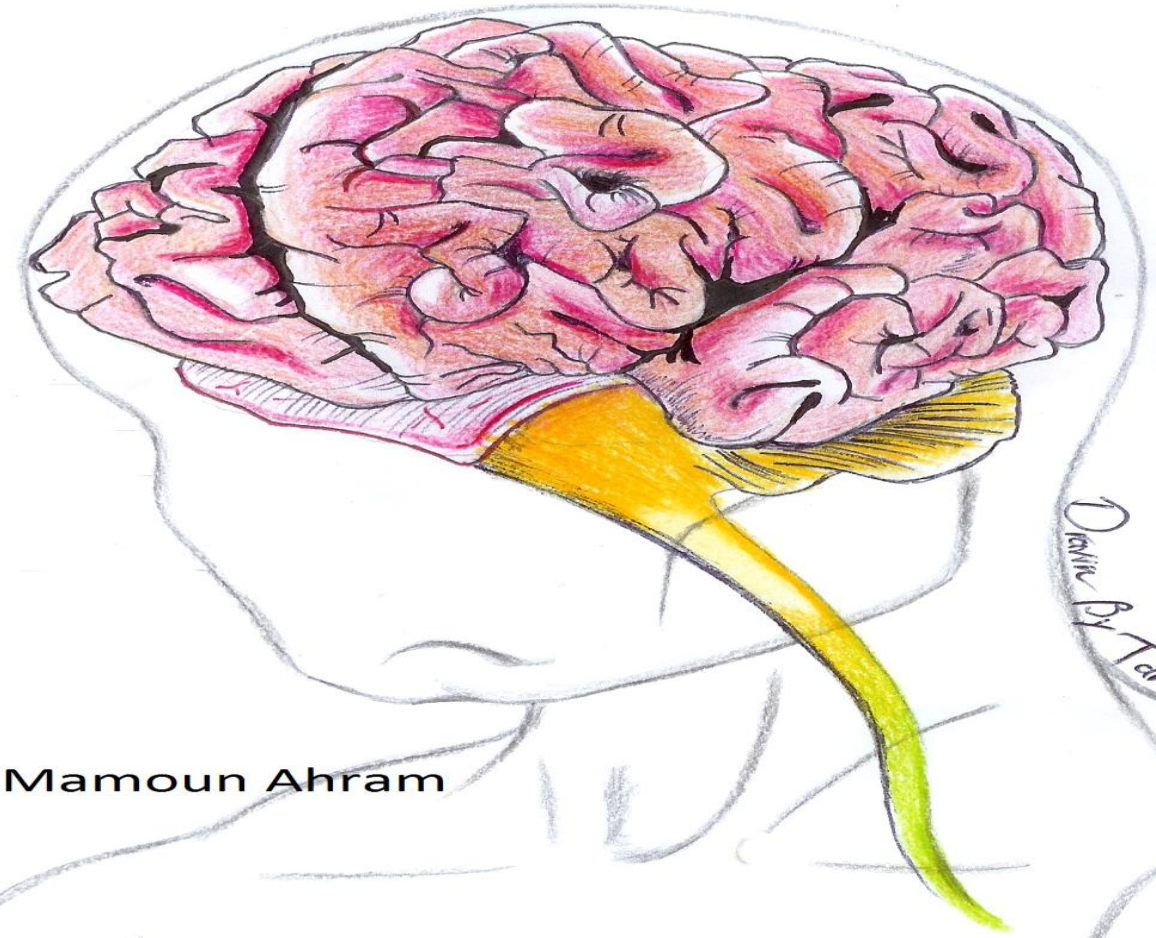
CENTRAL NERVOUS SYSTEM

- ○ Handout
- ○ Sheet
- ● Slide

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



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Biochemistry of neurotransmitters

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Neuroscience
2016

References

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

What is a neurotransmitter?

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

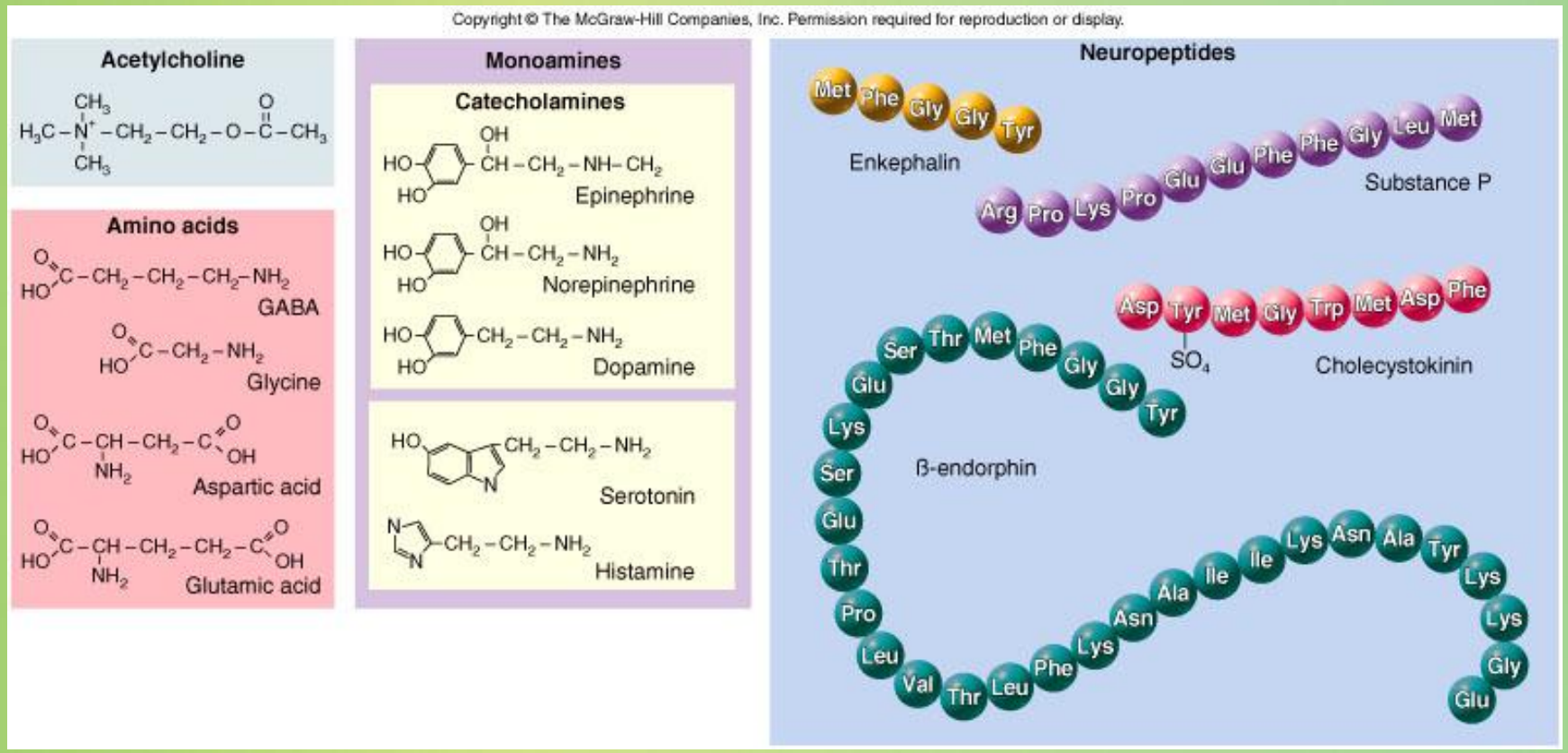
What is a neurotransmitter?

- **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 of neurotransmitters

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

Structures of neurotransmitters



NEUROPEPTIDES

Introduction

- **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 which may therefore exert its effects on distant peripheral targets.
- **Neurotransmitter:** a messenger released from a neuron at an anatomically specialised junction, which diffuses across a narrow cleft to affect one or sometimes two postsynaptic neurons, a muscle cell, or another effector cell.

Classification of neuropeptides

- **Peptides can be grouped by structural and functional similarity.**

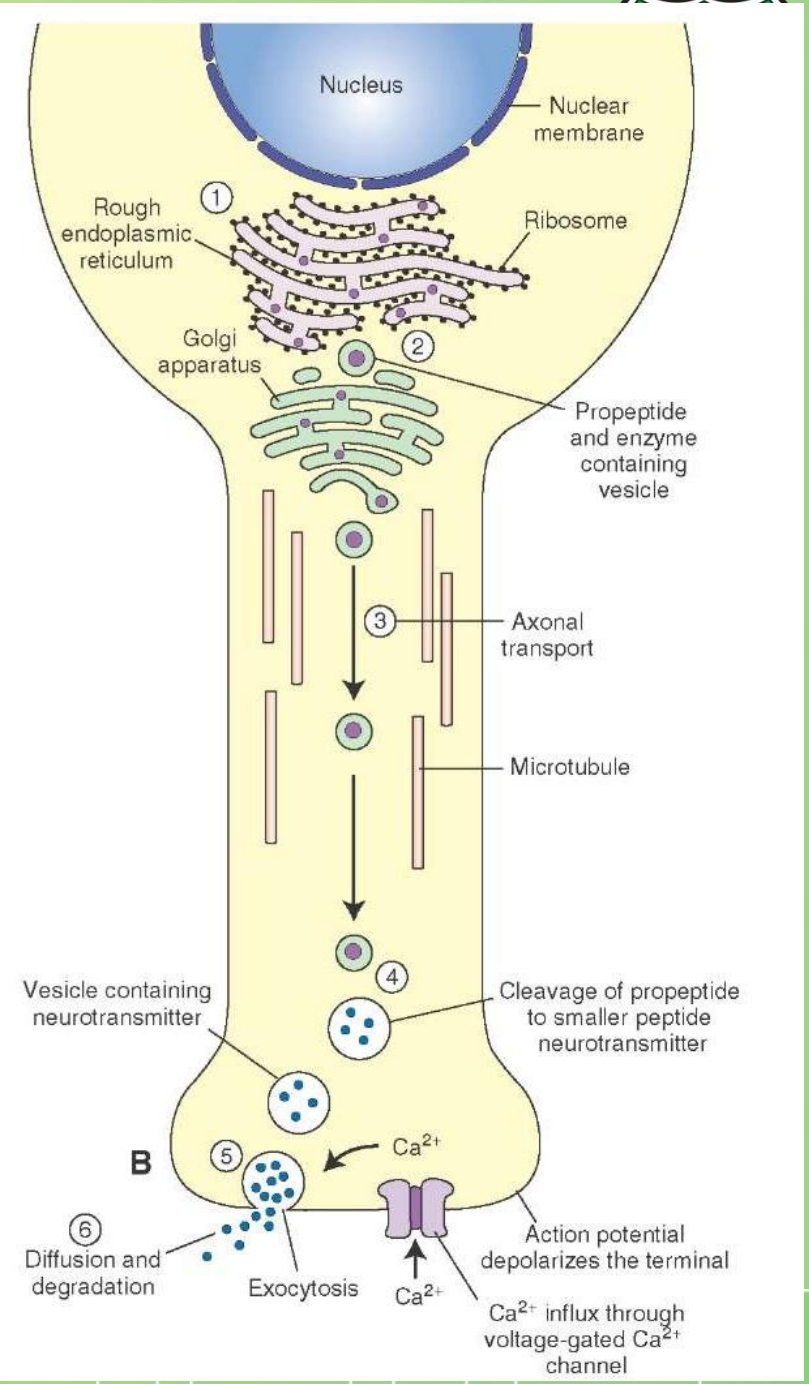
Neuropeptide Families
Tachykinins: substance P, bombesin, substance K
Insulins: insulin, insulin-like growth factors
Somatostatins: somatostatin, pancreatic polypeptide
Gastrins: gastrin, cholecystokinin
Opioids: opiocortins, enkephalins, dynorphin

- Vasopressin and oxytocin share 7 of 9 amino acids, but have different functions.
- opiate peptides share a common sequence and all are potent endogenous opiates but with distinct patterns of receptor selectivity.
- 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

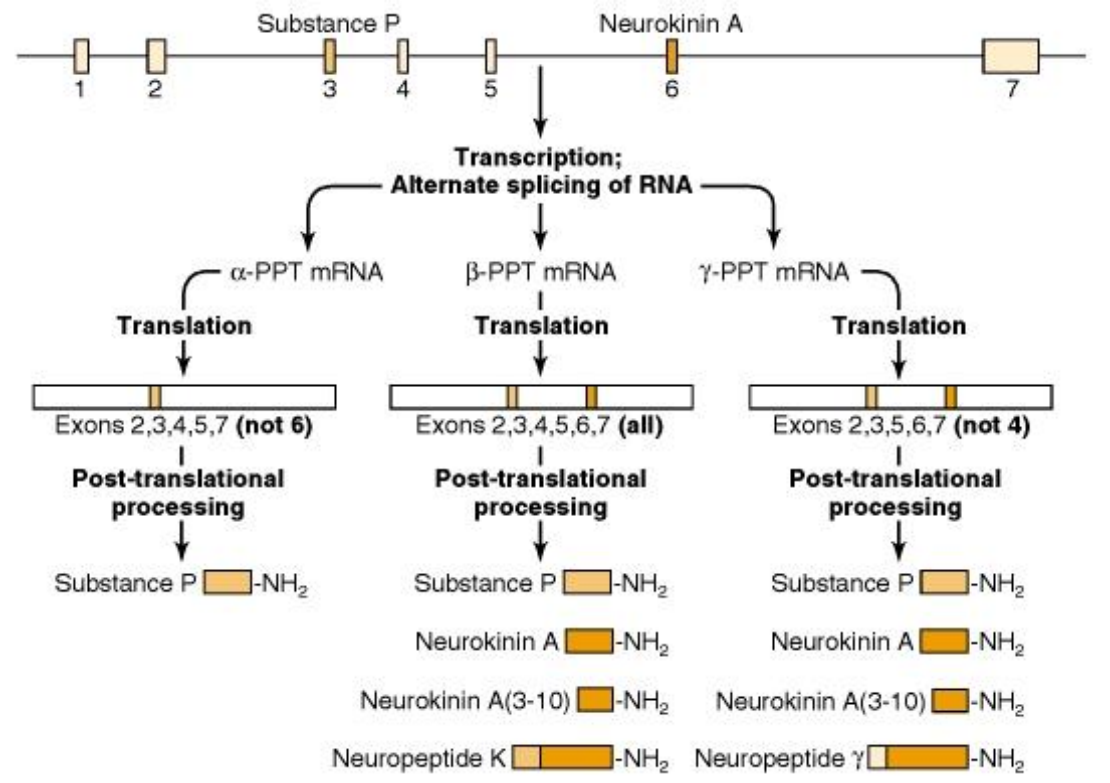
Stages of action

- 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



Diversity: alternative splicing

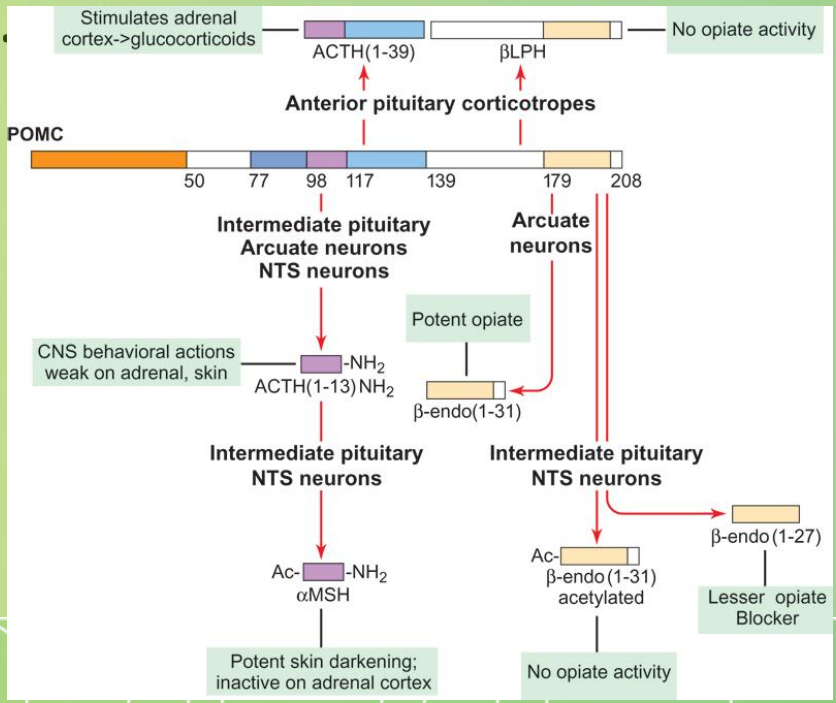
- **Alternative splicing of mRNA leads to translation of distinct precursors, and subsequent processing leads to unique mature peptides.**
  - Example is the substance P mRNA that normally also includes mRNA encoding substance K.



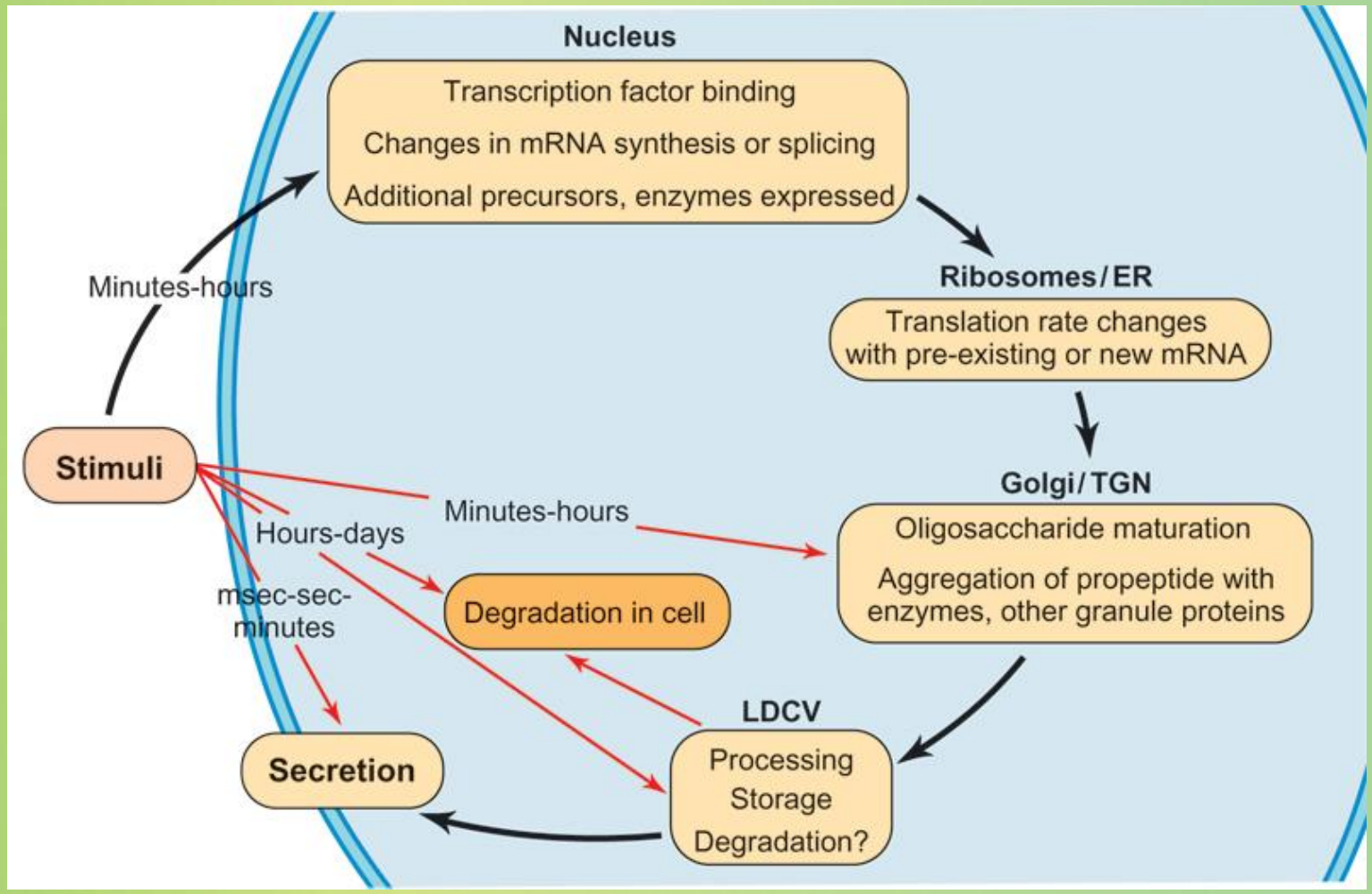
Diversity: proteolytic, differential, sequential processing

- **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

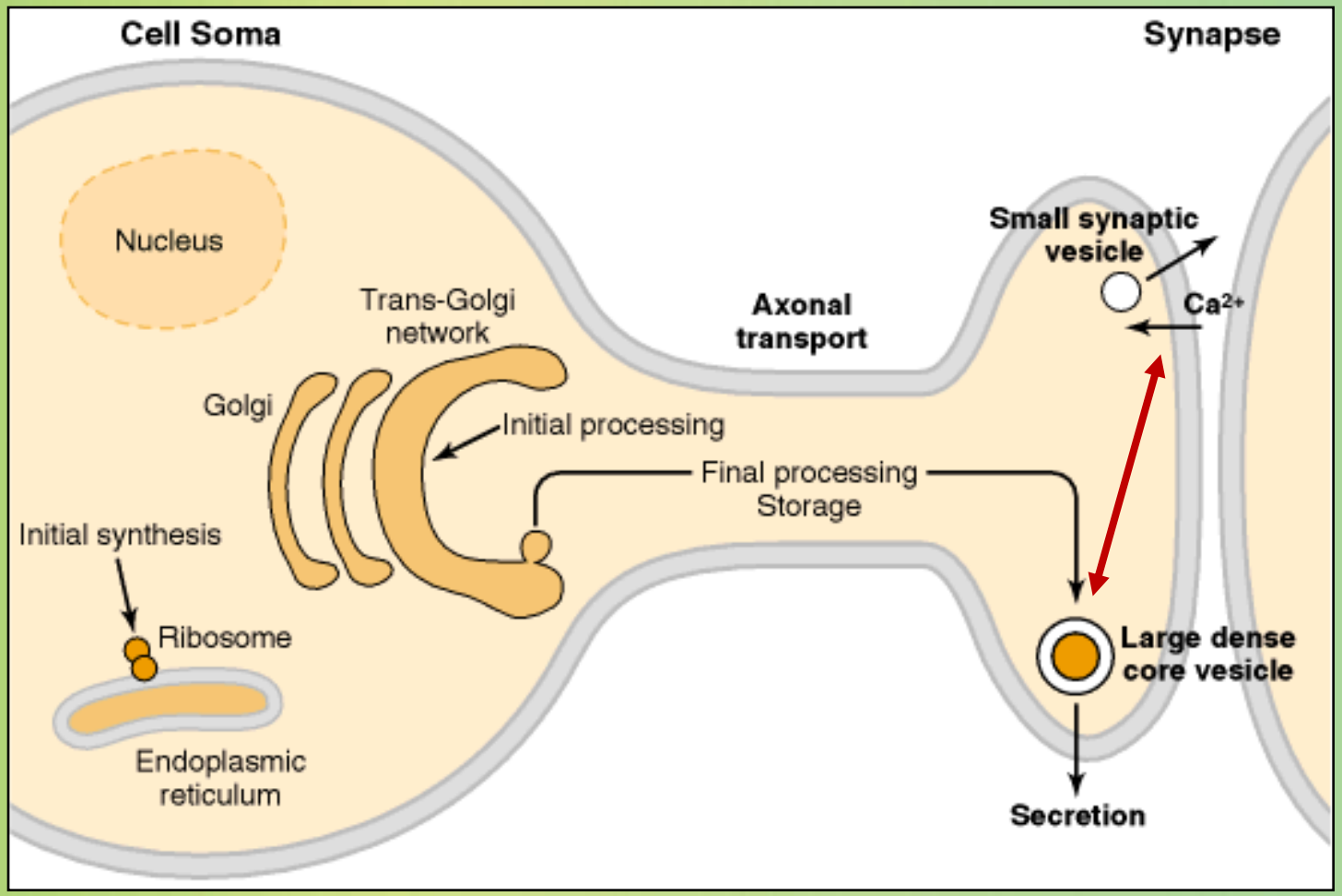
Processing of the pro-opiomelanocortin (*POMC)* precursor proceeds in an ordered, stepwise fashion. Some of the reactions are tissue specific. *ACTH,* adrenocorticotropic hormone; *CLIP,* corticotropin-like intermediate lobe peptide; *JP,* joining peptide; *LPH,* lipotropin; *MSH,* melanocyte-stimulating hormone; *PC,* prohormone convertase.



The levels of regulation of neuropeptide expression



Role of calcium



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

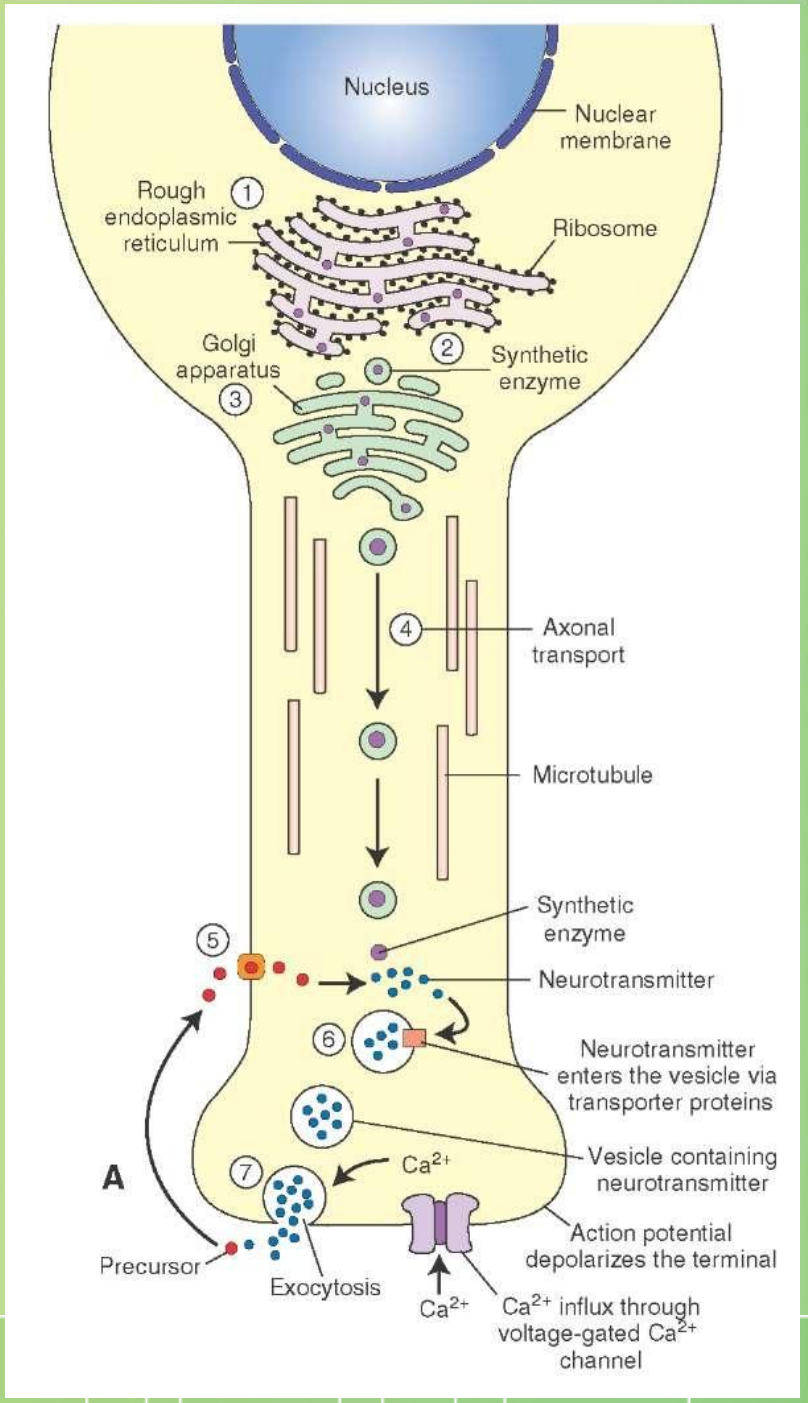
SMALL-MOLECULE NEUROTRANSMITTERS

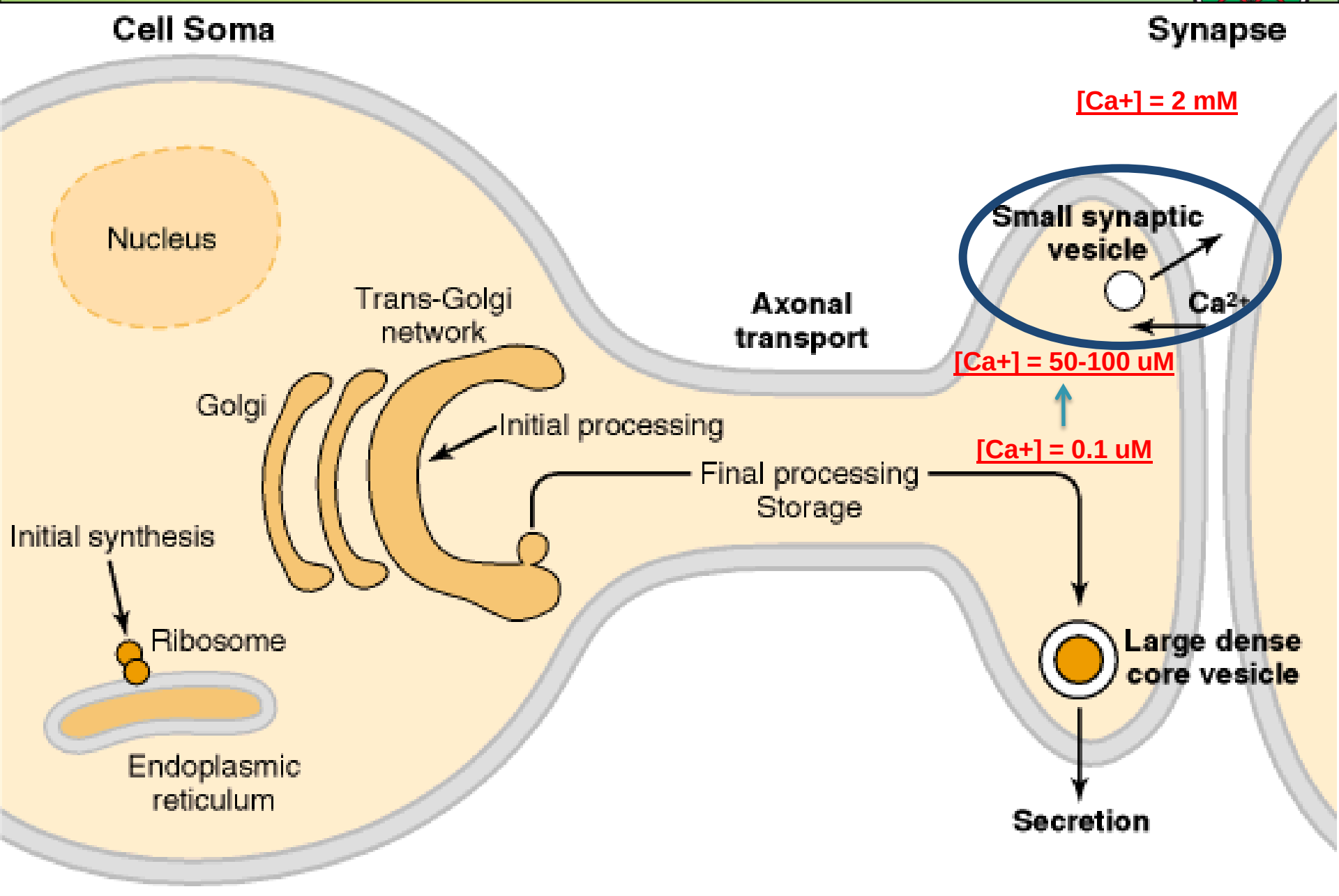
Types of small-molecule neurotransmitter

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

Stages of action

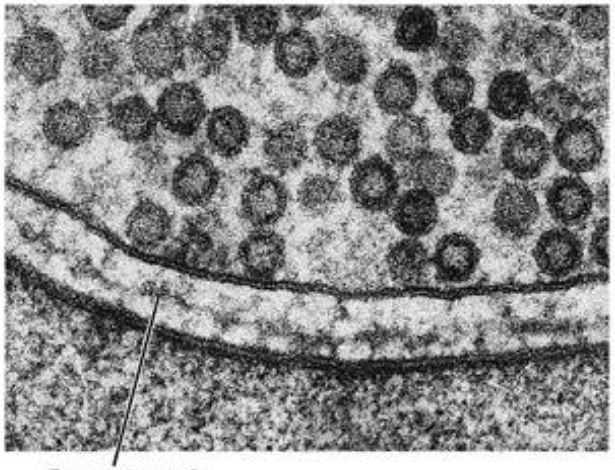
- Synthesis of enzymes
  - Cytosol
  - ER-Golgi apparatus (packaging into large-dense core vesicles)
- Transport of enzymes (slow and fast-axonal transport)
- Synthesis in pre-synaptic terminal
- Packaging in synaptic vesicles
- Release
  - They are released in brief pulses each time an action potential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



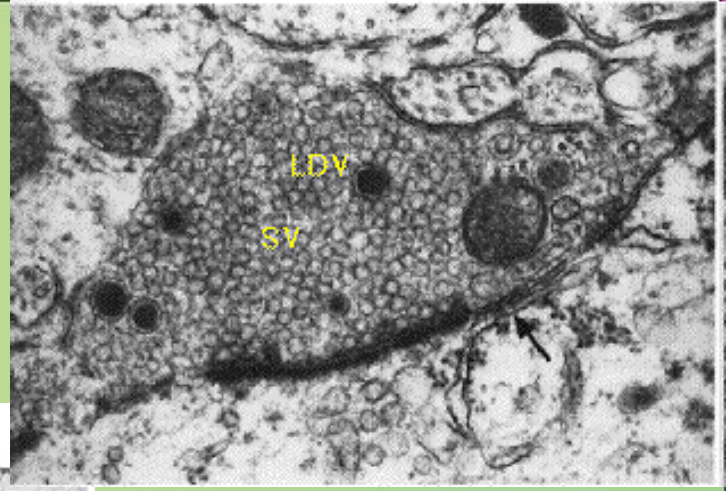

Cell Soma
Synapse
[Ca+] = 2 mM
Nucleus
Small synaptic vesicle
Ca2+
Trans-Golgi network
Axonal transport
[Ca+] = 50-100 uM
Golgi
Initial processing
[Ca+] = 0.1 uM
Final processing
Storage
Initial synthesis
Ribosome
Large dense core vesicle
Endoplasmic reticulum
Secretion

Presynaptic membrane (thin section)

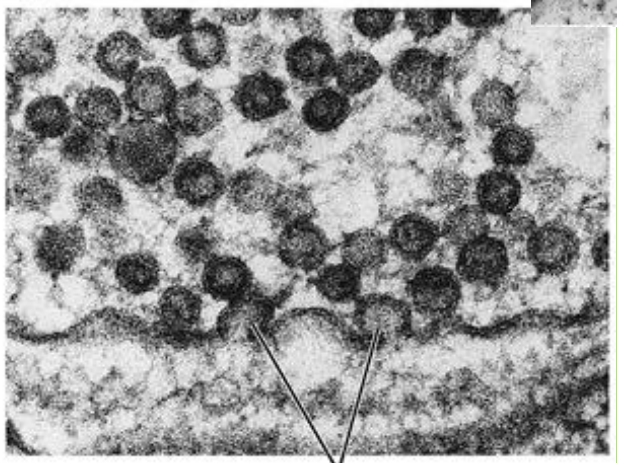
A_2



Synaptic cleft

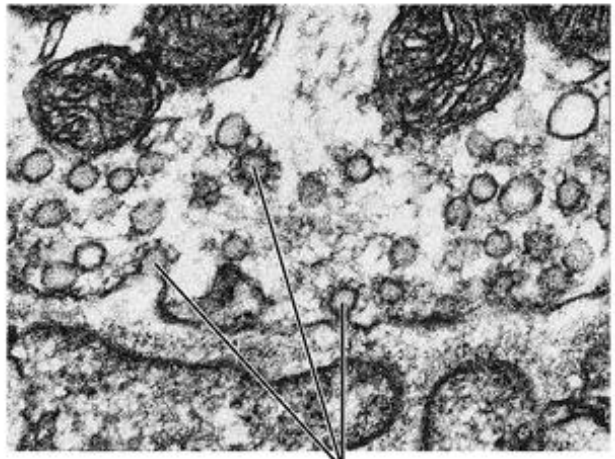


B_2



Vesicle fusions

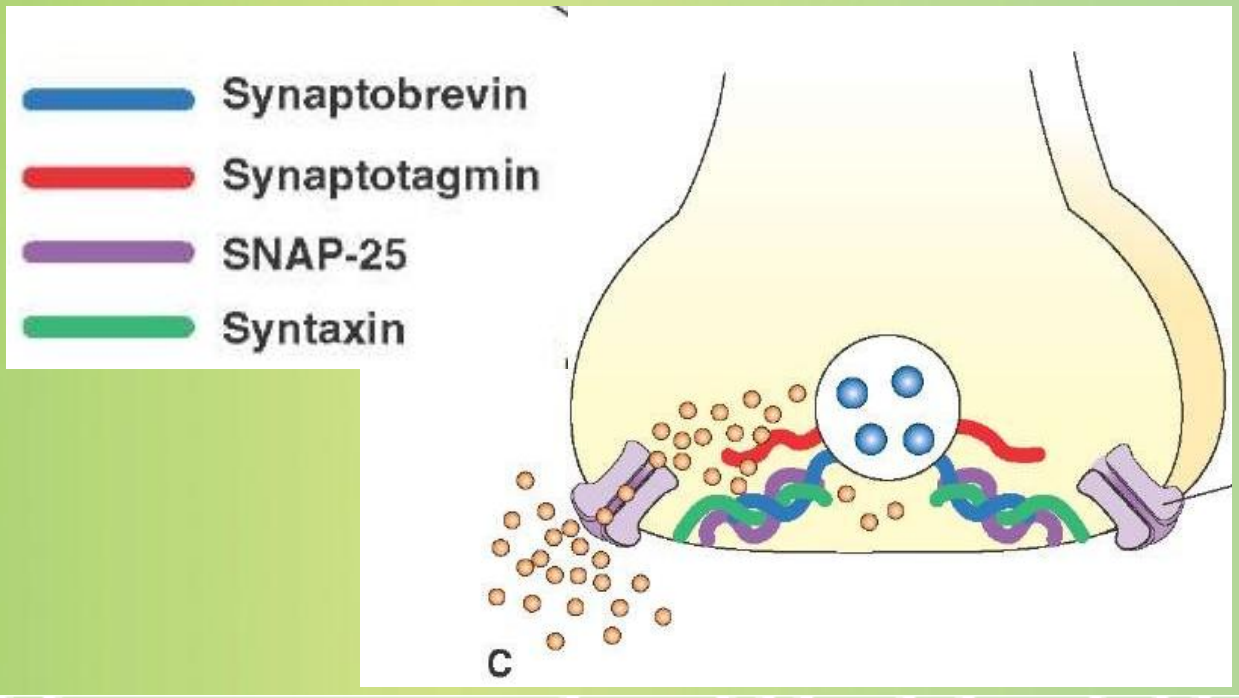
C_2



Coated vesicles

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.



Note the differences between neuropwptides and neurotransmitters

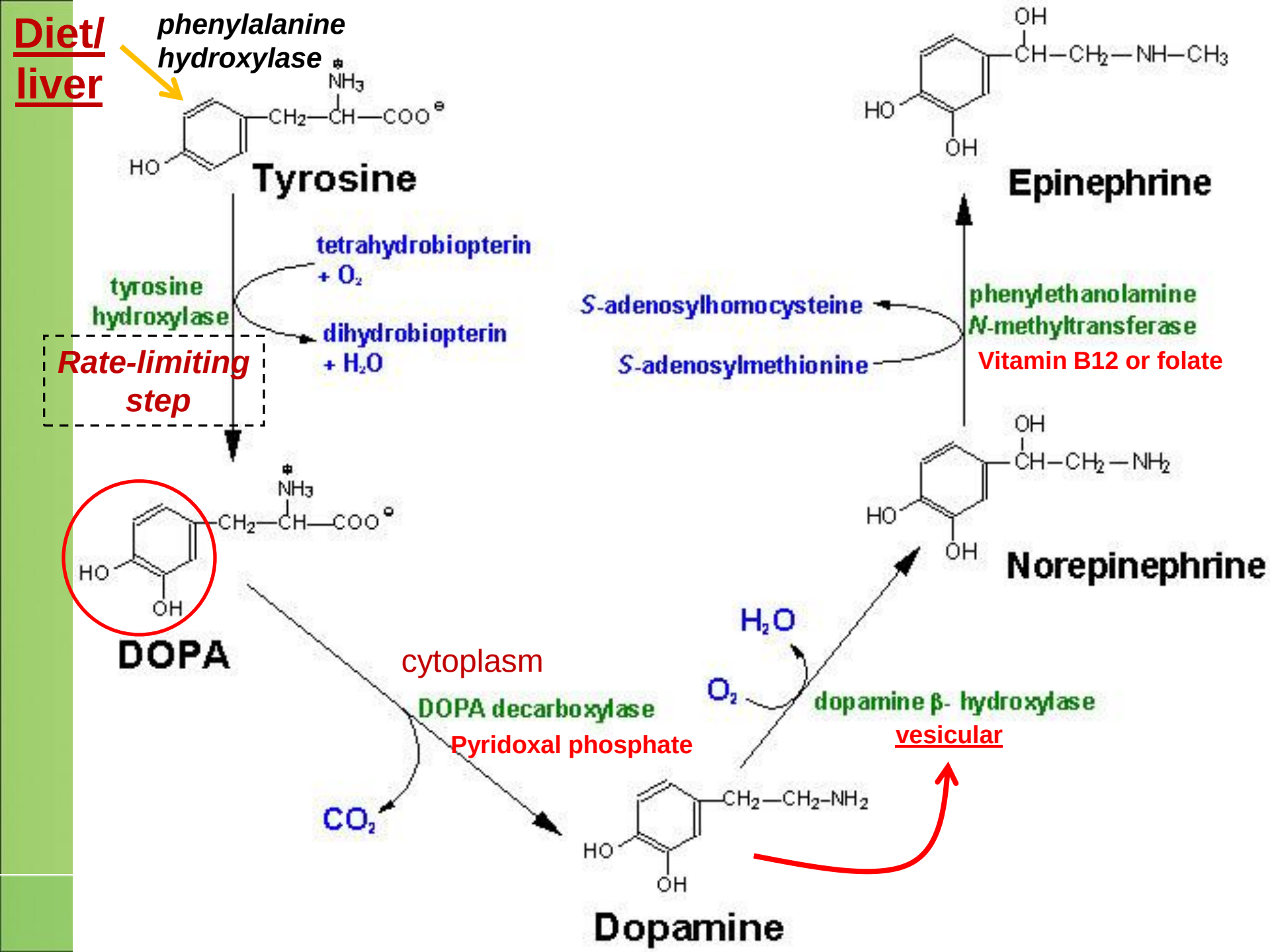
- Onset and duration of action
- Synthesis, transport, and packaging
- Concentration for action and receptor binding
- Concentration of [Ca+] for release
- Site of synthesis, modification
- Fate

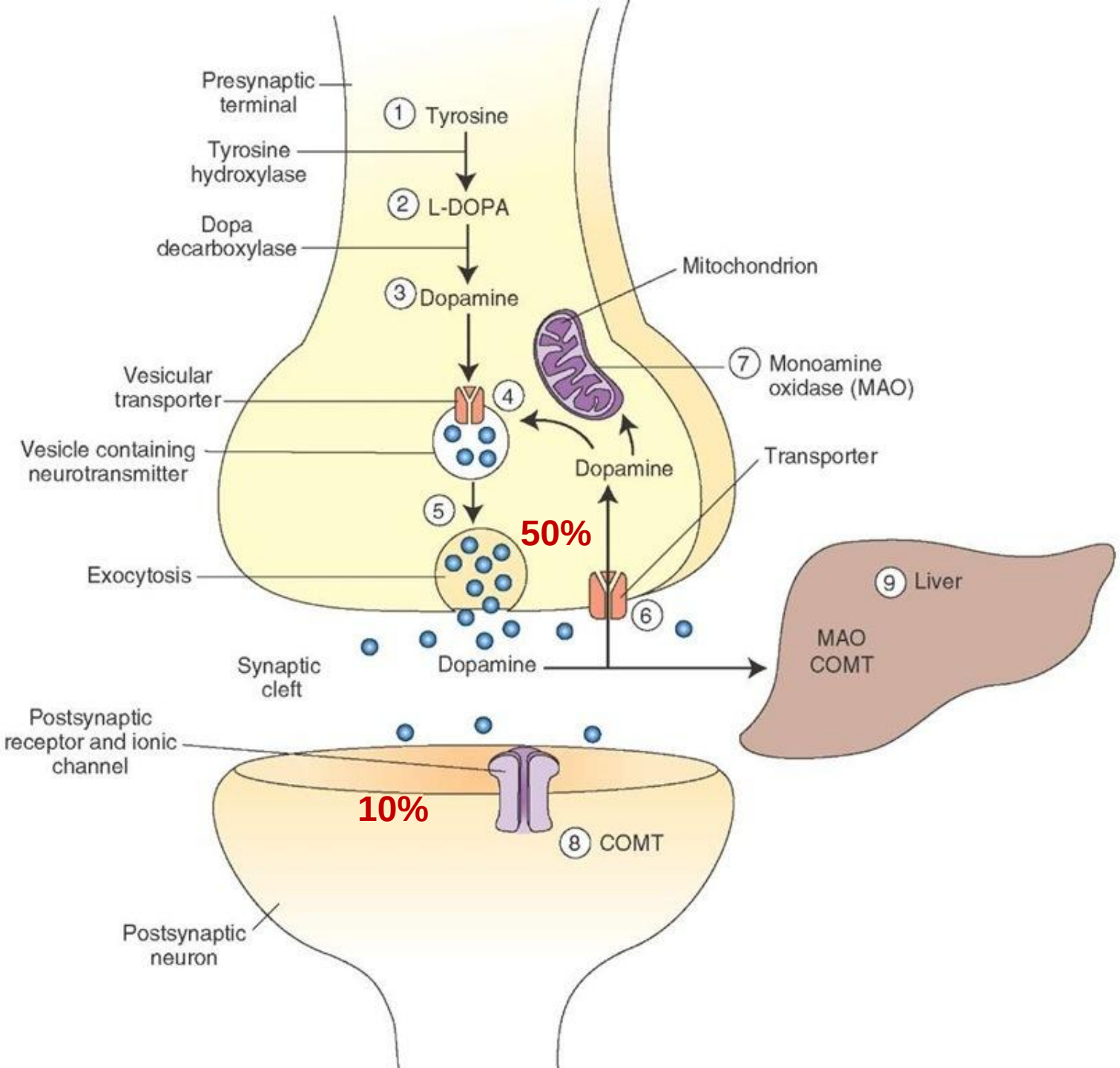
TYROSINE-DERIVED NEUROTRANSMITTERS

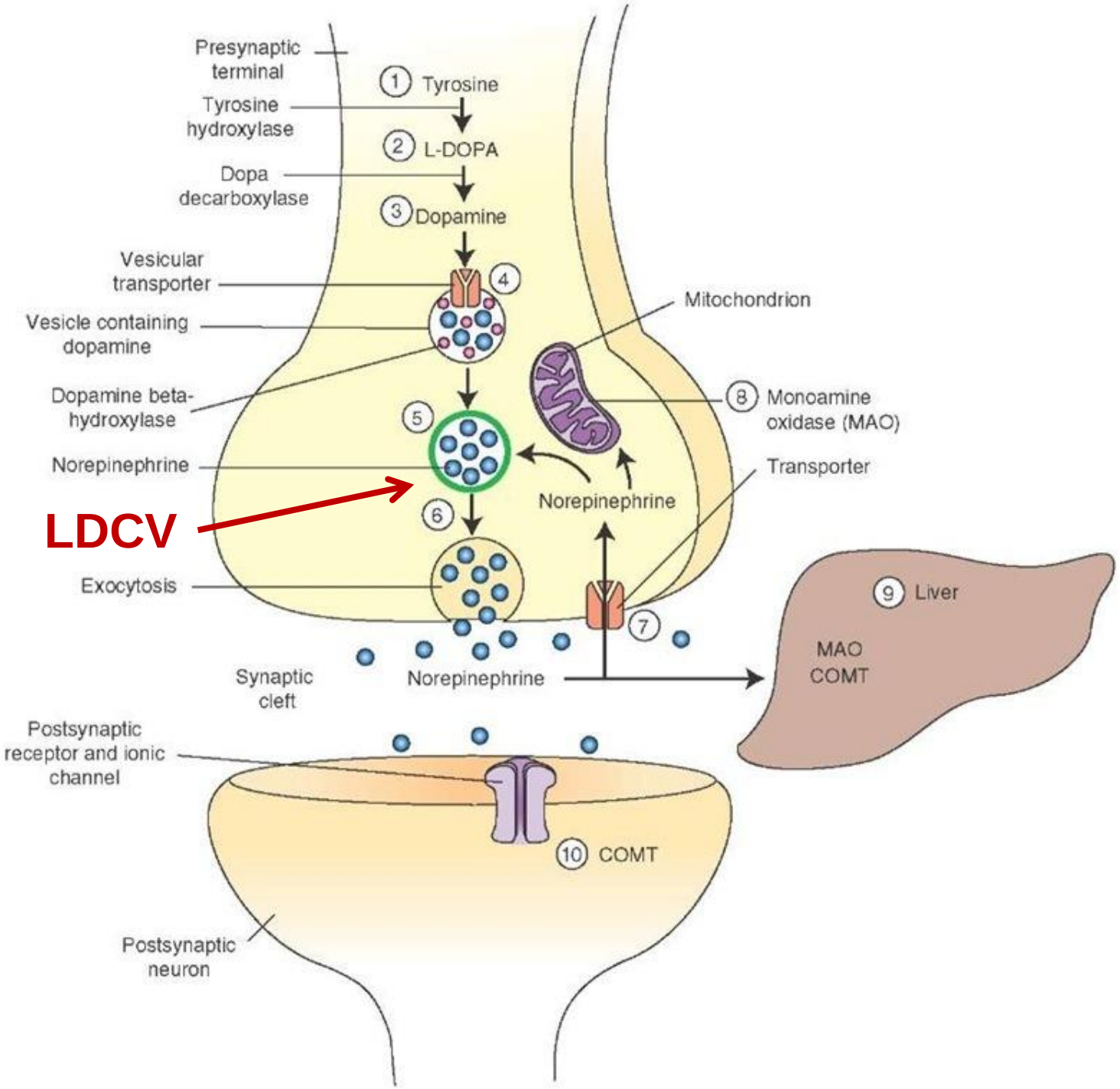
Dopamine, norepinephrine, and epinephrine

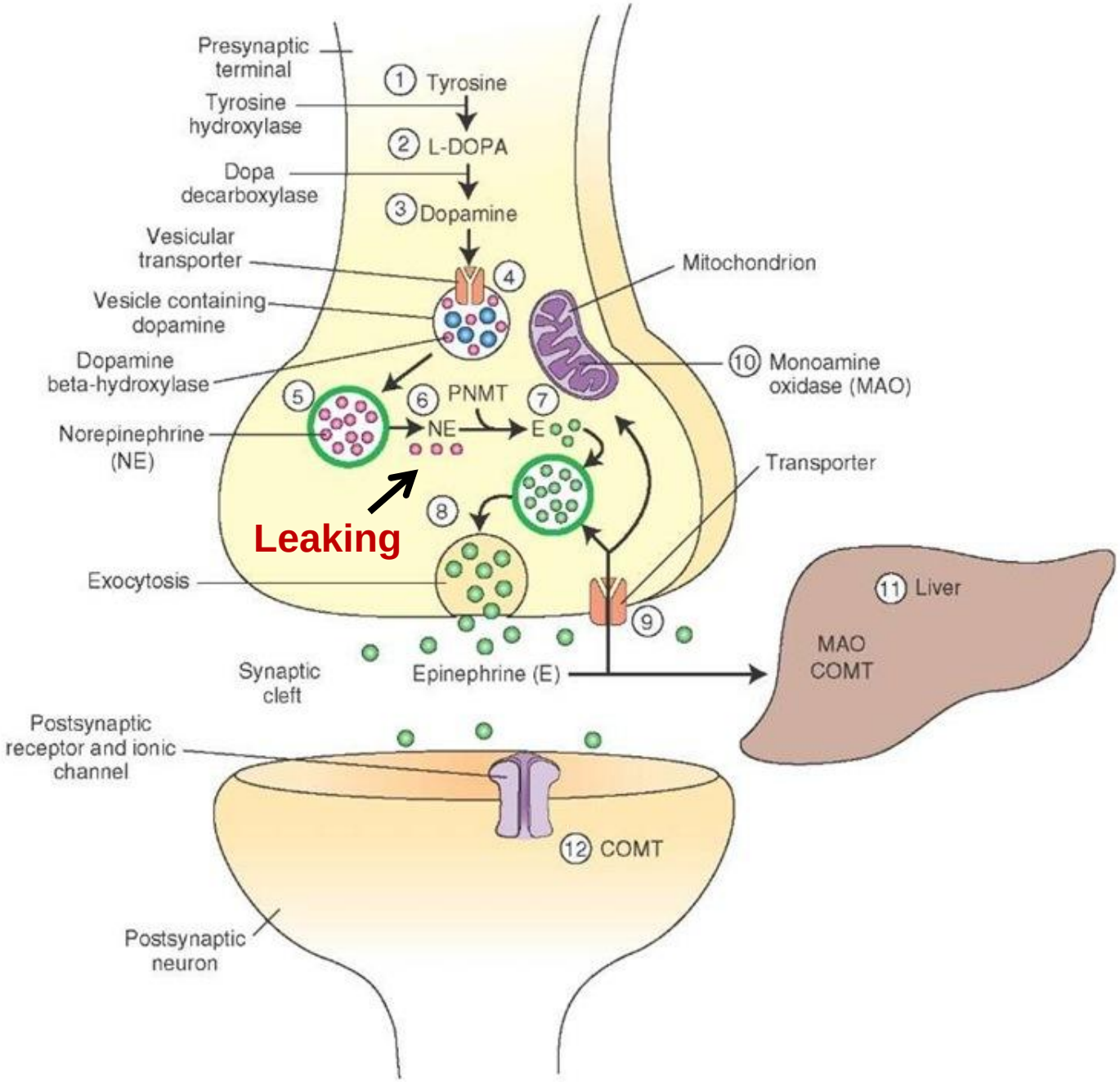
Notes

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


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

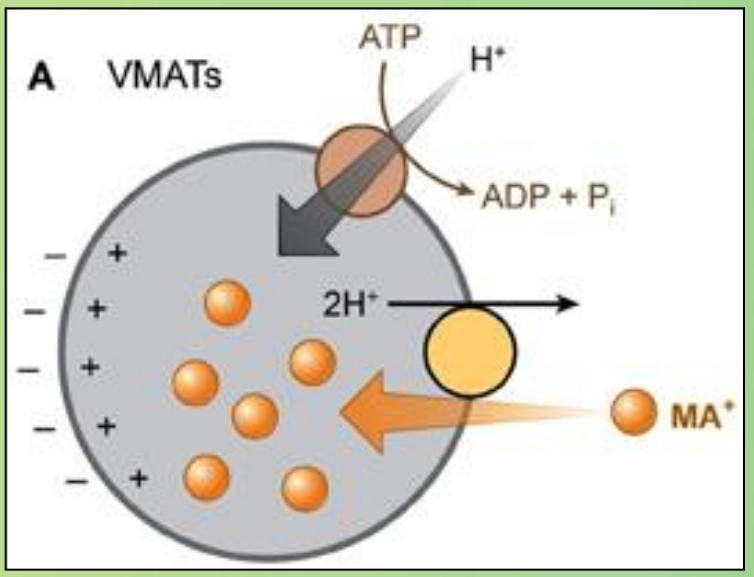

Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Mitochondrion
7 Monoamine oxidase (MAO)
Vesicular transporter
4
Vesicle containing neurotransmitter
Dopamine
Transporter
5
50%
Exocytosis
9 Liver
MAO
COMT
6
Synaptic cleft
Dopamine
Postsynaptic receptor and ionic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


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

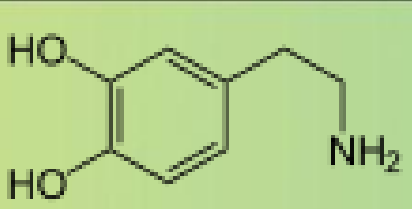

Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Vesicular transporter
4
Mitochondrion
Vesicle containing dopamine
Dopamine beta-hydroxylase
10 Monoamine oxidase (MAO)
5
6 PNMT
7
Norepinephrine (NE)
NE
E
Transporter
Leaking
8
Exocytosis
11 Liver
MAO
COMT
9
Synaptic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

Packaging 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).



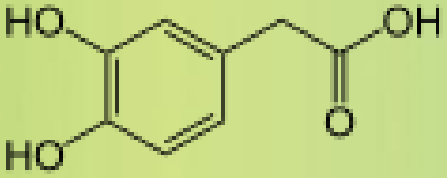
COMT and MAO



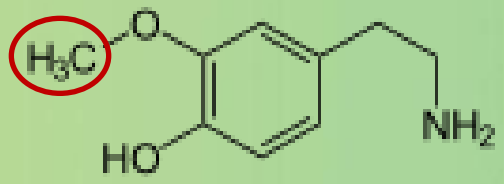
Dopamine (DA)

Monoamine oxidase (MAO), Aldehyde dehydrogenase

Catechol-O-methyl-transferase (COMT)



3,4-Dihydroxyphenyl-acetic acid (DOPAC)

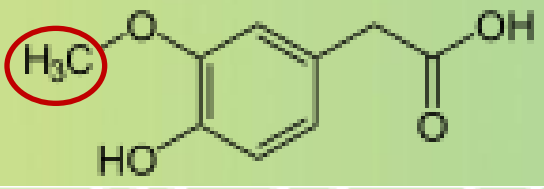


3-Methoxytyramine (3-MT)

Inactivation is dependent on SAM and vitamin B12 and folate

Catechol-O-methyl-transferase (COMT)

Monoamin oxidase (MAO), Aldehyde dehydrogenase



Homovanillic acid (HVA)

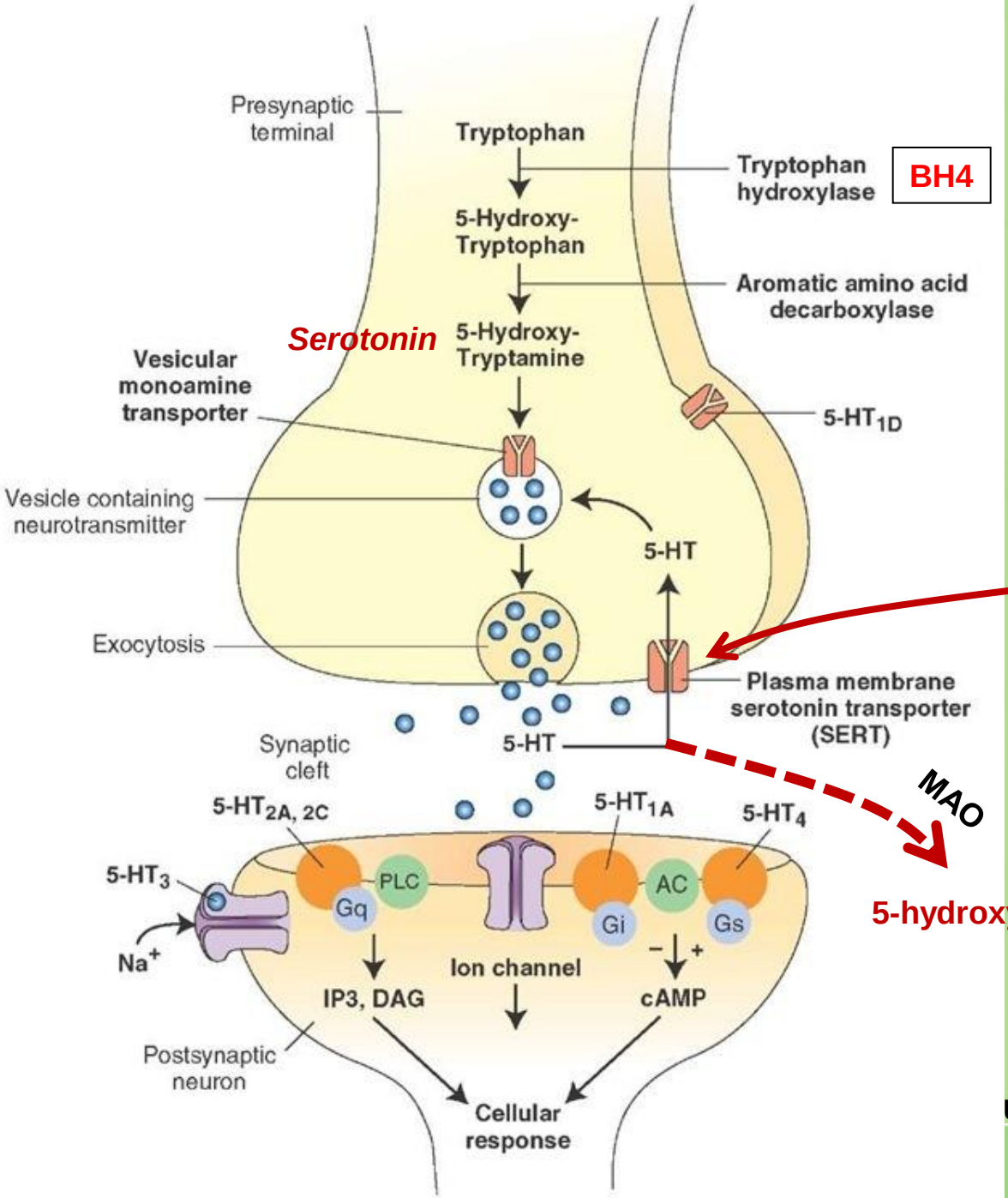
Parkinson's disease

Regulation

- Tyrosine hydroxylase
  - Short term
    - Inhibition by free cytosolic catecholamines
      - Catecholamines compete with BH4 binding to enzyme
    - Activation by depolarization
      - Tight binding to BH4 following phosphorylation by PKA, CAM kinases, PKC
  - Long-term (plus dopamine β-hyroxylase)

TRYPTOPHAN-DERIVED NEUROTRANSMITTERS

Serotonin and melatonin



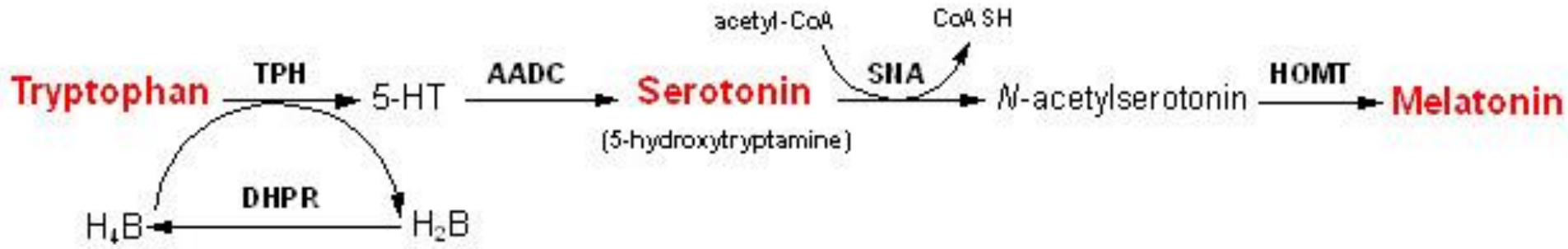
Antidepressants , called selective serotonin re-uptake inhibitors (SSRIs), like Prozac® inhibit the reuptake process resulting in prolonged serotonin presence in the synaptic cleft.

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) rythyms
  - Dark-light cycle



GLUTAMATE AND ASPARTATE

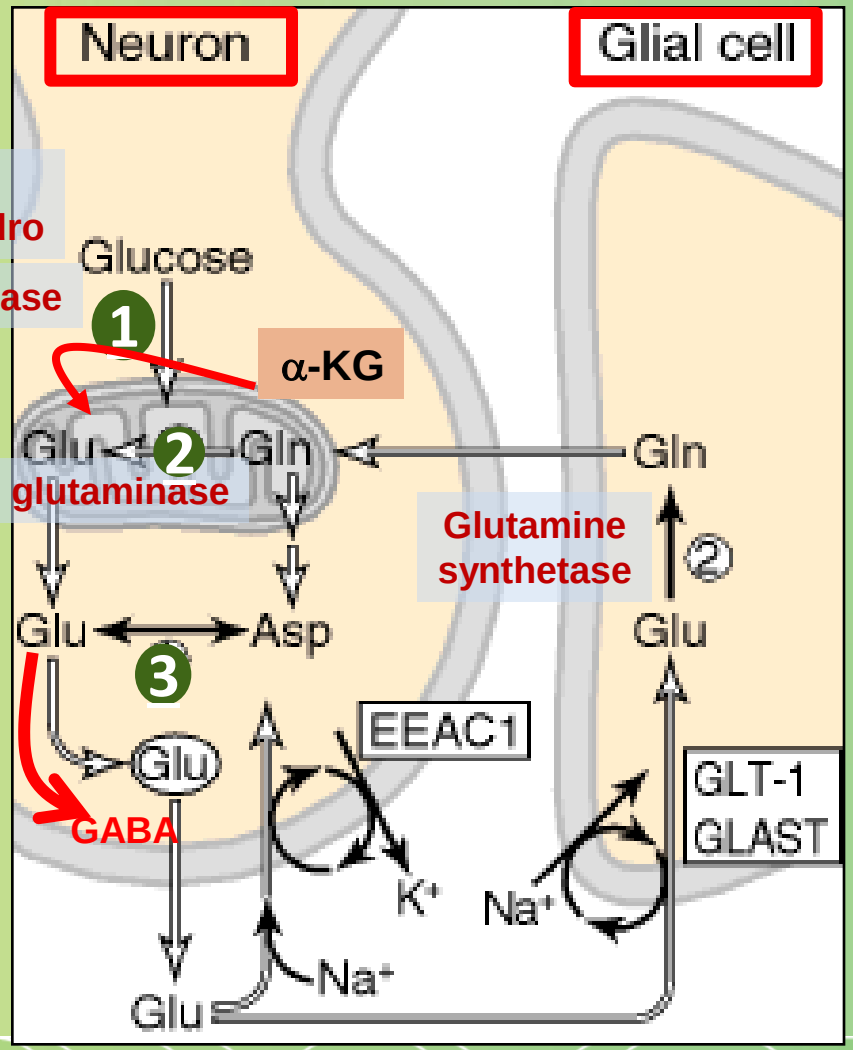
Glutamate and aspartate

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

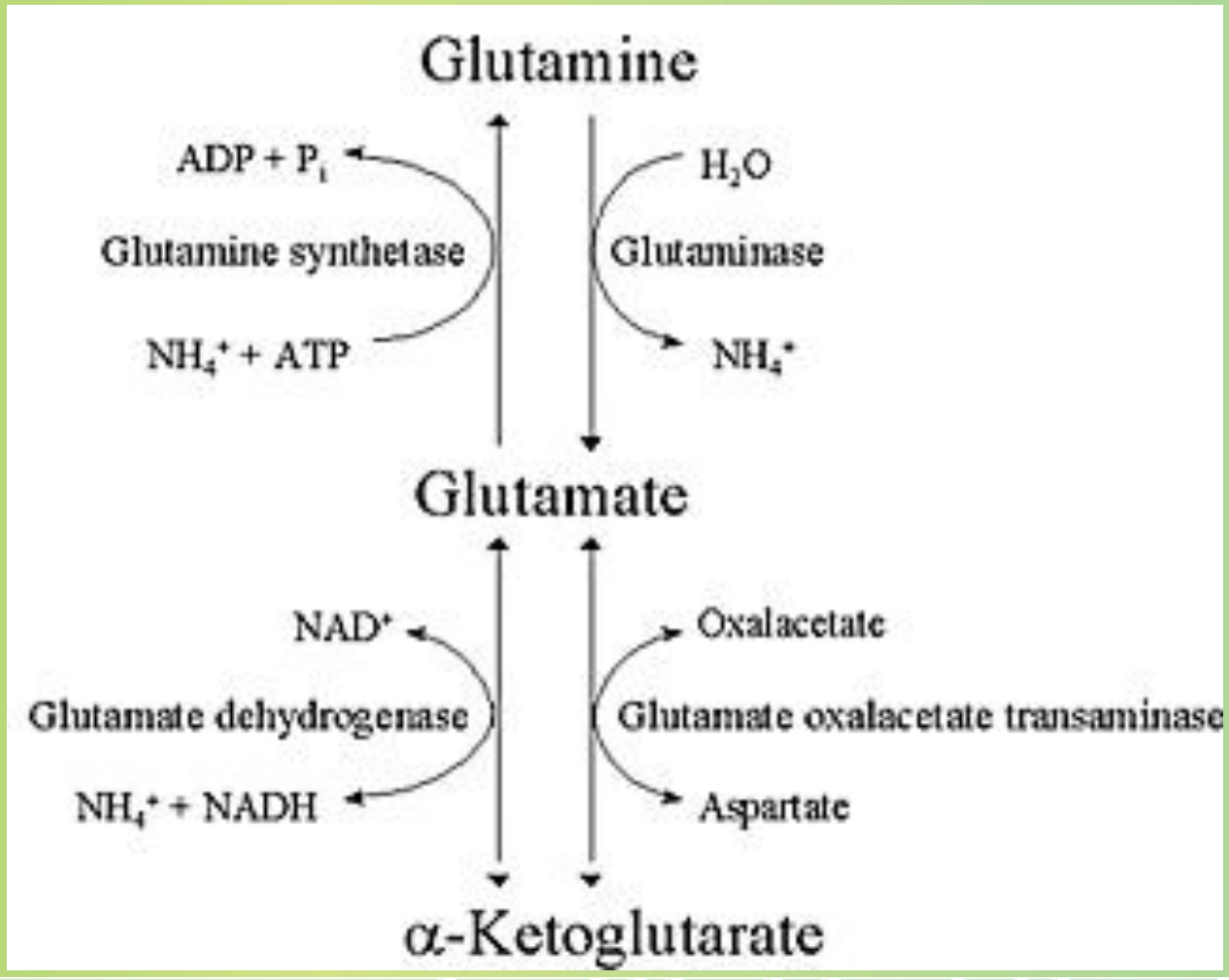
Synthesis of glutamate

Sources:

- **Glycolysis → Krebs cycle → Transamination or dehydrogenation**
- **Glutamine (deamination)**
- **Another source: aspartate**
- **Removal**
  - **excitatory amino acid carrier-1 *(EAAC1)***
  - **glutamate transporter-1 (GLT-1) and glutamate—aspartate transporter (GLAST)**



Sources of glutamate *(supplementary)*



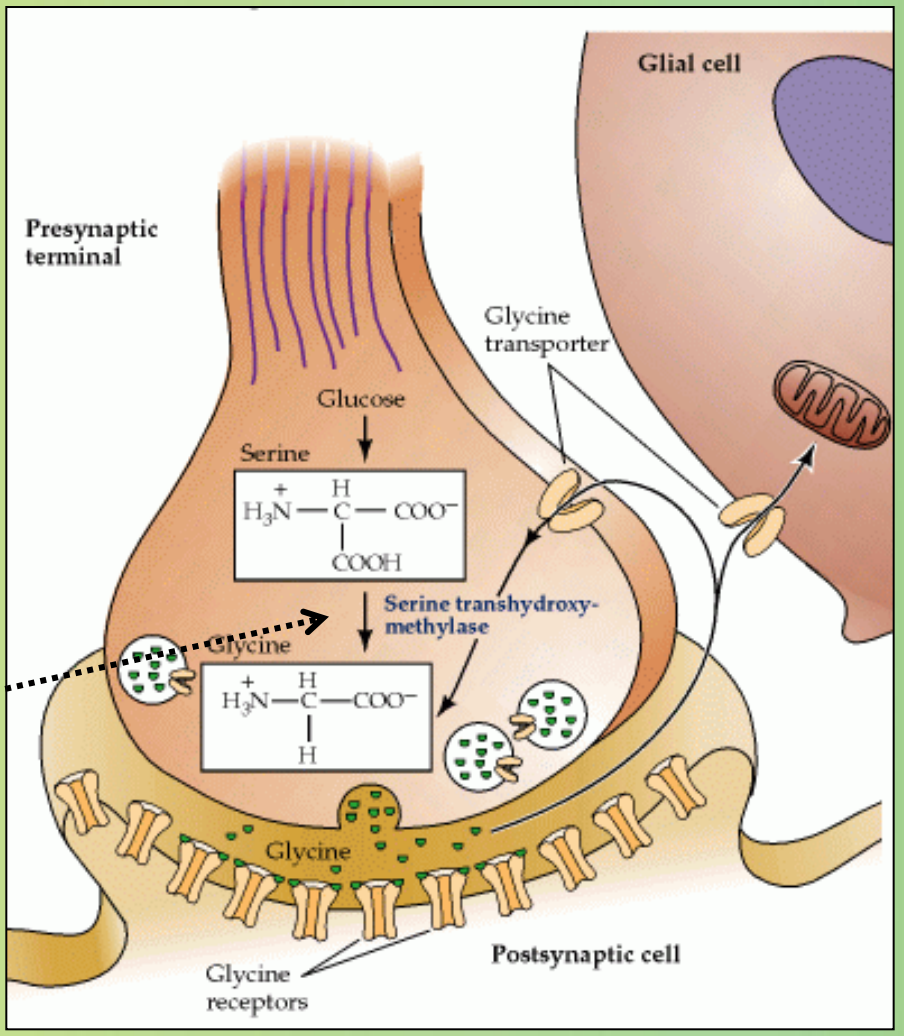
Aspartate

- 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 spical cord
- Synthesized from serine by serine hydroxymethyltransferase through 3-phosphoglycerate
- Removal: high-affinity transporter

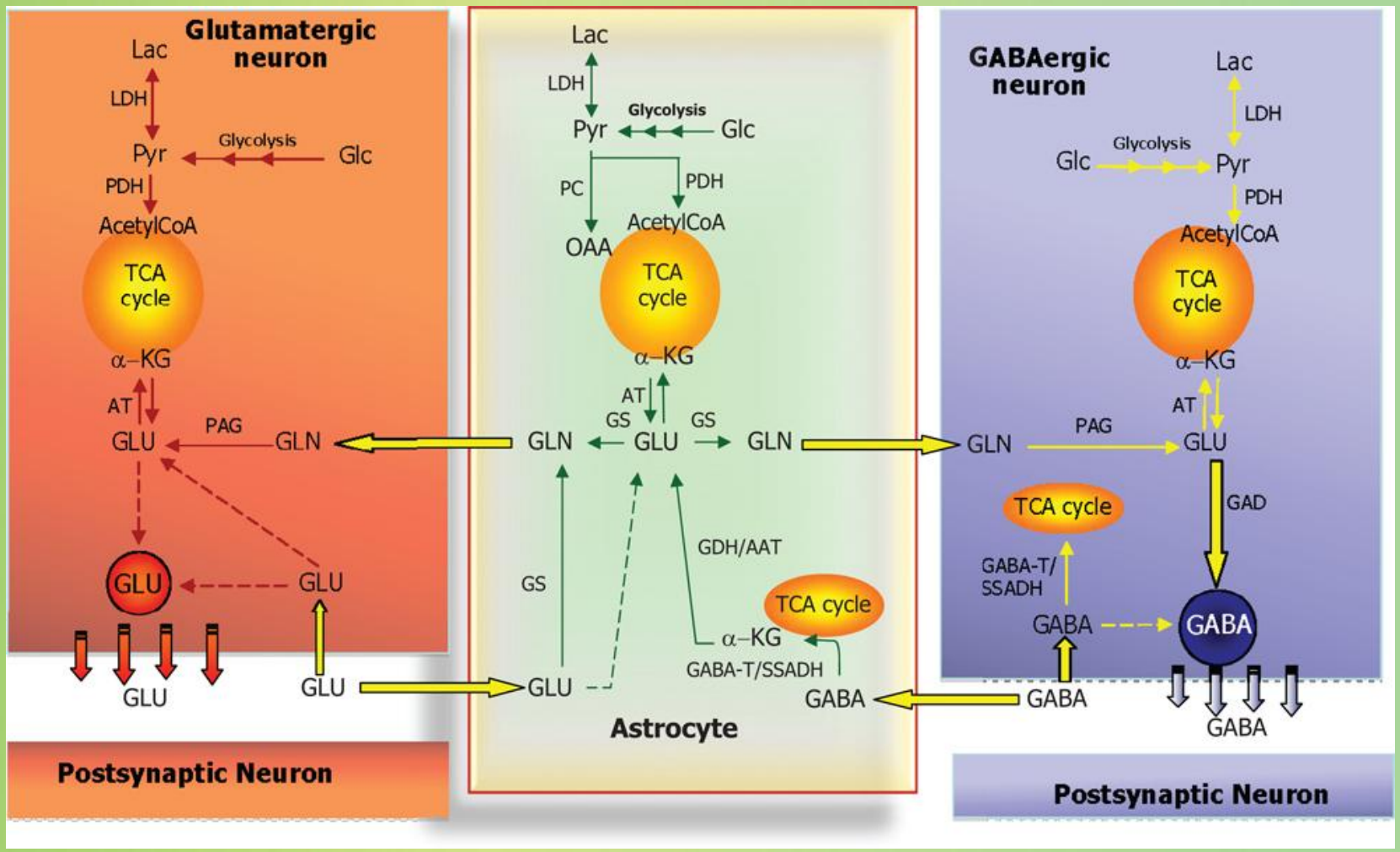
Folic acid



GABA

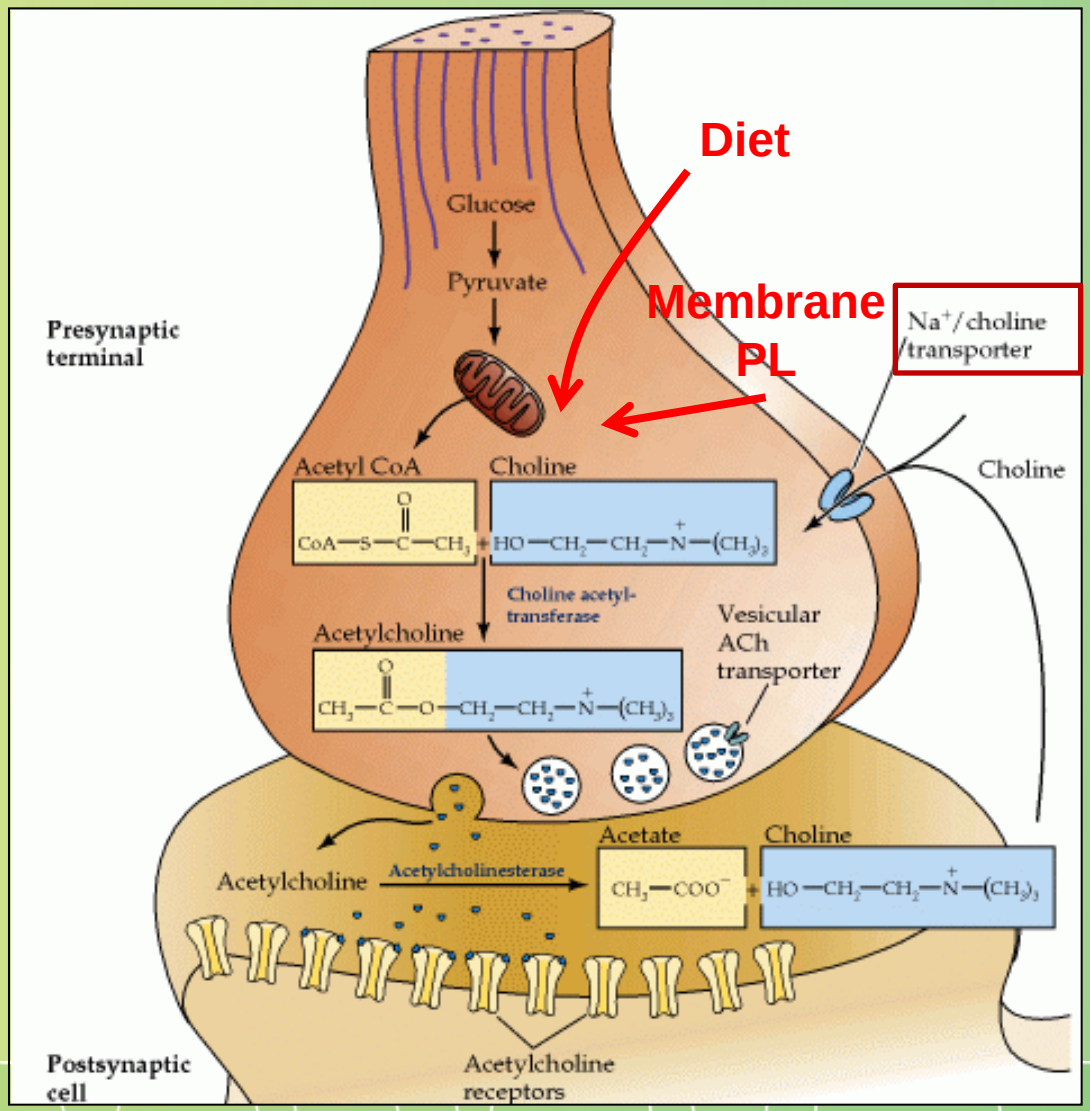
- GABA is present in high concentrations (millimolar) in many brain regions.
  - These concentrations are about 1,000 times higher than concentrations of the classical monoamine neurotransmitters in the same regions.
- The GABA shunt is a closed-loop process with the dual purpose of producing and conserving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

GABA shunt


Glutamatergic neuron
Lac
LDH
Pyr
Glycolysis
Glc
PDH
AcetylCoA
TCA cycle
α–KG
AT
GLU
PAG
GLN
GLU
GLU
GLU
Postsynaptic Neuron
Lac
LDH
Pyr
Glycolysis
Glc
PC
PDH
OAA
AcetylCoA
TCA cycle
α–KG
AT
GS
GS
GLN
GLU
GLN
GS
GDH/AAT
TCA cycle
α–KG
GABA-T/SSADH
GLU
GABA
Astrocyte
GABAergic neuron
Lac
LDH
Glc
Glycolysis
Pyr
PDH
AcetylCoA
TCA cycle
α–KG
AT
GLN
PAG
GLU
GAD
TCA cycle
GABA-T/ SSADH
GABA
GABA
GABA
Postsynaptic Neuron

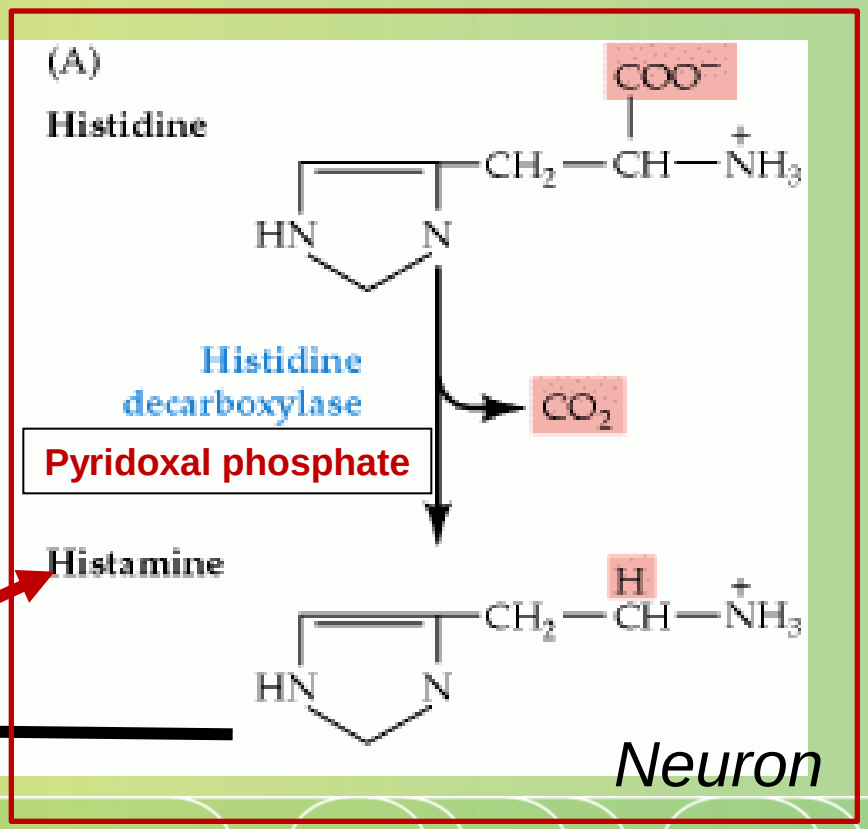
Synthesis of acetylcholine

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

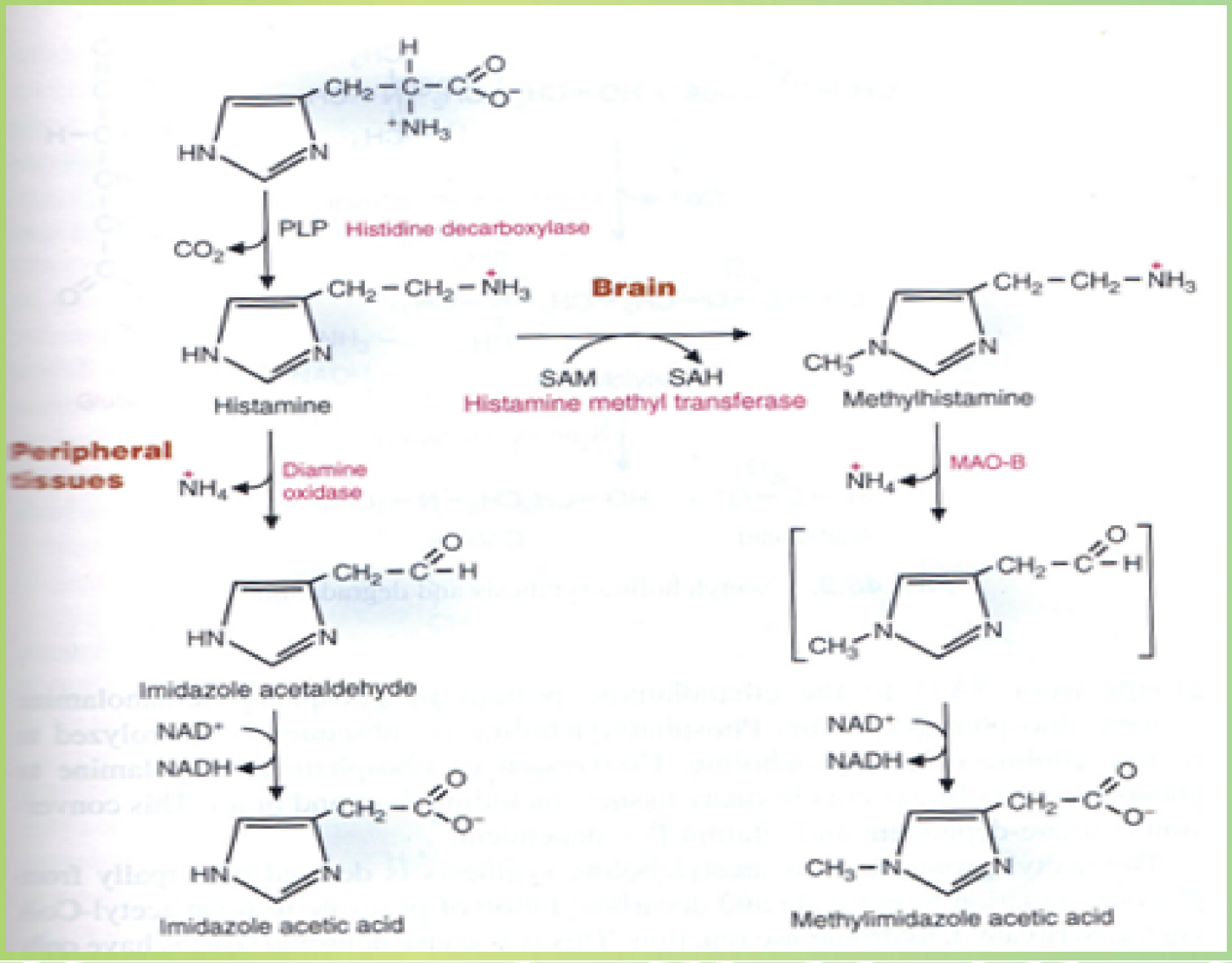


Histamine

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

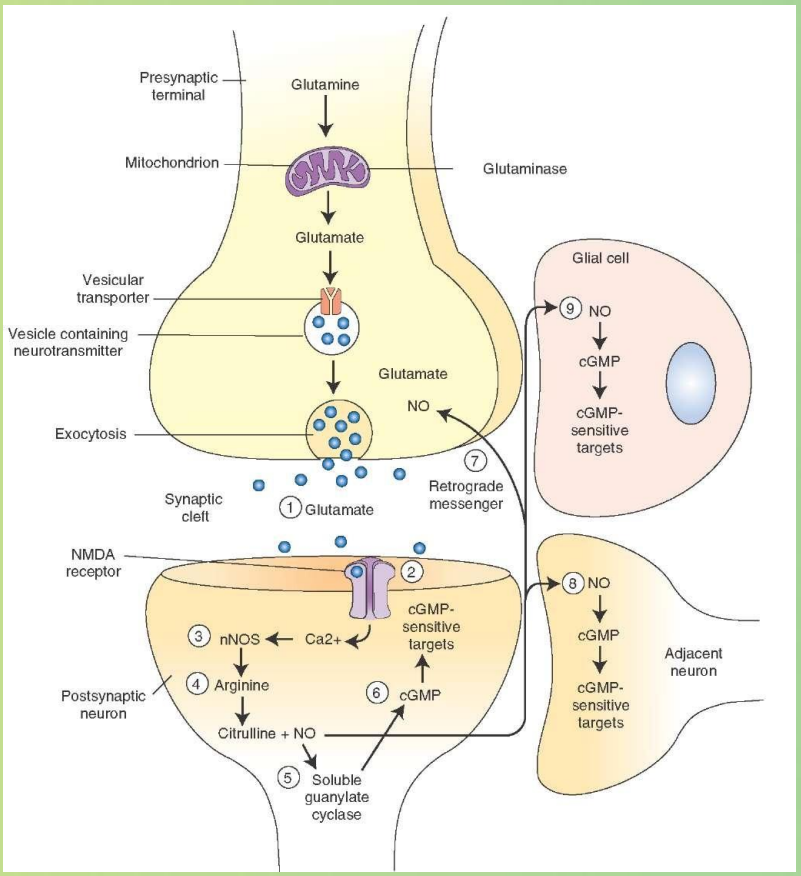


Inactivation of histamine



Nitric oxide (NO)

- Glutamate is released (1) and acts on NMDA receptors located on the post-synaptic neuron (2)
- Ca2+ enters the postsynaptic neuron and binds with calmodulin activating NOS (3) resulting in formation of NO and citrulline from L-arginine (4).
- NO stimulates guanylate cyclase forming cGMP (5), which results in a physiological response (6)
- No can diffuse out: a) to the presynaptic terminal (***retrograde messenger***) (7) prolonging effect and b) into adjacent neurons (8) and glial cells (9) stimulating guanylate cyclase.

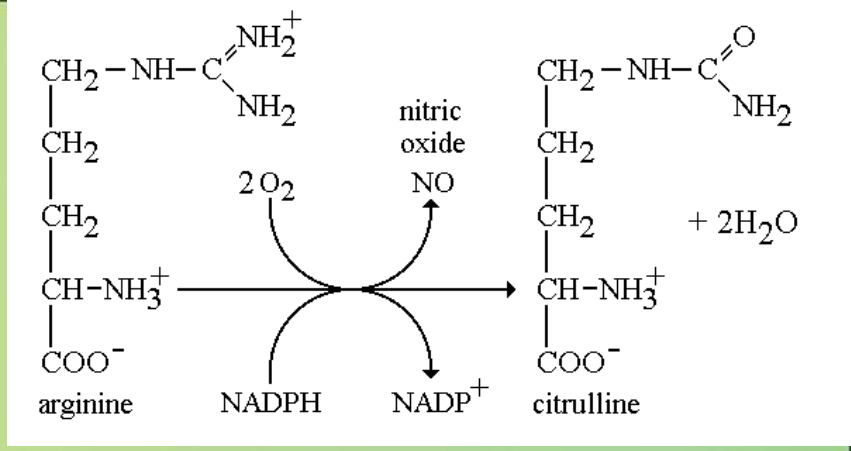


Half-life: 2-4 seconds
NO is inhibited by hemoglobin and other heme proteins which bind it tightly

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.

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