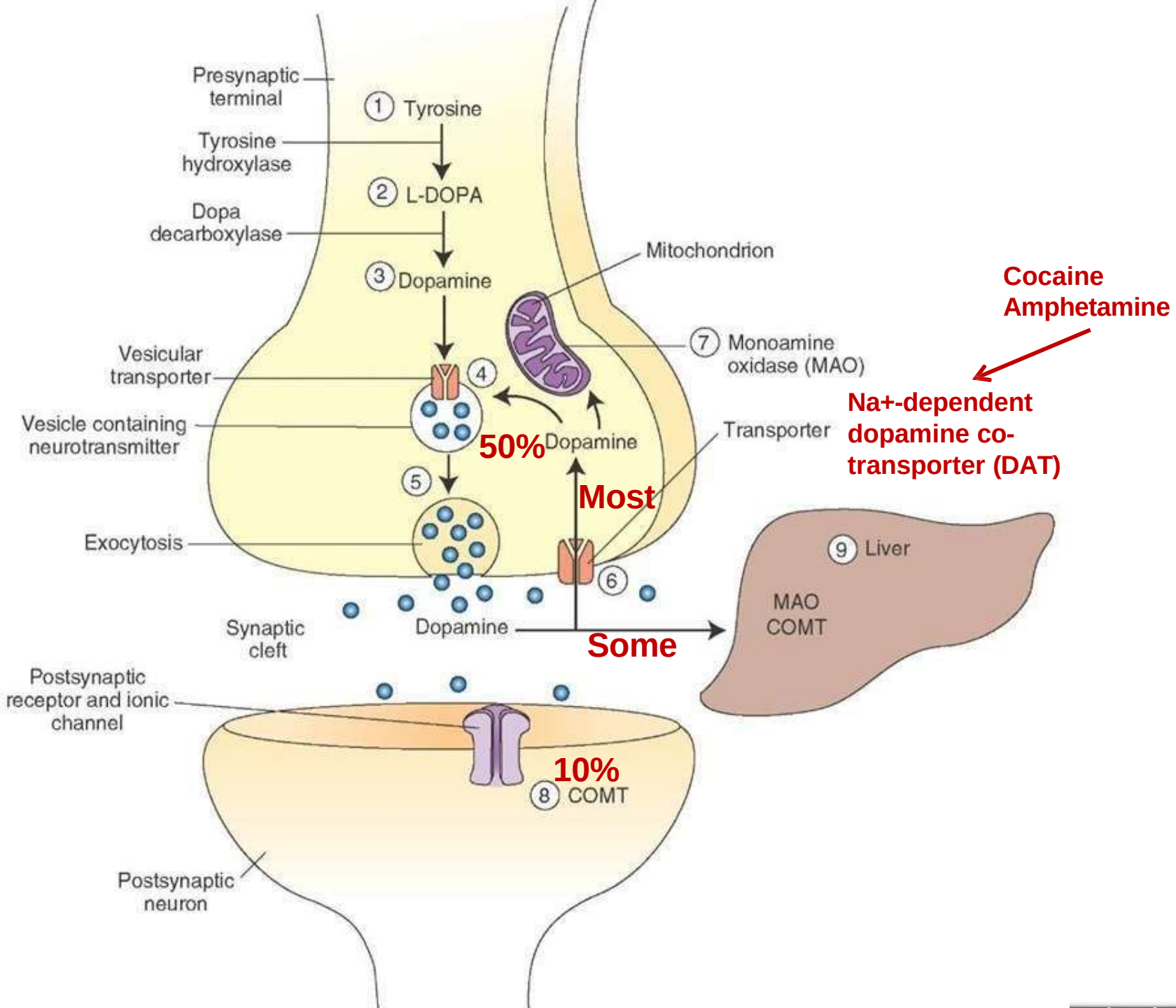
O_2

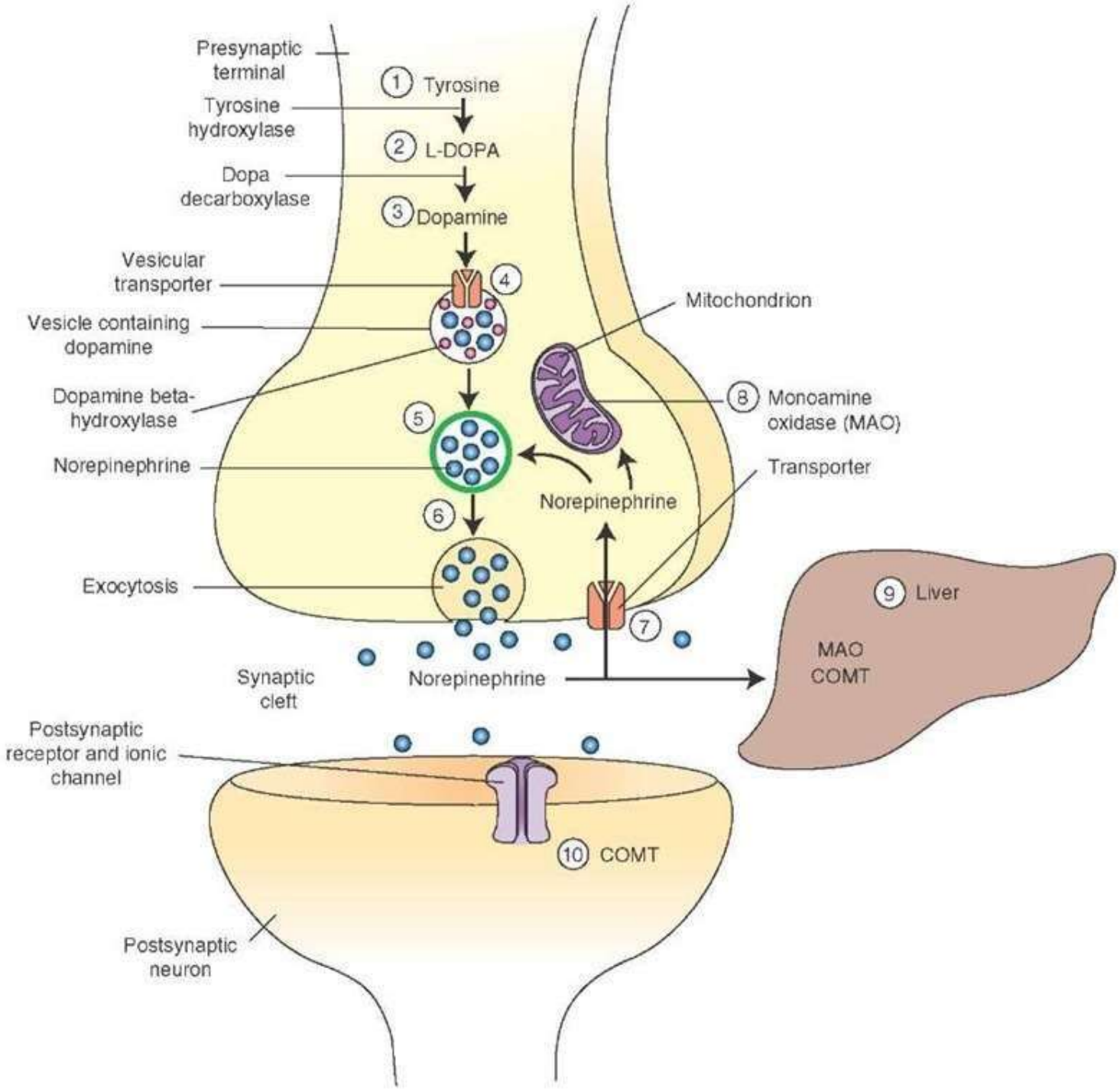
dopamine β- hydroxylase

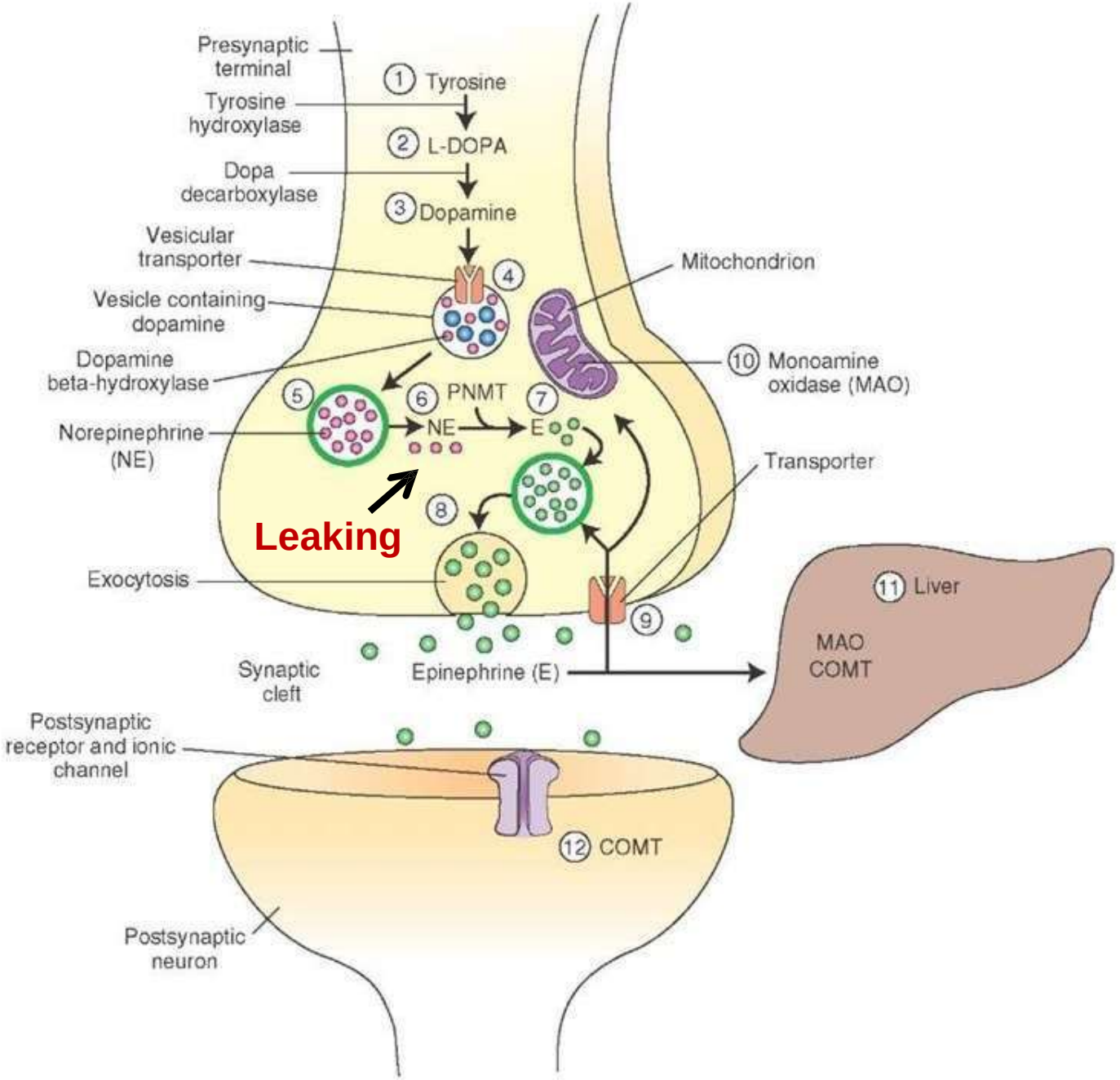
vesicular



Dopamine

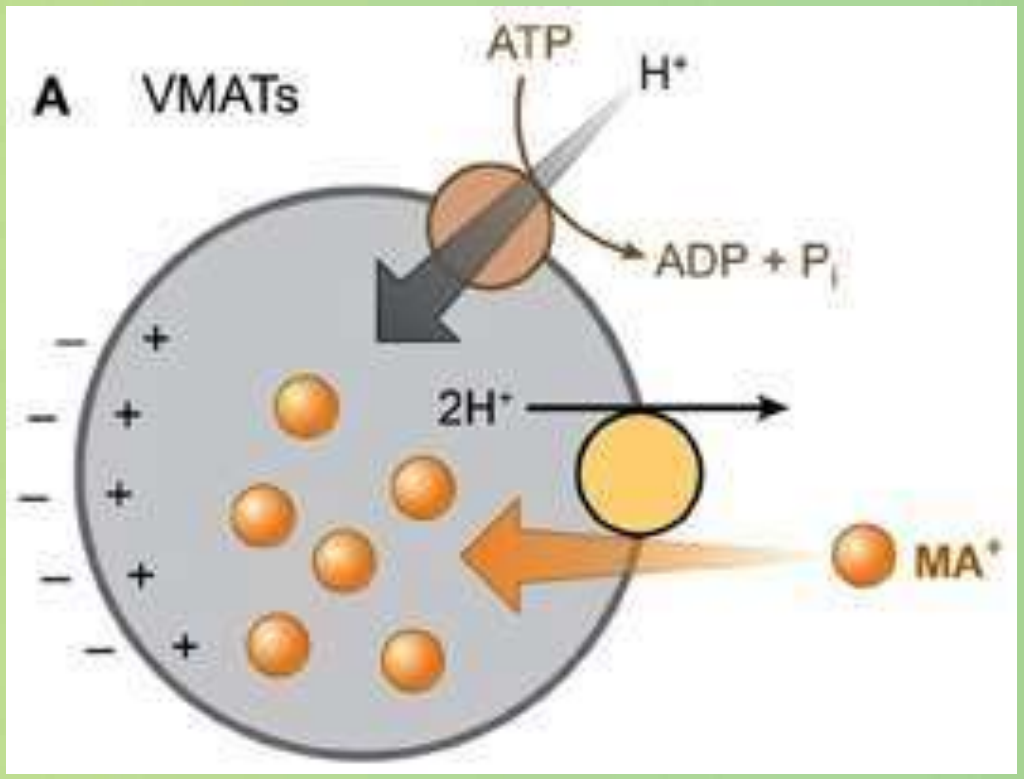

Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Mitochondrion
7 Monoamine oxidase (MAO)
Vesicular transporter
4
Vesicle containing neurotransmitter
50% Dopamine
Transporter
5
Most
Exocytosis
6
9 Liver
MAO
COMT
Synaptic cleft
Dopamine
Some
Postsynaptic receptor and ionic channel
10%
8 COMT
Postsynaptic neuron
Cocaine
Amphetamine
Na+-dependent dopamine co-transporter (DAT)


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

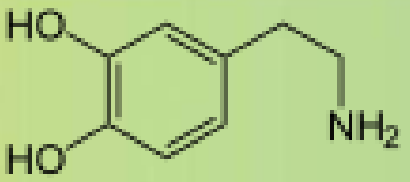

Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Vesicular transporter
4
Vesicle containing dopamine
Dopamine beta-hydroxylase
5
6 PNMT
7
Norepinephrine (NE)
NE
E
Leaking
8
Exocytosis
Synaptic cleft
Epinephrine (E)
9
Transporter
Mitochondrion
10 Monoamine oxidase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

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 then exchange for the positively-charged catecholamine via the transporter VMAT (vesicular monoamine transporter).
  - Targeting VMATs causes depletion of the neurotransmitters.

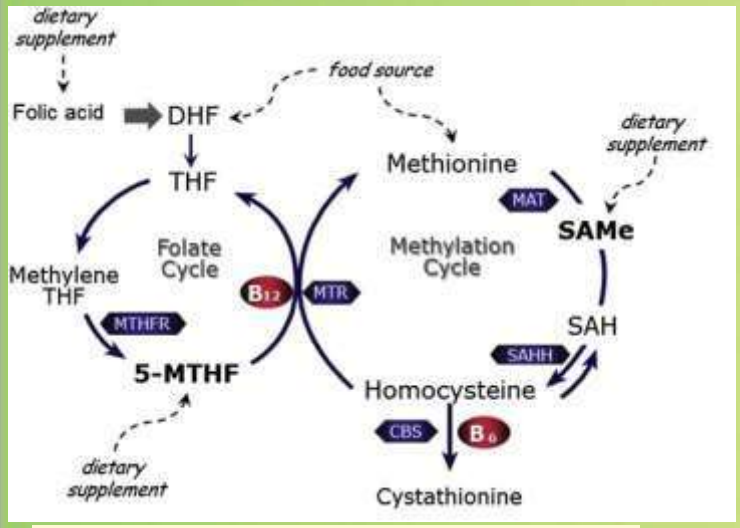


COMT and MAO



Dopamine (DA)

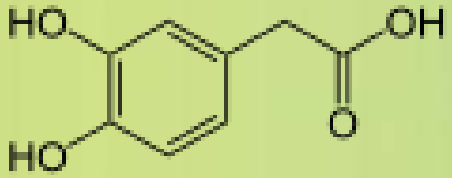
Degradative enzymes are present in the presynaptic terminal and in adjacent cells.



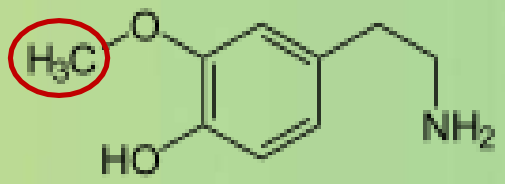
Inactivation is dependent on S-adenosylmethionine (SAM) and, indirectly, vitamin B12 and folate

Monoamine oxidase (MAO), Aldehyde dehydrogenase

Catechol-O-methyl-transferase (COMT)



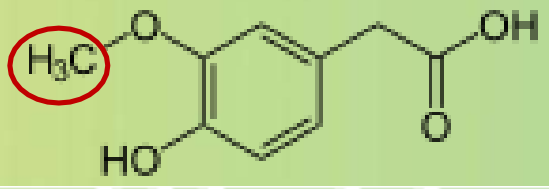
3,4-Dihydroxyphenyl-acetic acid (DOPAC)



3-Methoxytyramine (3-MT)

Catechol-O-methyl-transferase (COMT)

Monoamin oxidase (MAO), Aldehyde dehydrogenase



Homovanillic acid (HVA)

Parkinson's disease

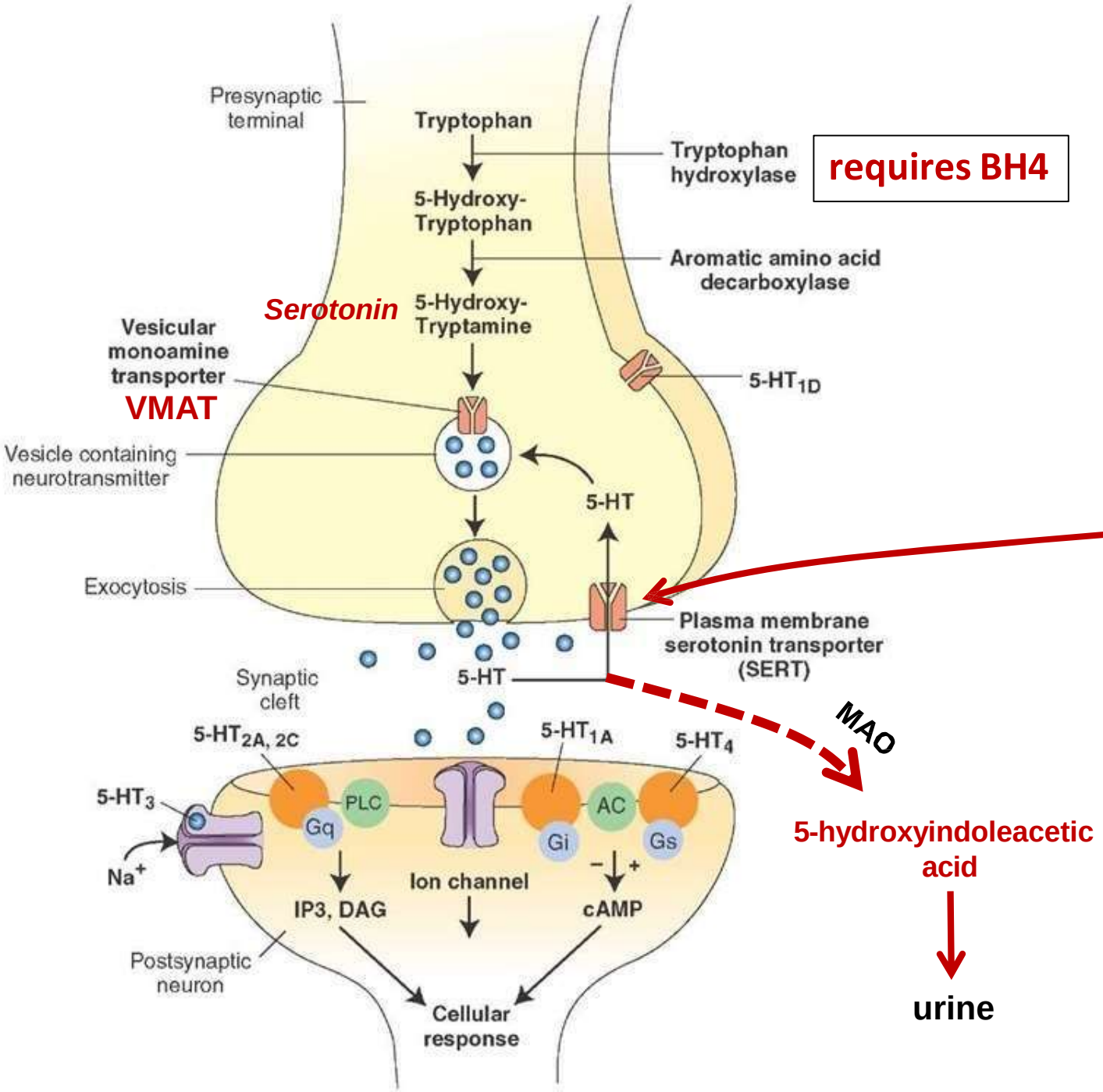
Drugs that deplete storage vesicles indirectly increase catecholamine degradation.

Regulation

- Tyrosine hydroxylase
  - Short-term:
    - Inhibition by free cytosolic catecholamines, which compete with BH_4 binding to the enzyme.
    - Activation by depolarization, which activates several protein kinases including PKC, PKA, Ca^{2+}-calmodulin–dependent kinases that phosphorylate tyrosine hydroxylase. This makes the enzyme bind more tightly to BH4 and, consequently, less sensitive to end-product inhibition.
  - Long-term (plus dopamine β-hydroxylase)
    - Prolonged sympathetic neuronal activity increases the transcription of tyrosine hydroxylase and dopamine β–hydroxylase.

TRYPTOPHAN-DERIVED NEUROTRANSMITTERS

Serotonin and melatonin

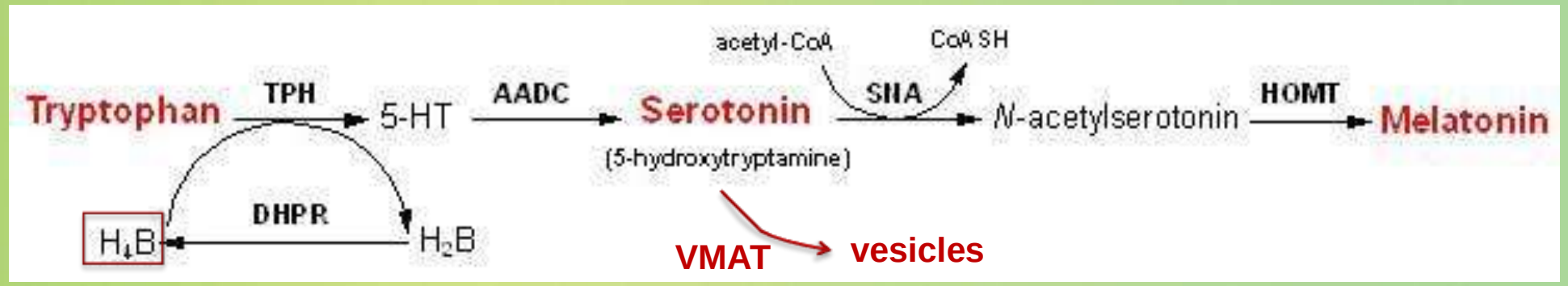


Antidepressants, called selective serotonin reuptake inhibitors (SSRIs) like Prozac® inhibit the reuptake process resulting in prolonged serotonin presence in the synaptic cleft.

Serotonin is packaged into vesicles by VMAT.

Melatonin

- Serotonin is synthesized in the pineal gland and 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

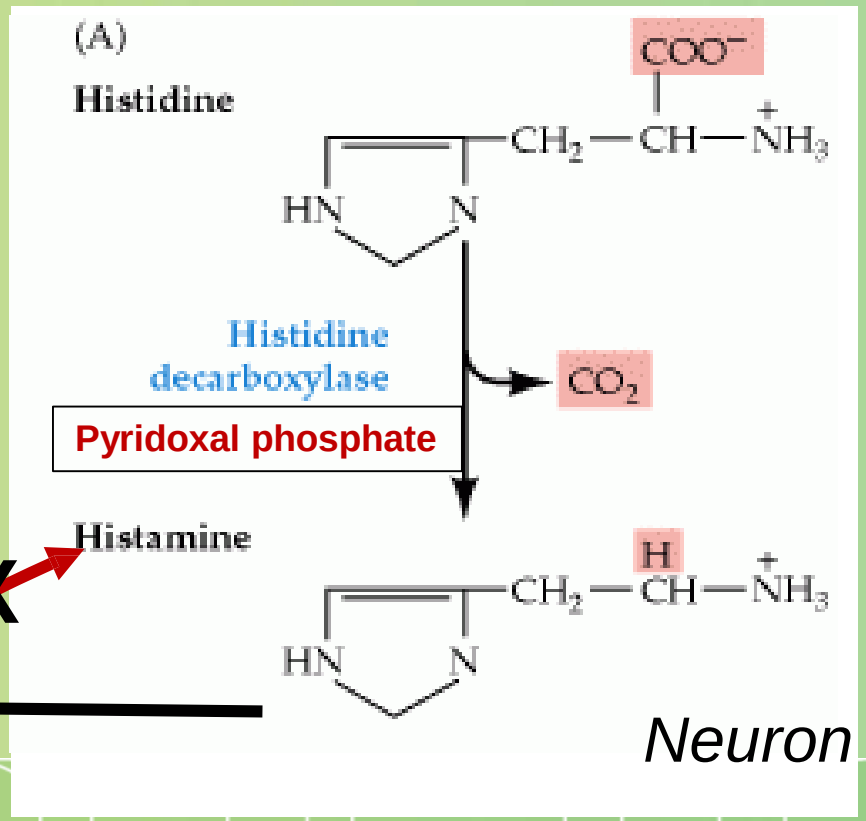


AMINO ACID-BASED NEUROTRANSMITTERS

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 and diamine oxidase (histaminase).

Histamine is packaged into vesicles by VMAT.



No recycling

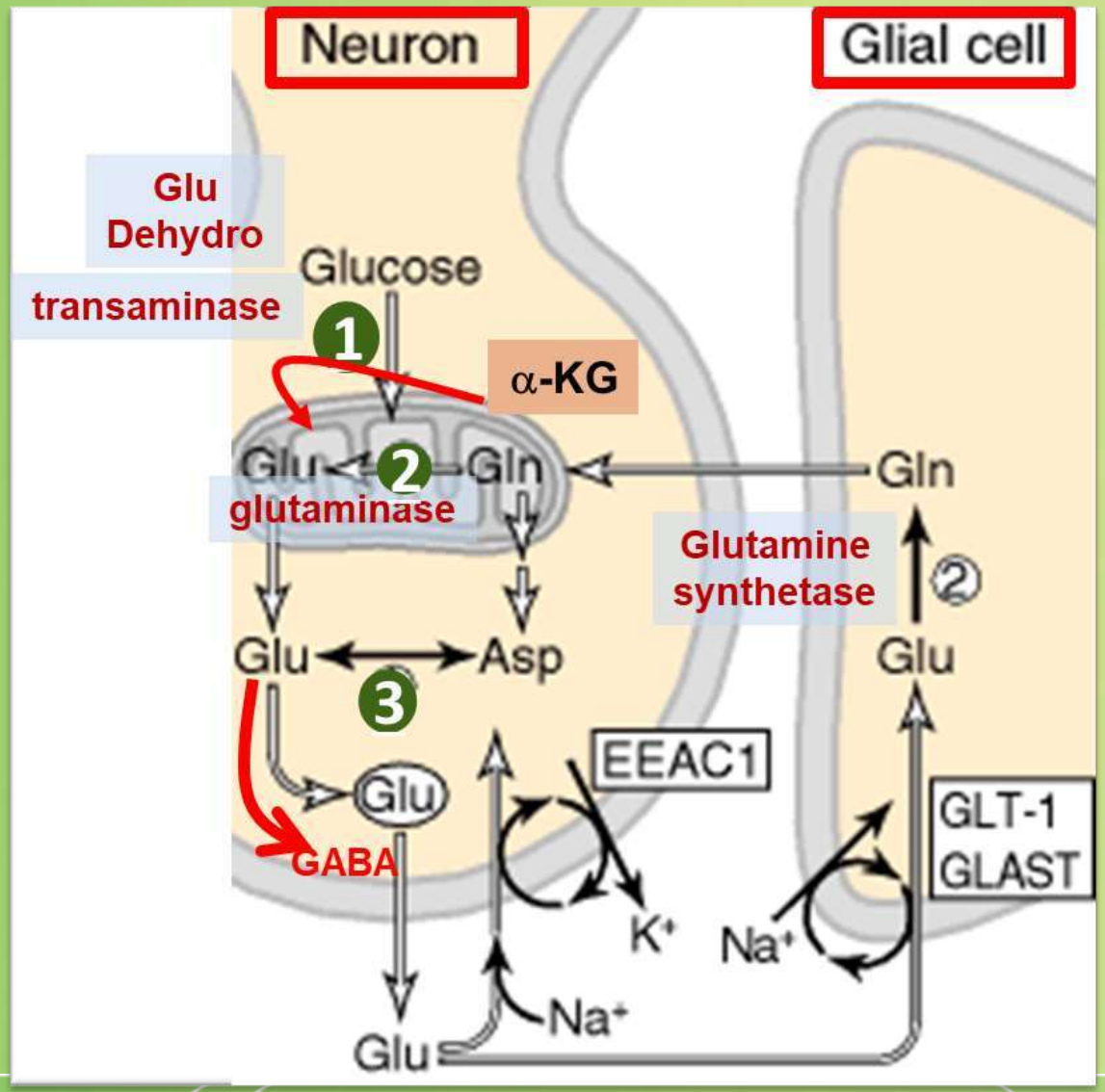
X

Astrocytes (MAO)

Glutamate and aspartate

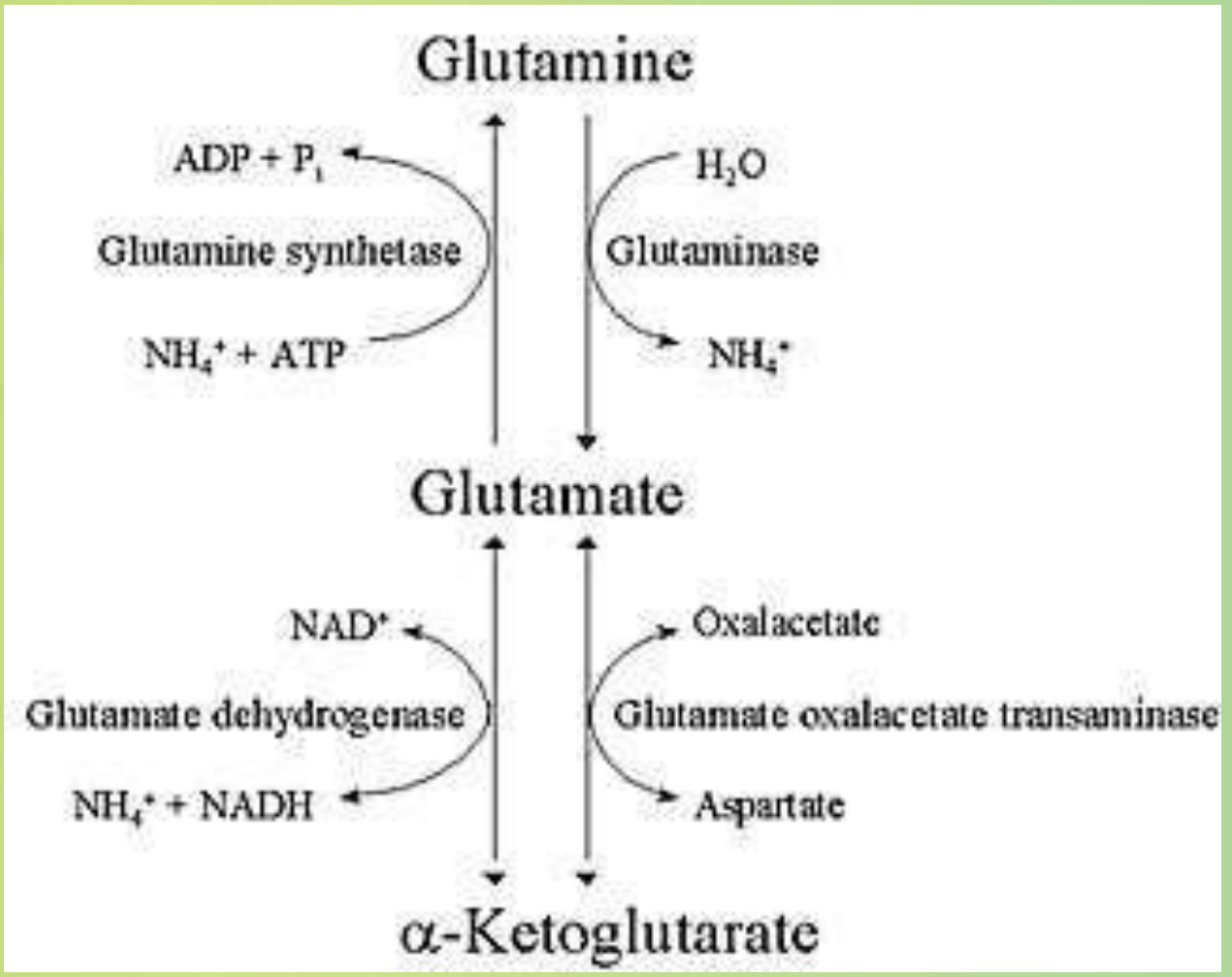
- Nonessential amino acids
- Do not cross BBB
  - must be synthesized in neurons
- Main synthetic compartments
  - neurons
  - glial cells
- Both are excitatory neurotransmitters.

Synthesis of glutamate



- Sources:
  1. Glycolysis → Krebs cycle → dehydrogenation of α-ketoglutarate
  2. Glutamine (deamination)
  3. Aspartate (transamination)
- Removal
  - Uptake and re-uptake by high affinity transport systems in the nerve
  - terminal and glial cells.

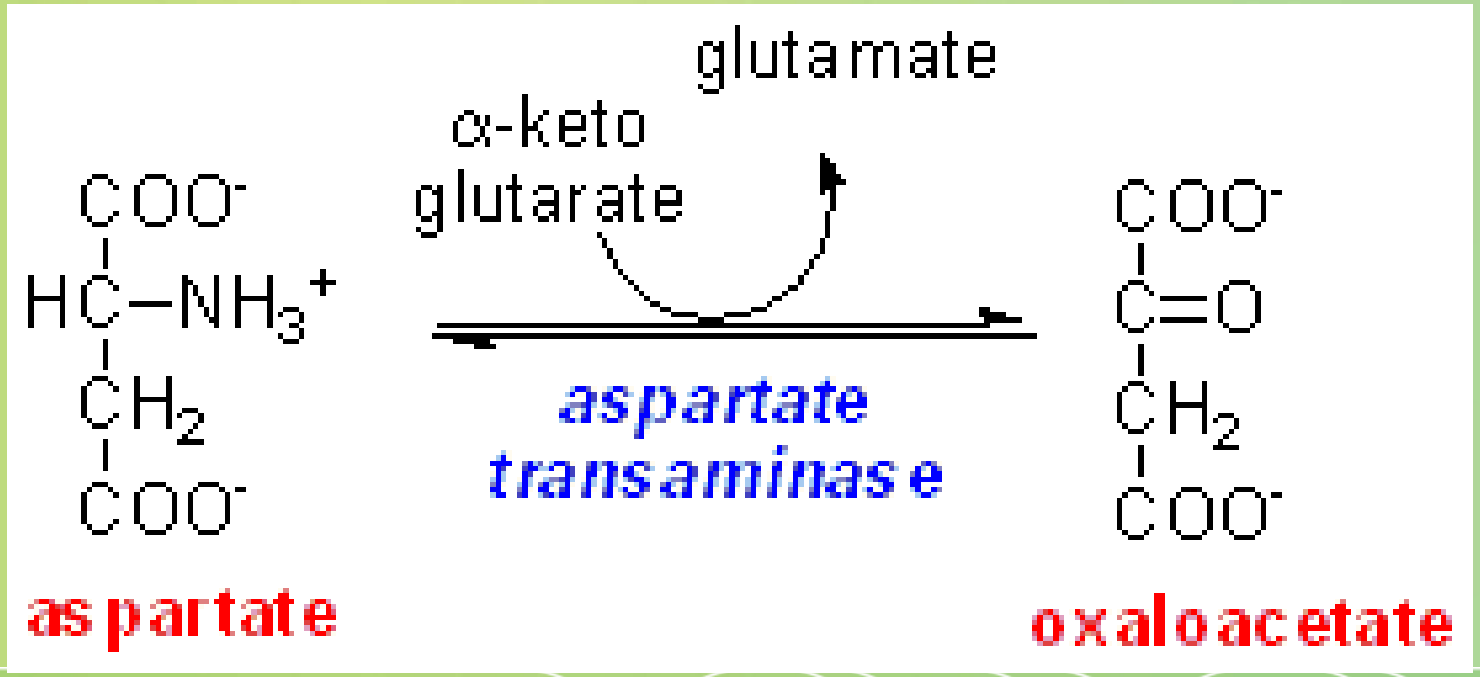
Sources of glutamate *(supplementary)*



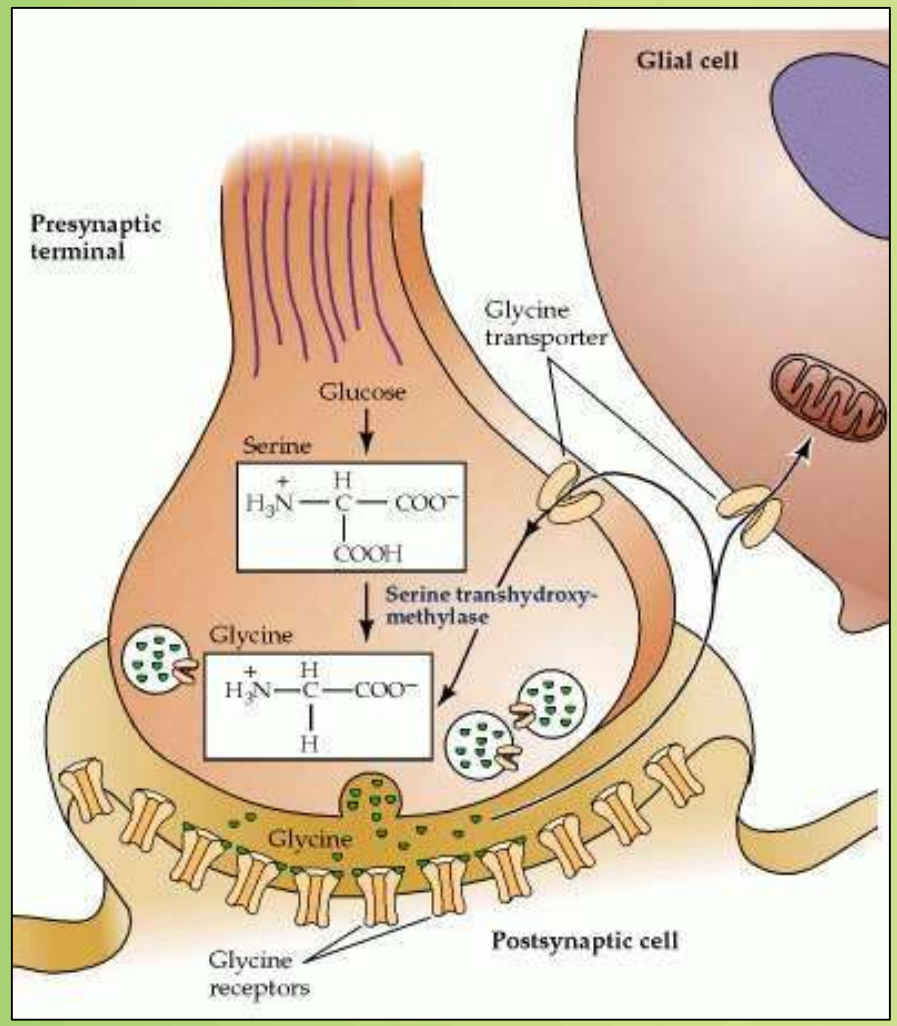
Aspartate

- Note: Similar to glutamate

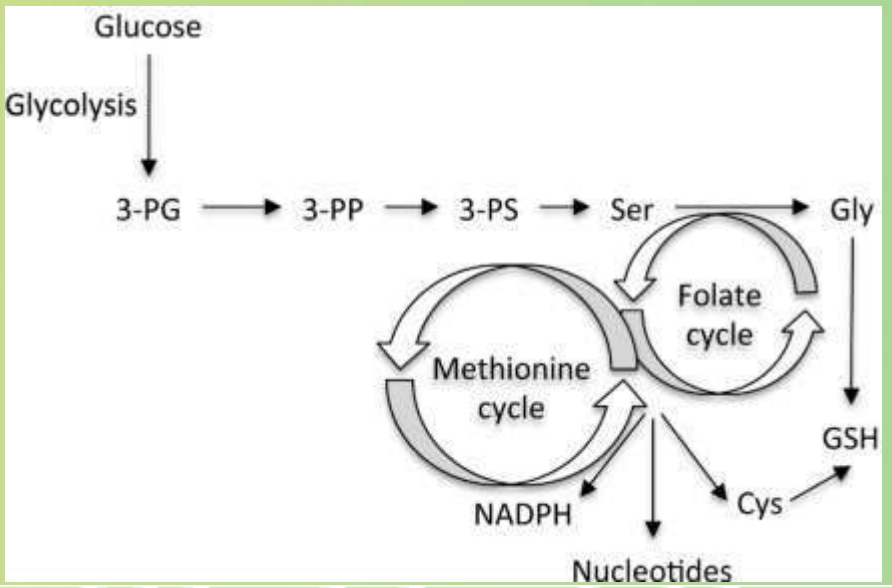
  Precursor: oxaloacetate (transamination)



Glycine



- A major inhibitory neurotransmitter
- It is synthesized from serine by serine hydroxymethyltransferase through 3-phosphoglycerate.
- Removal: high-affinity transporter



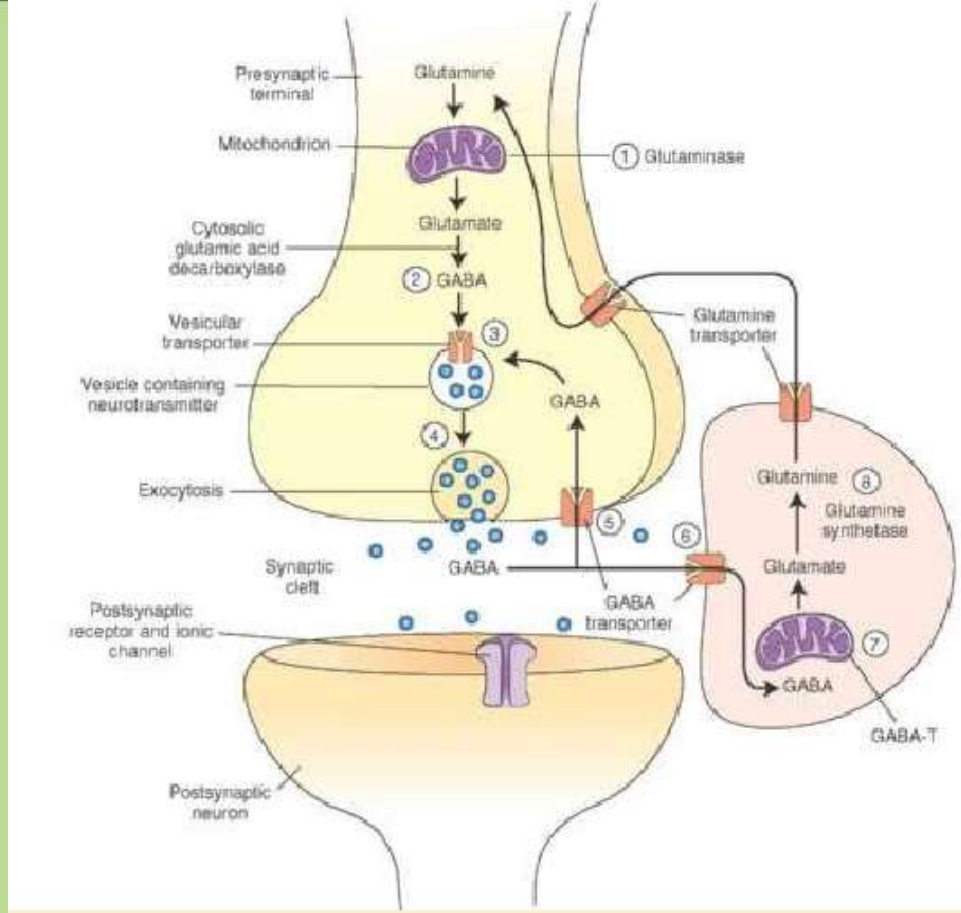
Gamma- aminobutyric acid (GABA)

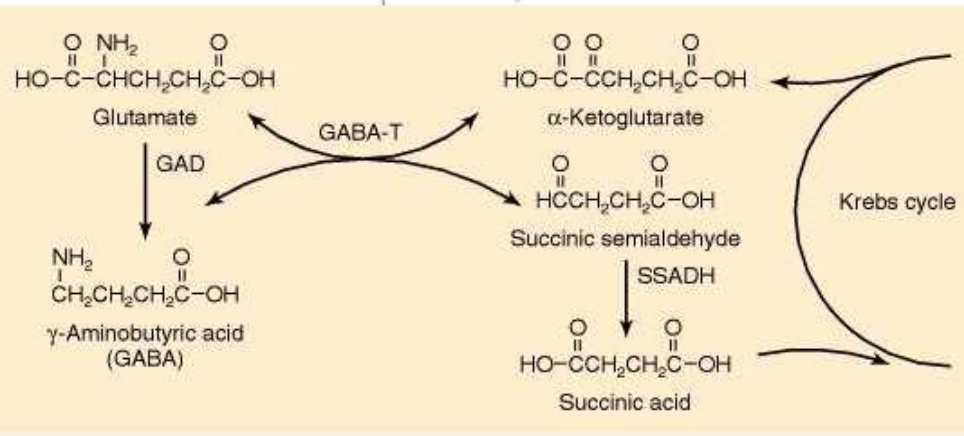
- GABA is present in high concentrations (millimolar) in many brain regions.
  - These concentrations are about 1,000 times higher than concentrations of the classical monoamine neurotransmitters in the same regions.
- The GABA shunt is a closed-loop process with the dual purpose of producing and conserving the supply of GABA.

GABA shunt

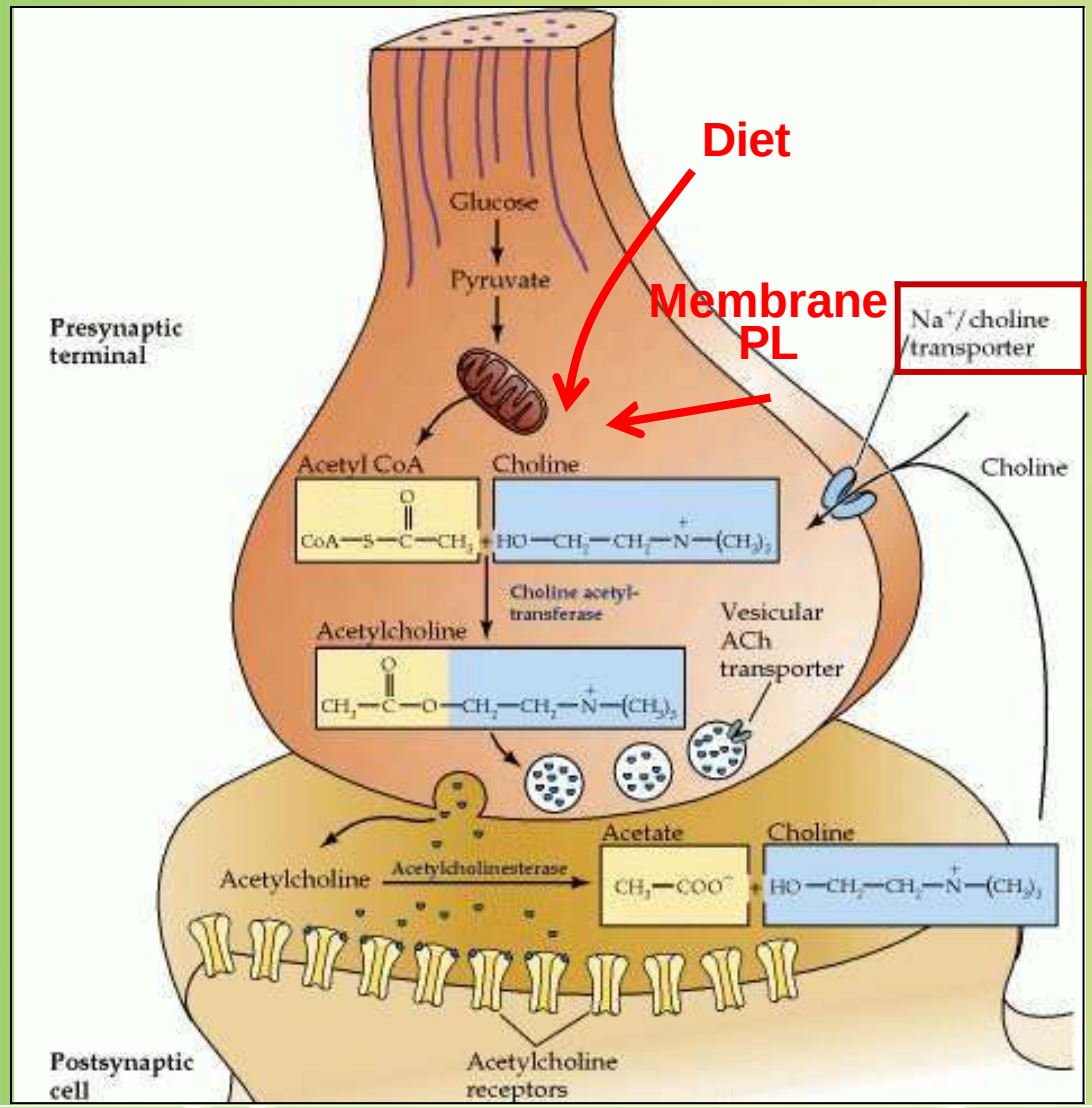
A conservation mechanisms of glutamate and GABA

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





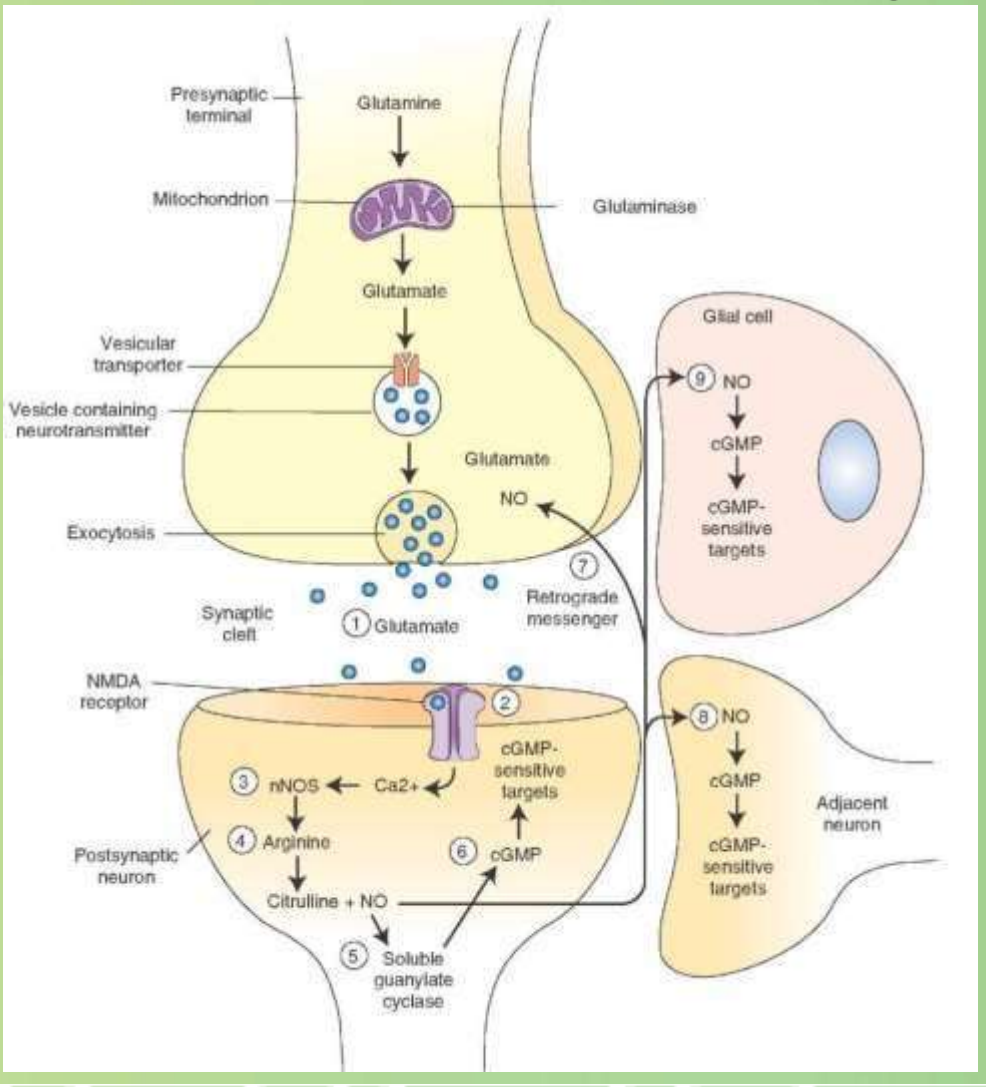
Synthesis of acetylcholine



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

Nitric oxide (NO)

- Glutamate is released (1) and acts on the N-methyl-D-aspartate (NMDA) receptors located on the post-synaptic neuron (2)
- Ca^{2+} enters the postsynaptic neuron activating nitric oxide synthase (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) and
  - b) into adjacent neurons (8) and glial cells (9) stimulating guanylate cyclase.
- It has a half-life of 2-4 seconds.
- NO is inhibited by hemoglobin and other heme proteins which bind to 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.

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.