Biochemistry of neurotransmitters

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Neuroscience
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References

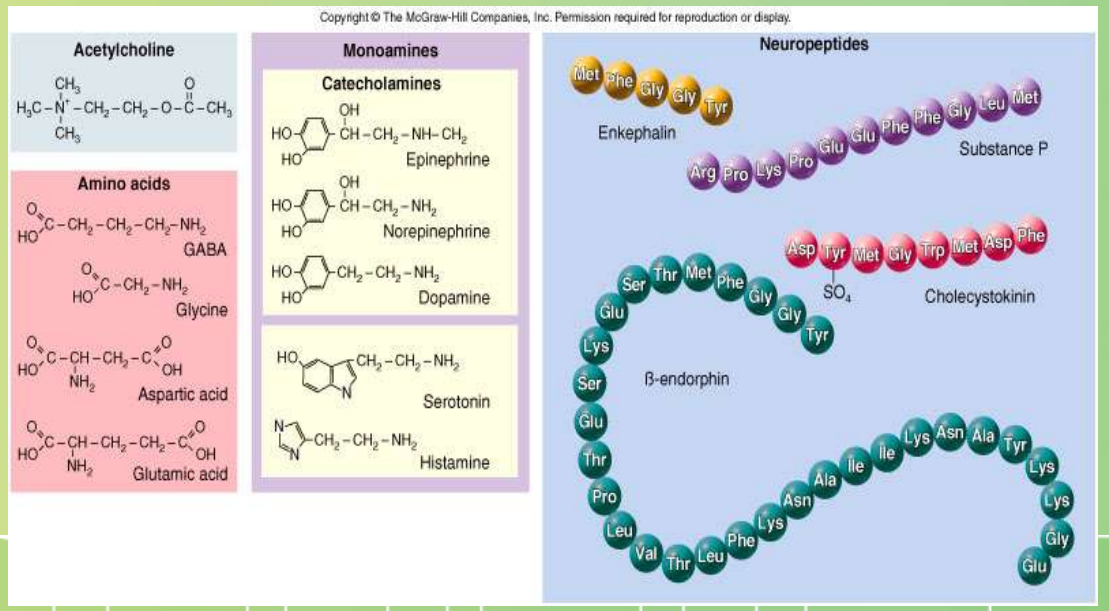
- This lecture
- Mark's Basic Medical Biochemistry, 4th ed, pp. 908-918
- http://what-when-how.com/neuroscience/neurotransmitters-the-neuron-part-1/

What is a neurotransmitter?

- **A 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)**



Note the differences

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

NEUROPEPTIDES

Introduction

- **More than 50 neuropeptides have been described**
  - **Behavior**
  - **Pain perception**
  - **Memory**
  - **Appetite**
  - **Thirst**
  - **Temperature**
  - **Homeostasis**
  - **Sleep**

Neuropeptides: neurohormones or neurotransmitters?

- **Neurohormones:** when neurons secrete their peptides into the vascular system to be transported to a relatively distant target

- **Neurotransmitter:** Many axon terminals of neurosecretory cells secrete their products at the synapse to directly affect a post synaptic cell

- Neuropeptides can do both – depends on nerve terminal

Classification of neuropeptides

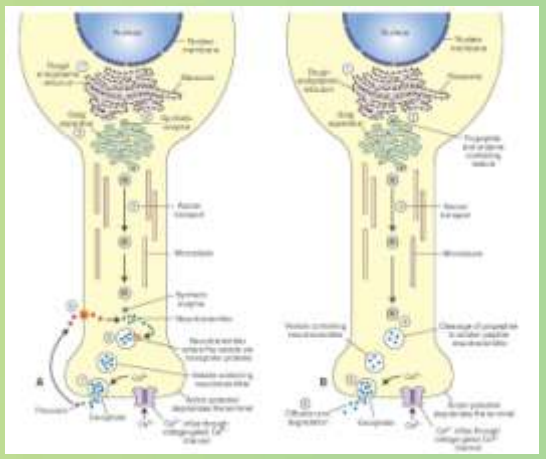
- **Neuropeptides can be grouped into families based on similarities in their amino acid sequences.**

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

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

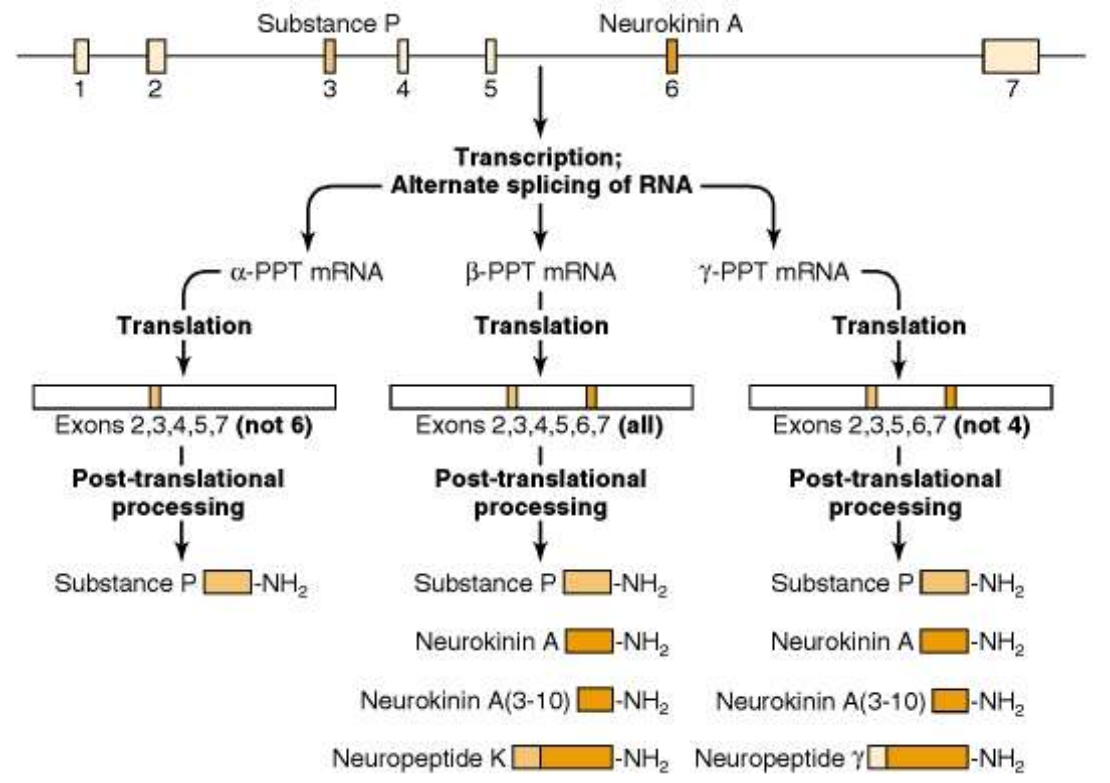
Stages of action

- 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



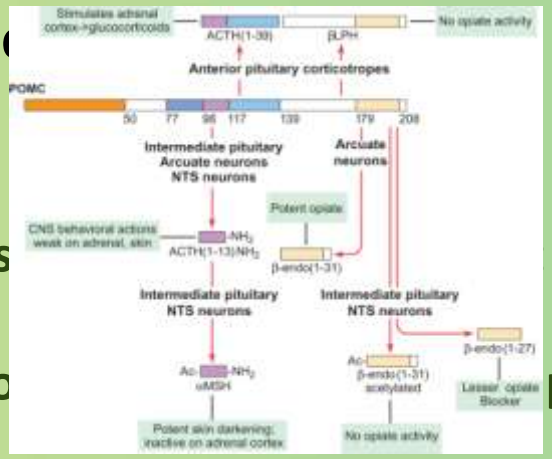
Diversity: alternative splicing

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



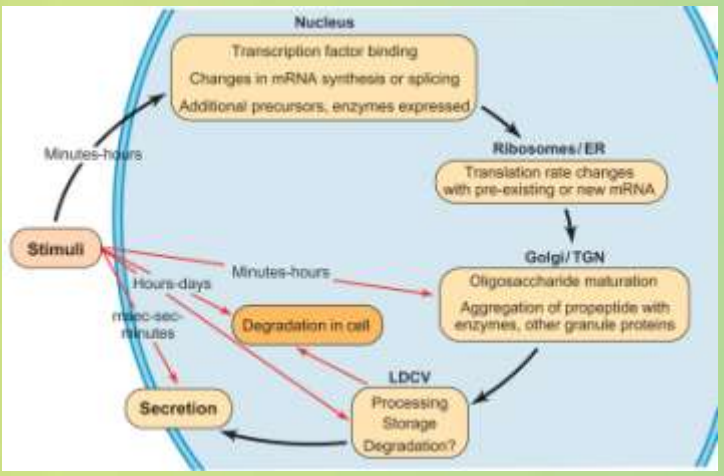
Diversity: proteolytic, differential, sequential processing

- **Neuropeptides are produced from a l protein by**
  - Proteolytic processing.
  - Vesicular packaging of different proteases cleavage sequences
  - Hiding a proteolytic site by post-translatio ple: addition of a carbohydrate side chain.)
  - Tissue-specific

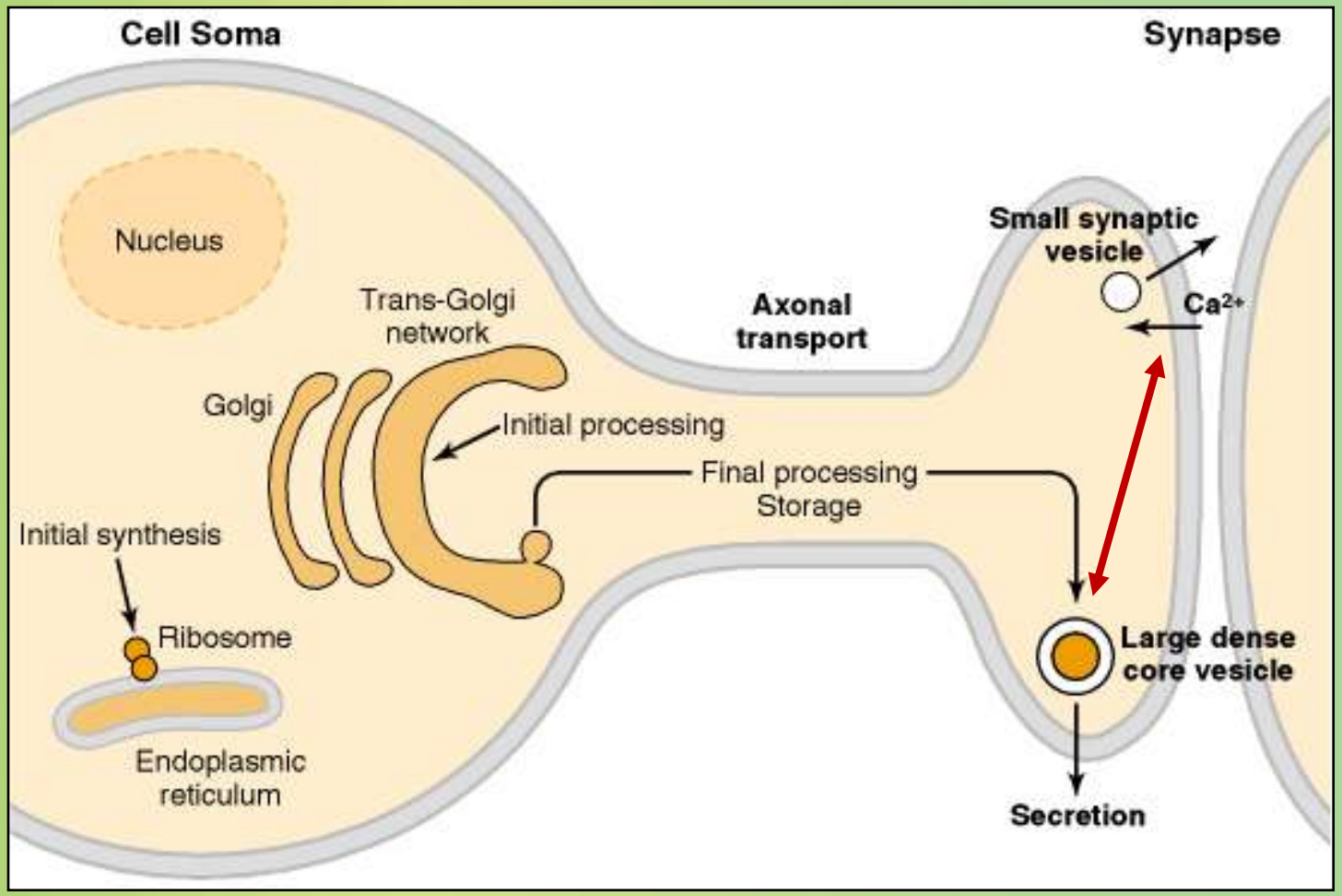


Processing of the pro-opiomelanocortin (*POMC)* precursor proceeds in an ordered, stepwise fashion. Some of the reactions are tissue specific. *ACTH,* adrenocorticotropic hormone; *CLIP,* corticotropin-like intermediate lobe peptide; *JP,* joining peptide; *LPH,* lipotropin; *MSH,* melanocyte-stimulating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

Neuropeptides

- The endogenous opiates
- Neuropeptide Y
- Galanin
- Pituitary adenylate cyclase–activating peptide (PACAP)
- Melanocyte-stimulating hormone (MSH)
- Neurokinin A (NKA)
- Substance P (SP)
- Neurotensin
- Calcitonin-gene–related protein (CGRP)
- Vasoactive intestinal polypeptide (VIP)

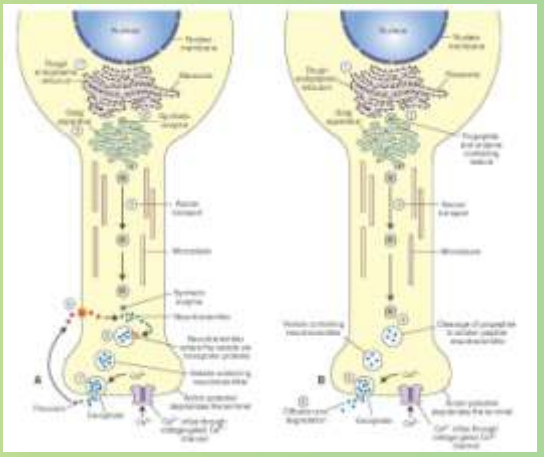
SMALL-MOLECULE NEUROTRANSMITTERS

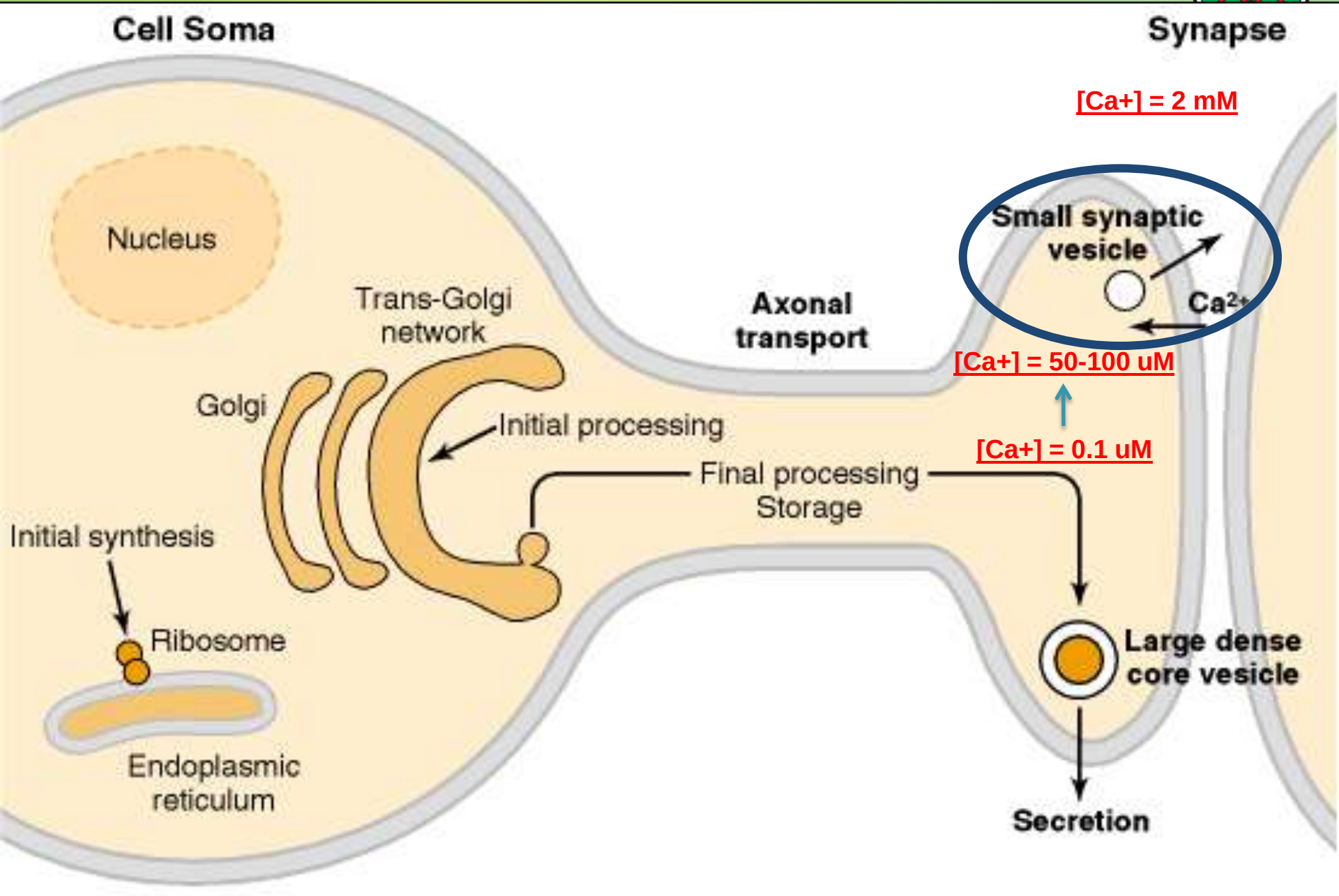
Types of small-molecule neurotransmitter

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

Stages of action

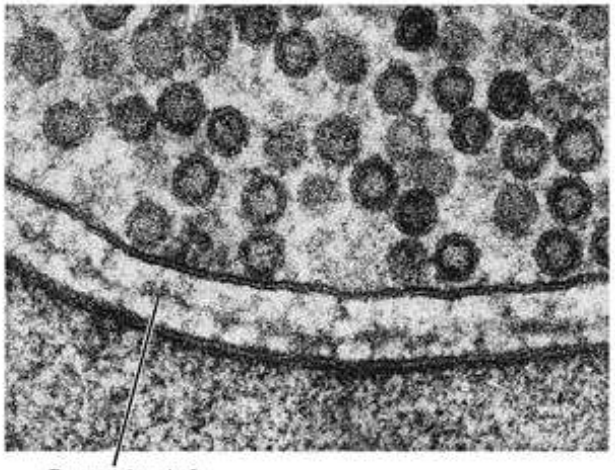
- Synthesis of enzymes
  - Cytosol
  - ER-Golgi apparatus (packaging into large-dense core vesicles)
- Transport of enzymes (slow and fast-axonal transport)
- Synthesis in pre-synaptic terminal
- Packaging in synaptic vesicles
- Release
  - They are released in brief pulses each time an action potential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



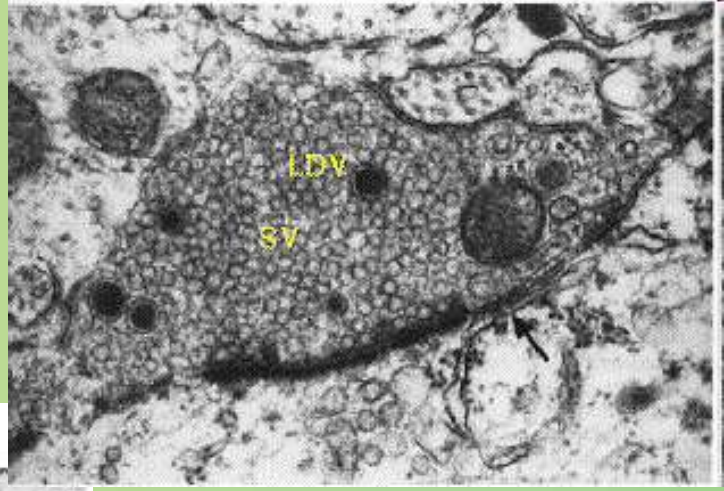

Cell Soma
Synapse
[Ca+] = 2 mM
Nucleus
Small synaptic vesicle
Ca2+
Trans-Golgi network
Axonal transport
[Ca+] = 50-100 uM
Golgi
Initial processing
[Ca+] = 0.1 uM
Final processing
Storage
Initial synthesis
Ribosome
Large dense core vesicle
Endoplasmic reticulum
Secretion

Presynaptic membrane (thin section)

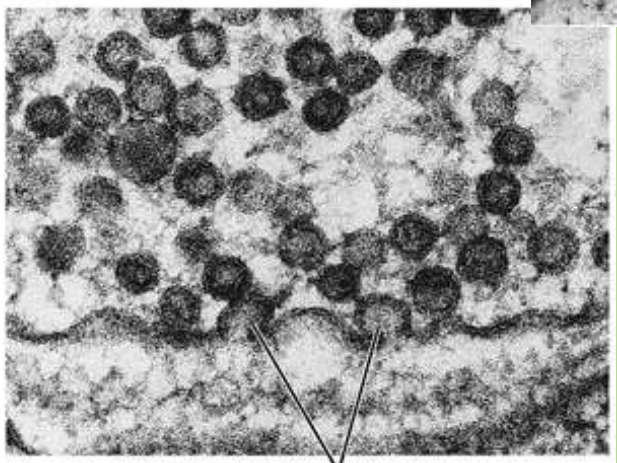
A_2



Synaptic cleft

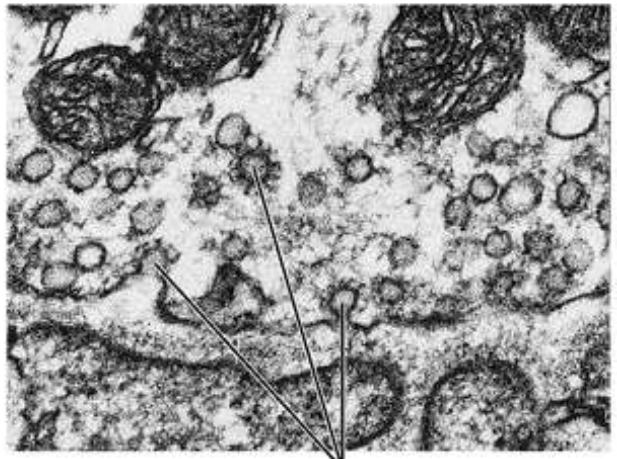


B_2



Vesicle fusions

C_2



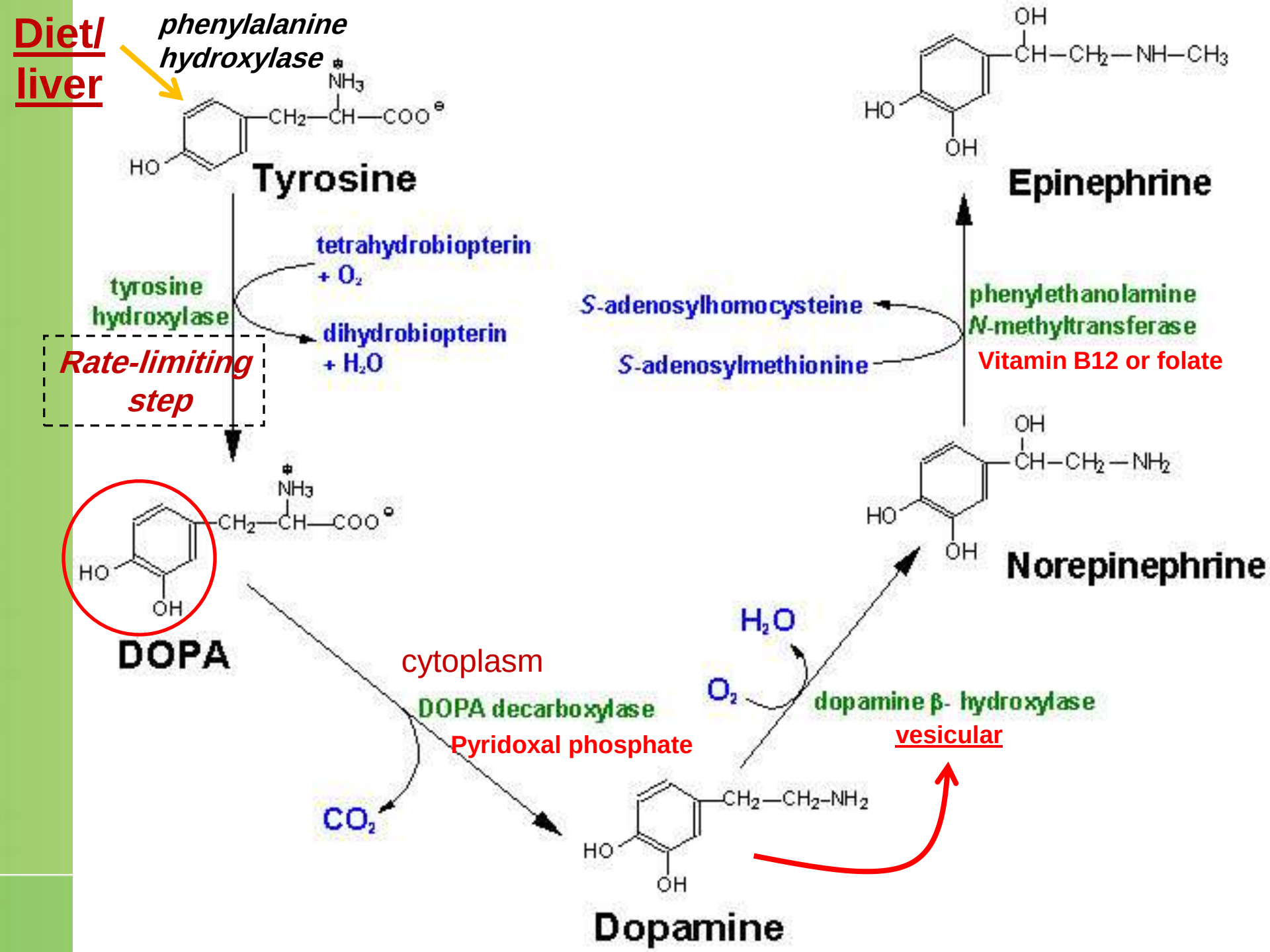
Coated vesicles

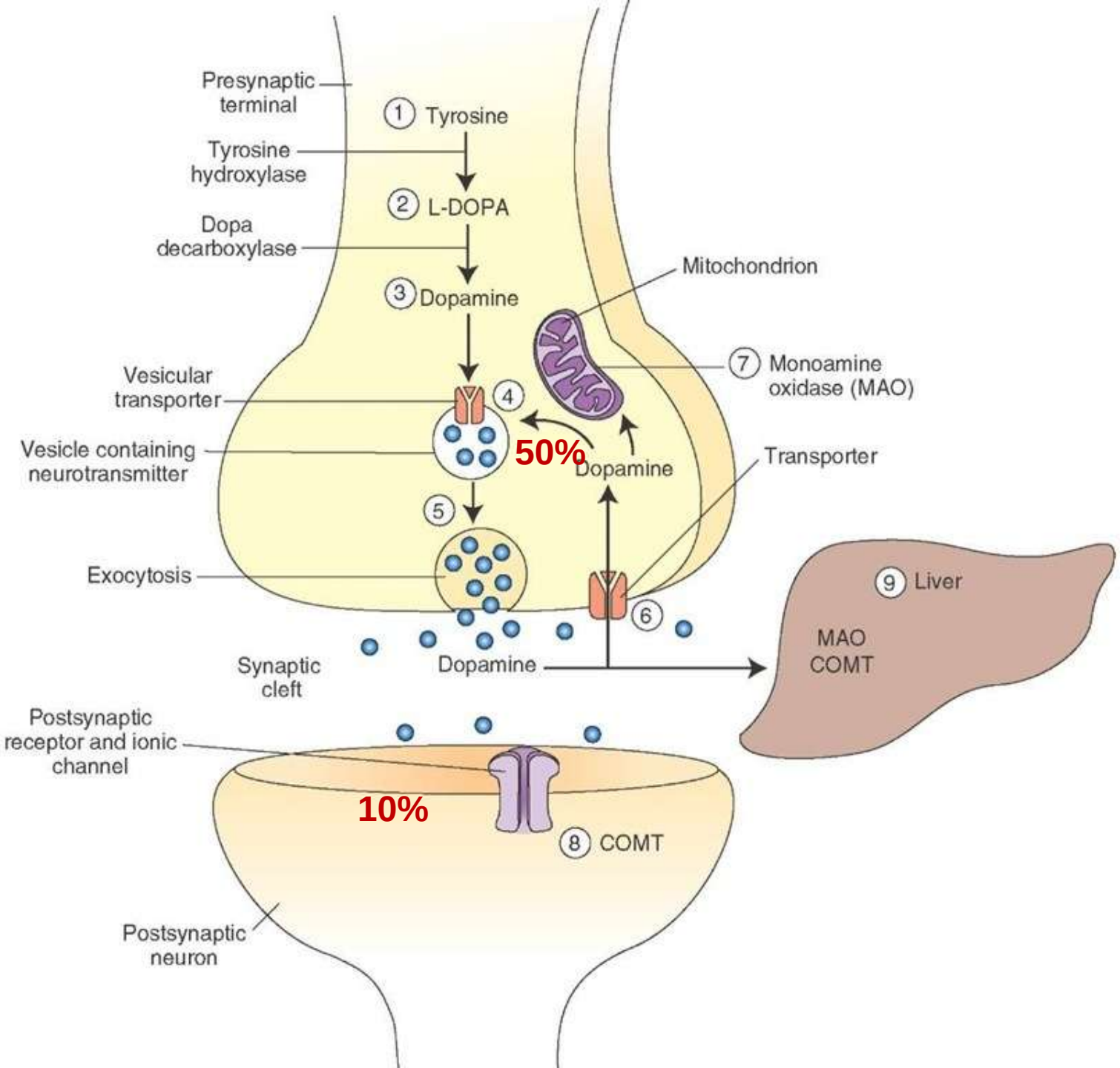
Notes

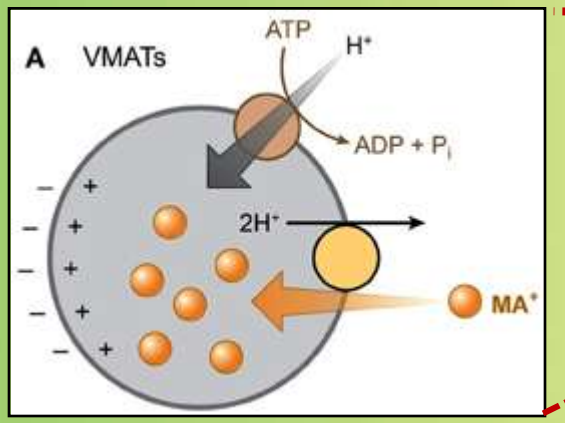
- **Role of cofactors**
  - **S-adenosylmethionine (methyl transfer)**
  - **Pyrodoxal phosphate (vitamin B6): transamination, decarboxylation**
  - **Tetrahydrobiopterin (BH4)**

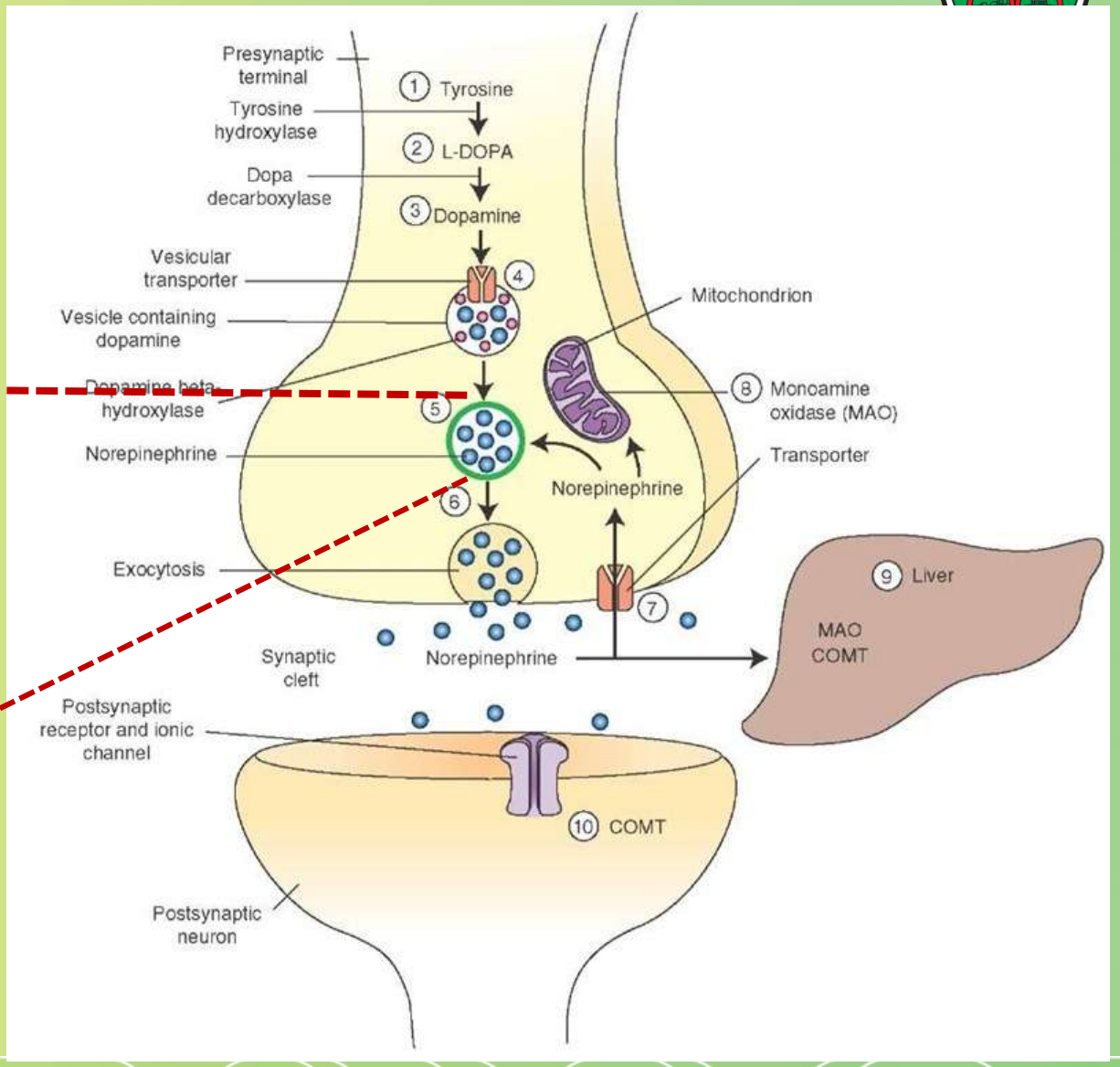
TYROSINE-DERIVED NEUROTRANSMITTERS

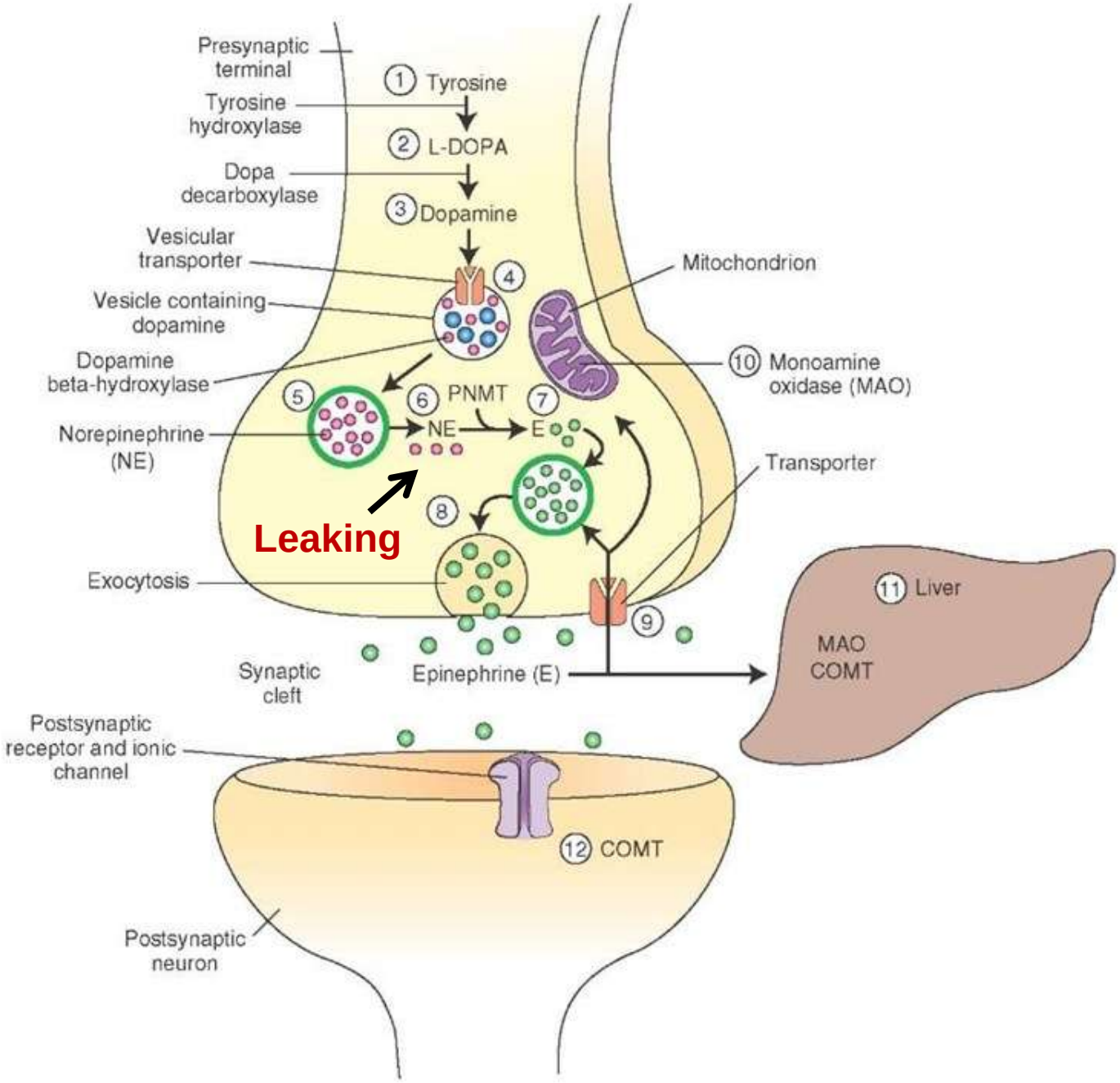
Dopamine, norepinephrine, and epinephrine


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

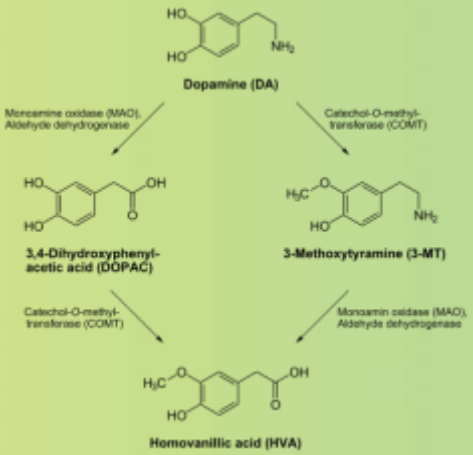

Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Mitochondrion
7 Monoamine oxidase (MAO)
Vesicular transporter
4
Vesicle containing neurotransmitter
50%
Dopamine
Transporter
5
Exocytosis
9 Liver
MAO
COMT
6
Synaptic cleft
Dopamine
Postsynaptic receptor and ionic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


A VMATs
ATP
H+
ADP + Pi
2H+
MA+


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

COMT and MAO



Inactivation is dependent on SAM and vitamin B12 and folate

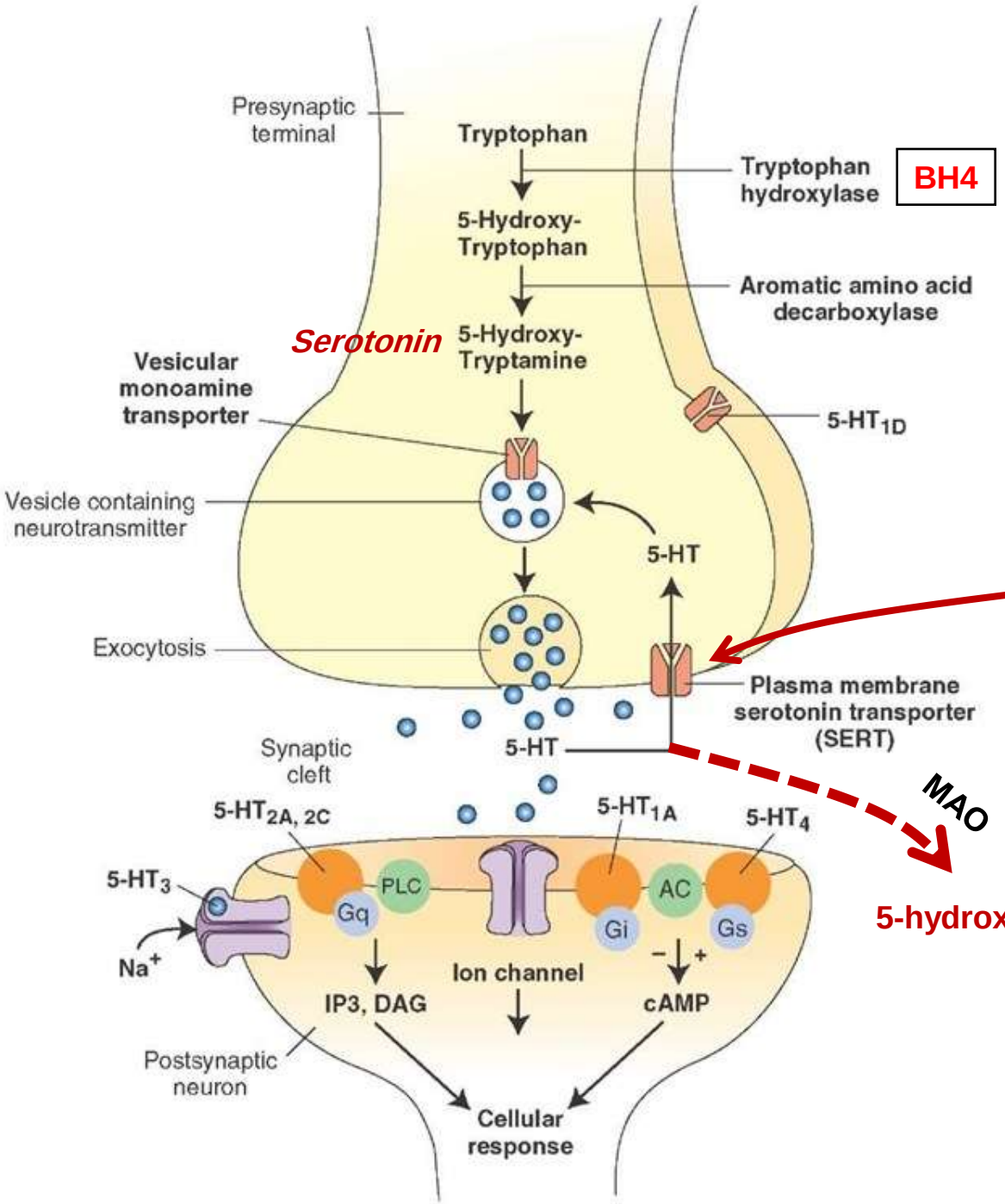
Parkinson's disease

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



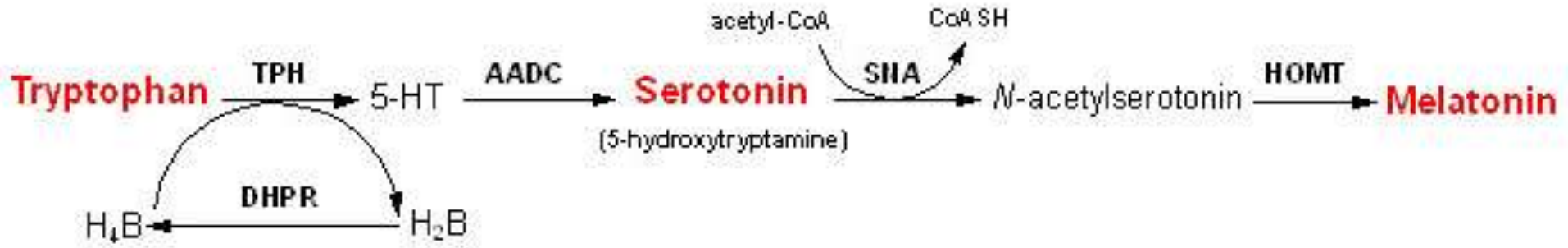
Antidepressants , called selective serotonin re-uptake inhibitors (SSRIs), like Prozac® inhibit the reuptake process resulting in prolonged serotonin presence in the synaptic cleft.

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

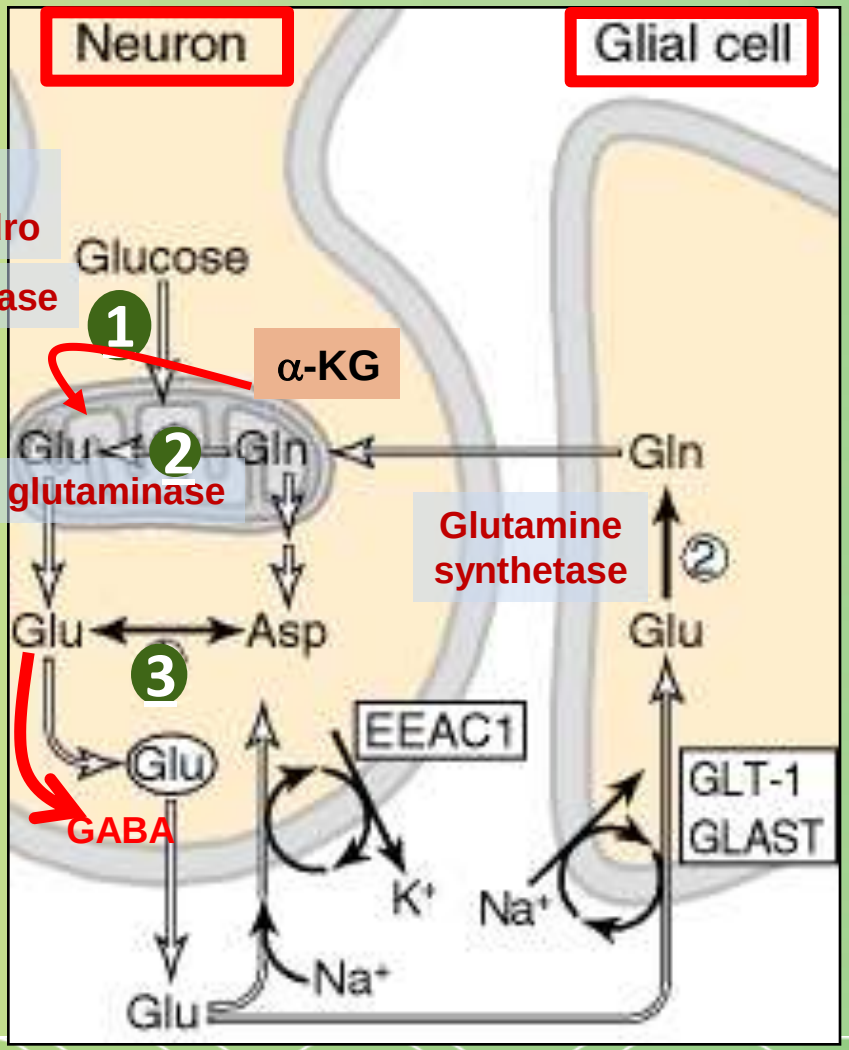
Glutamate and aspartate

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

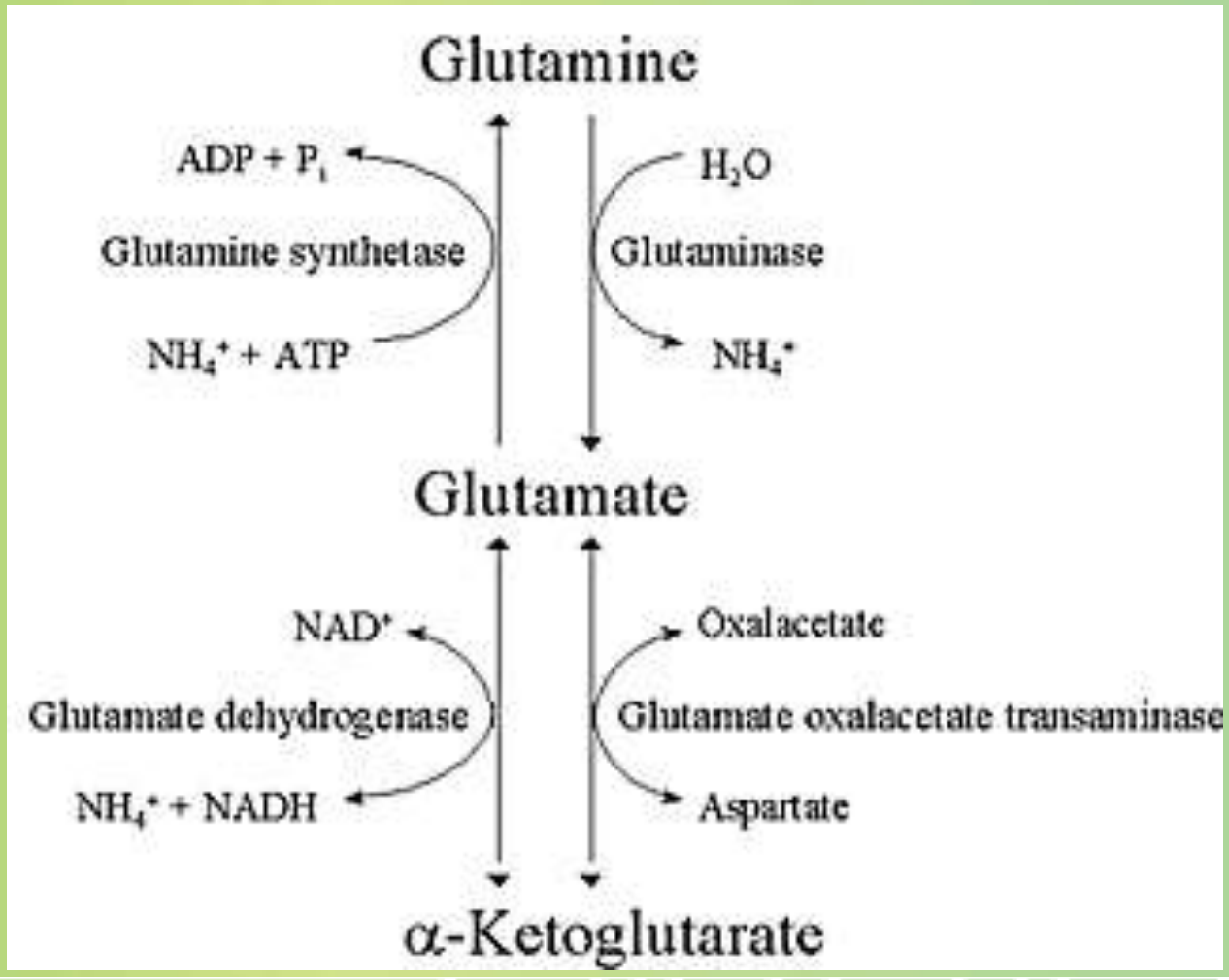
Synthesis of glutamate

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)*



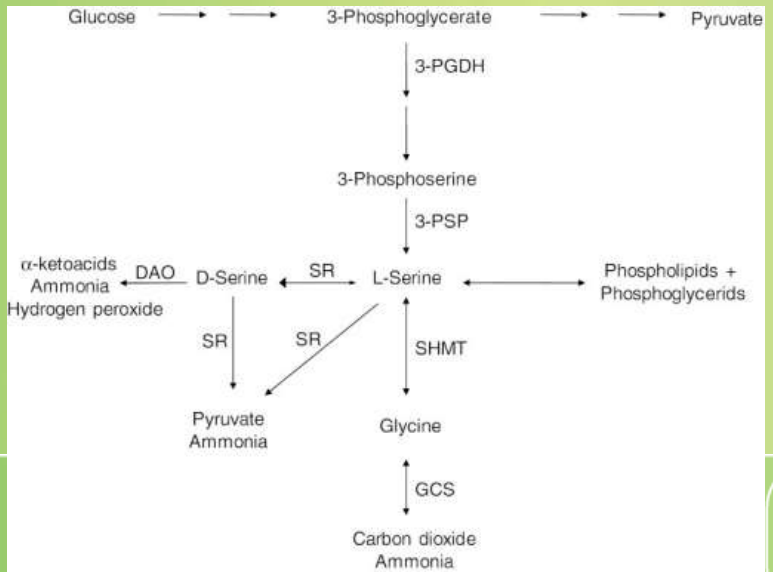
Aspartate

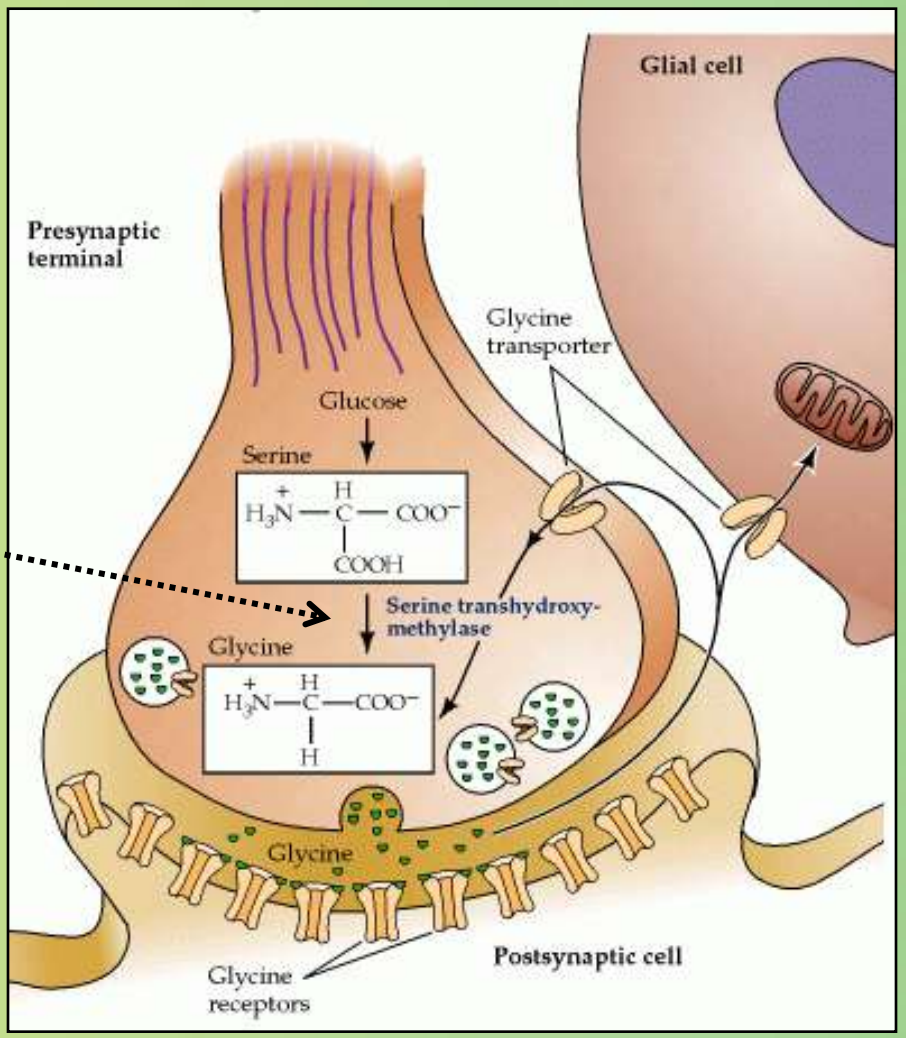
- A vesicular uptake mechanism for aspartate has not yet been demonstrated, somewhat weakening the case for considering aspartate to be a neurotransmitter
- Precursor: oxaloacetate (transamination)

Glycine

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

Folic acid



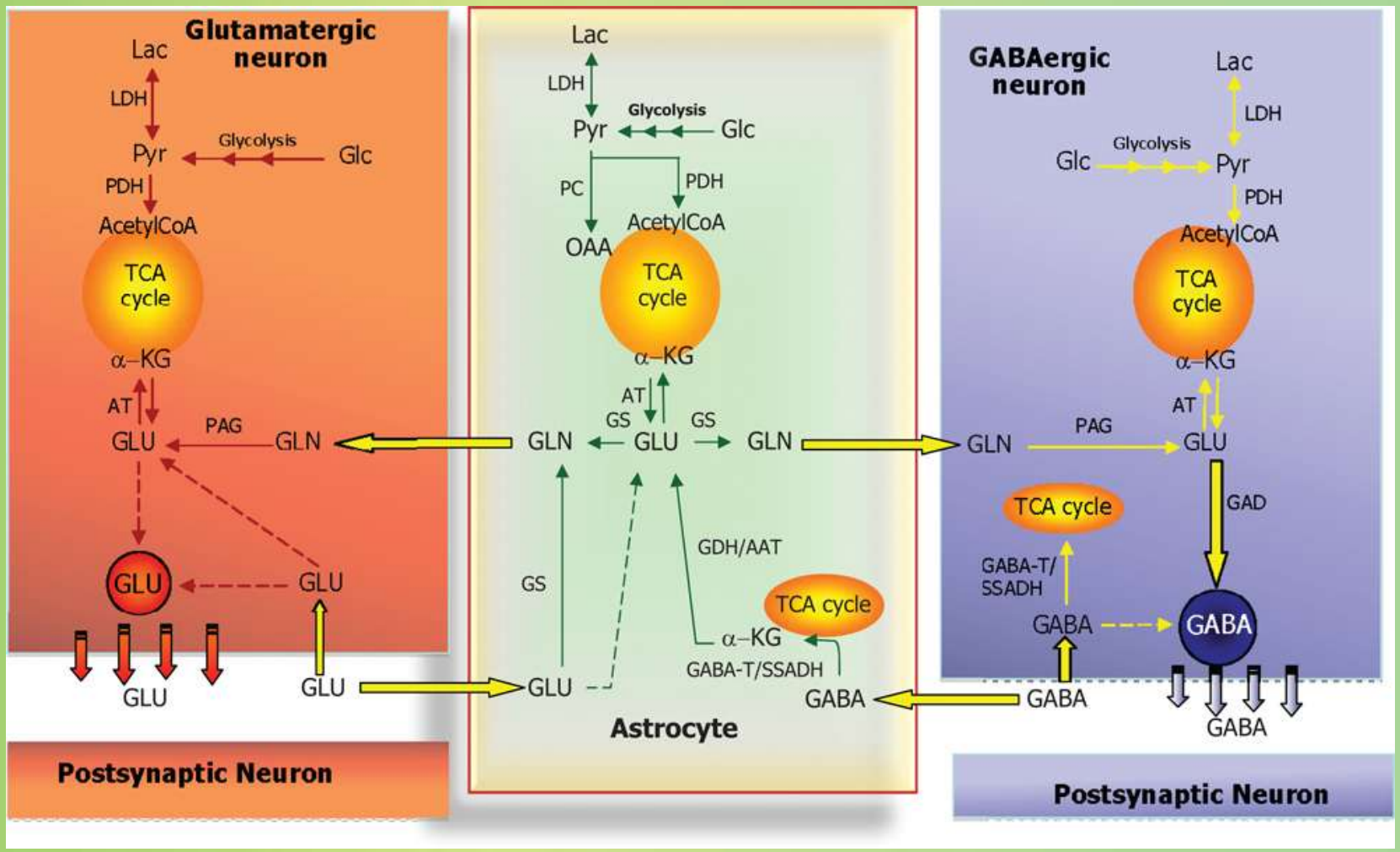


OTHERS

GABA

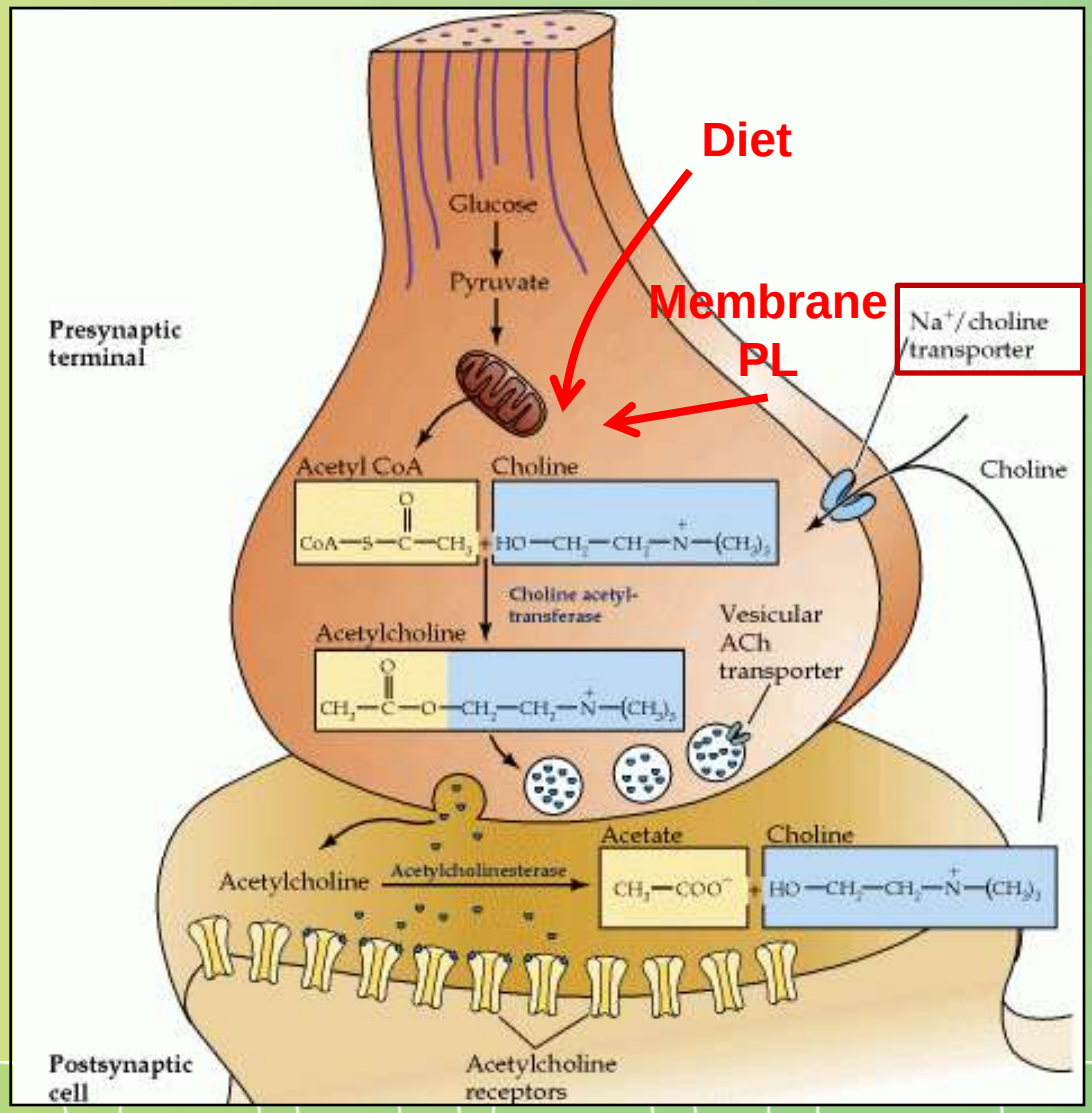
- GABA is present in high concentrations (millimolar) in many brain regions.
  - These concentrations are about 1,000 times higher than concentrations of the classical monoamine neurotransmitters in the same regions.
- The GABA shunt is a closed-loop process with the dual purpose of producing and conserving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

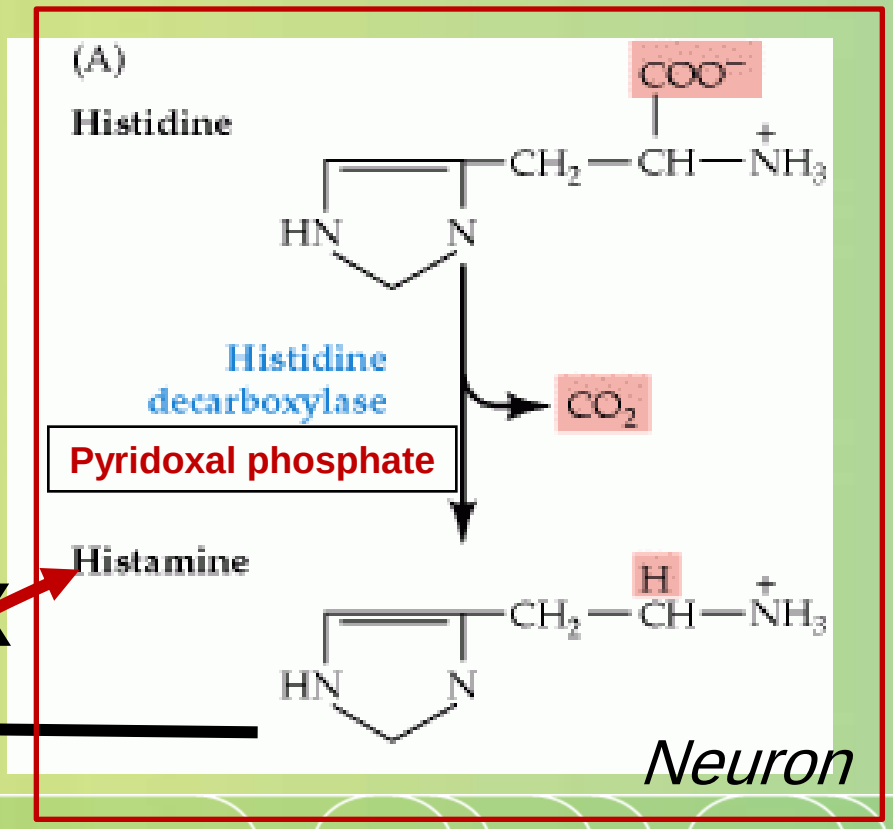
Synthesis of acetylcholine

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

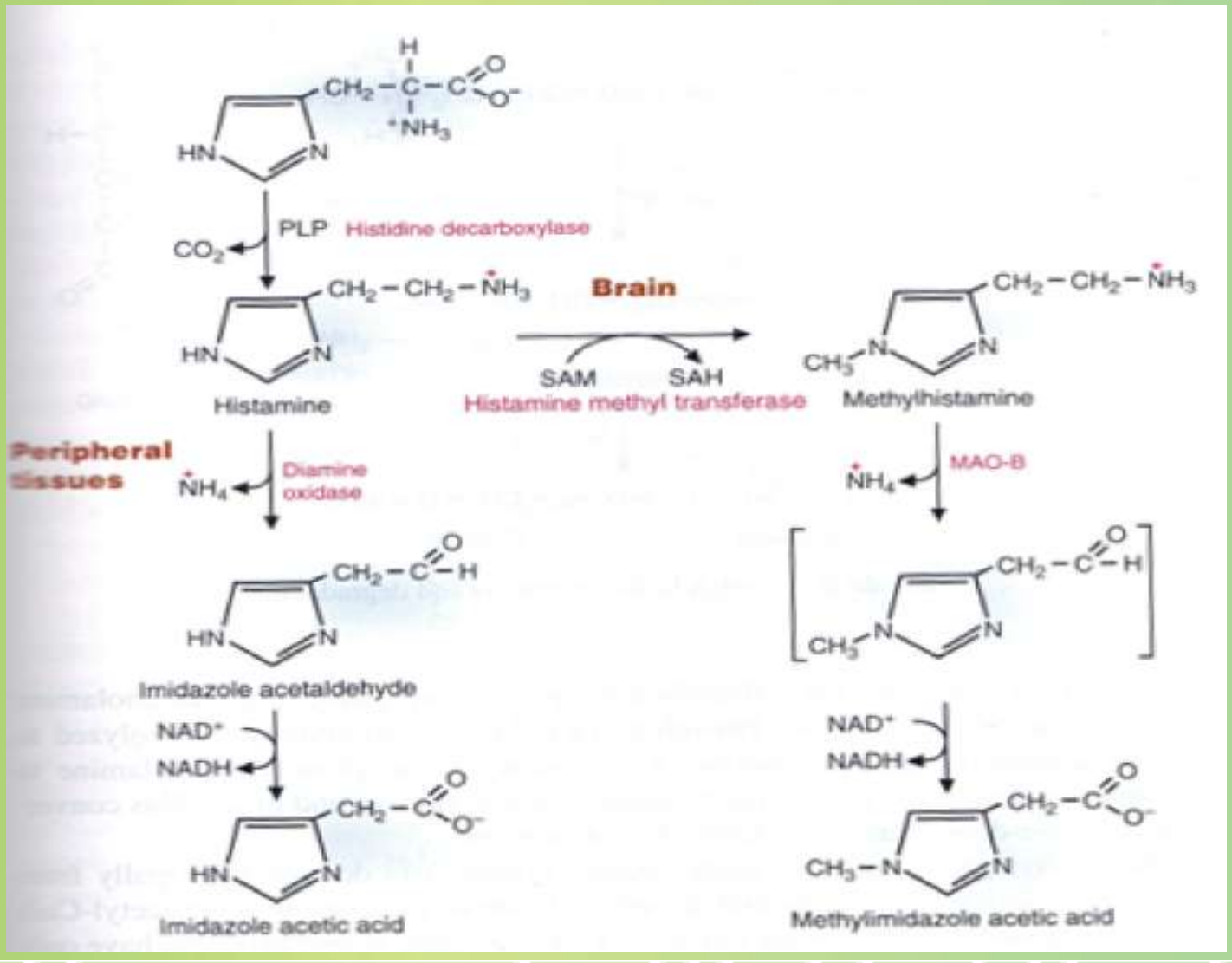


Histamine

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

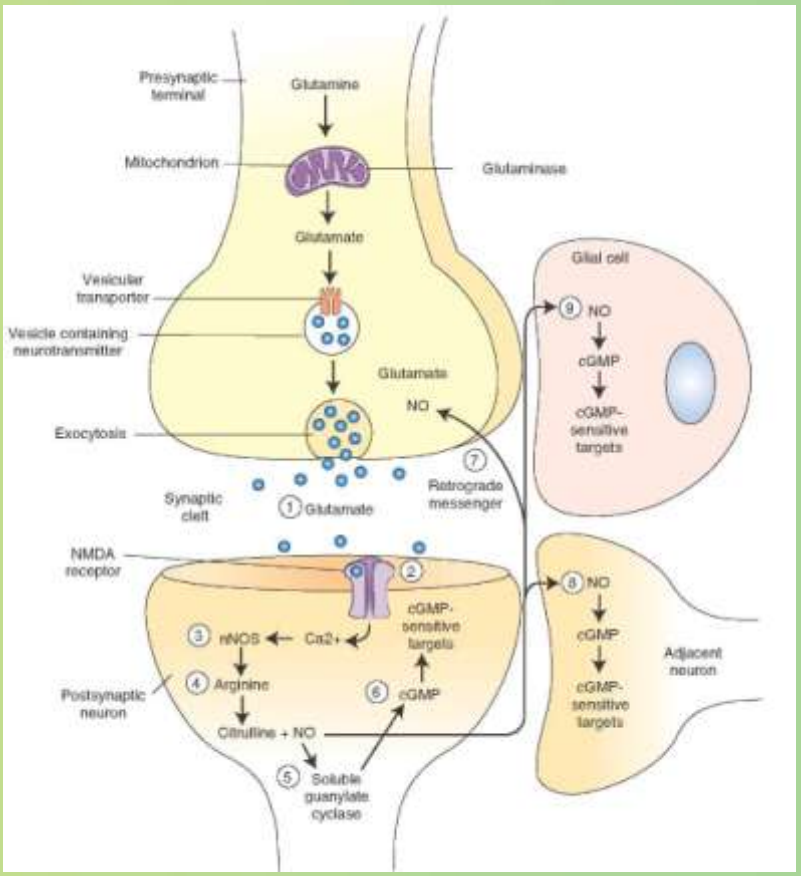


Inactivation of histamine



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.

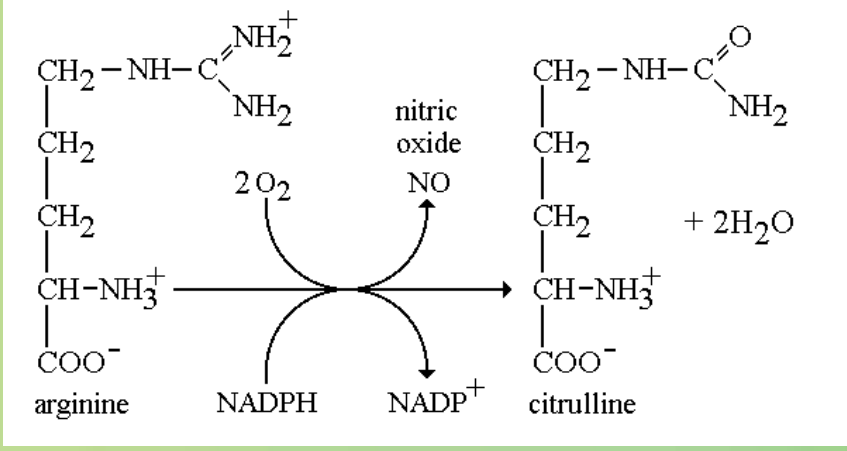


Half-life: 2-4 seconds
NO is inhibited by hemoglobin and other heme proteins which bind it tightly

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