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/

Definition of a neurotransmitter

- **A neurotransmitter is 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.**

Characteristics of a neurotransmitter

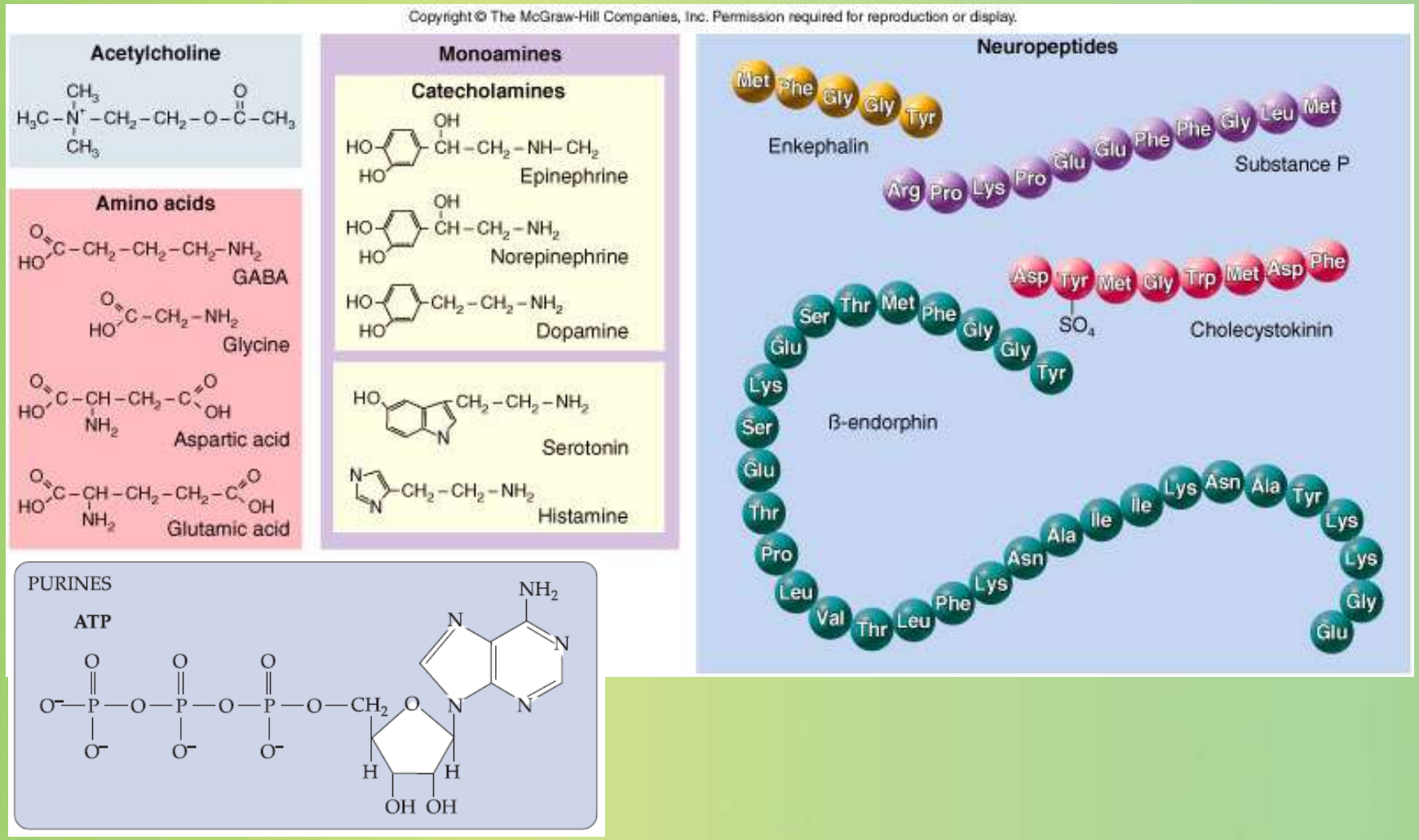
- **A chemical substance that:**
  - **Is synthesized and stored in a presynaptic neuron (the enzymes needed for its synthesis must be present in the neuron),**
  - **Is released at a synapse following depolarization of the nerve terminal (usually dependent on influx of calcium ions),**
  - **binds to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

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

Two or more transmitters (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).

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:** when released by neurons into the haemolymph and exert its effects on distant peripheral targets.
- **Neurotransmitter:** when released from a neuron at a specialized junction and diffuses across a narrow cleft to affect one or two postsynaptic neurons, a muscle cell, or another effector cell.

Classification of neuropeptides

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

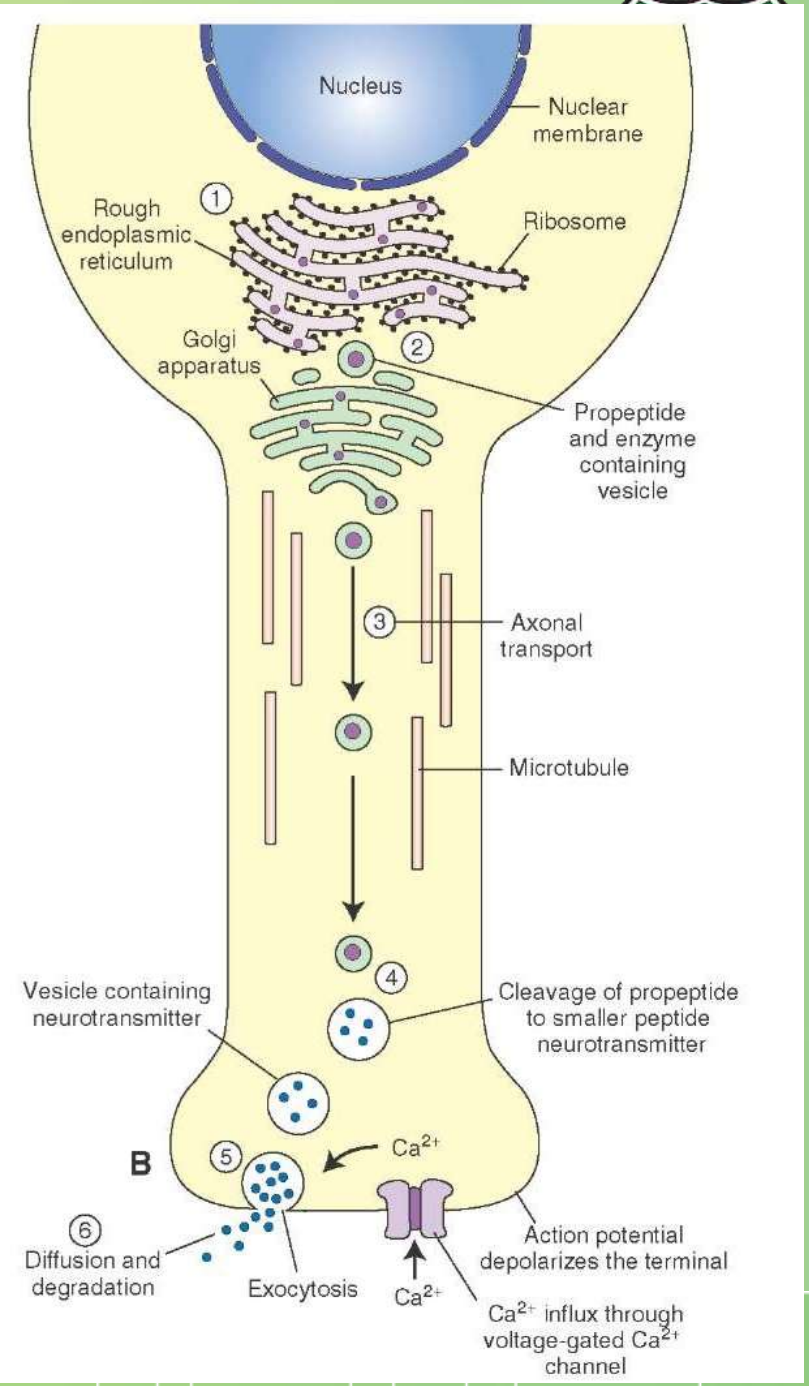
Neuropeptide Families
Tachykinins: substance P, bombesin, substance K **Insulins**: insulin, insulin-like growth factors **Somatostatins**: somatostatin, pancreatic polypeptide **Gastrins**: gastrin, cholecystokinin **Opioids**: opiocortins, enkephalins, dynorphin

- 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

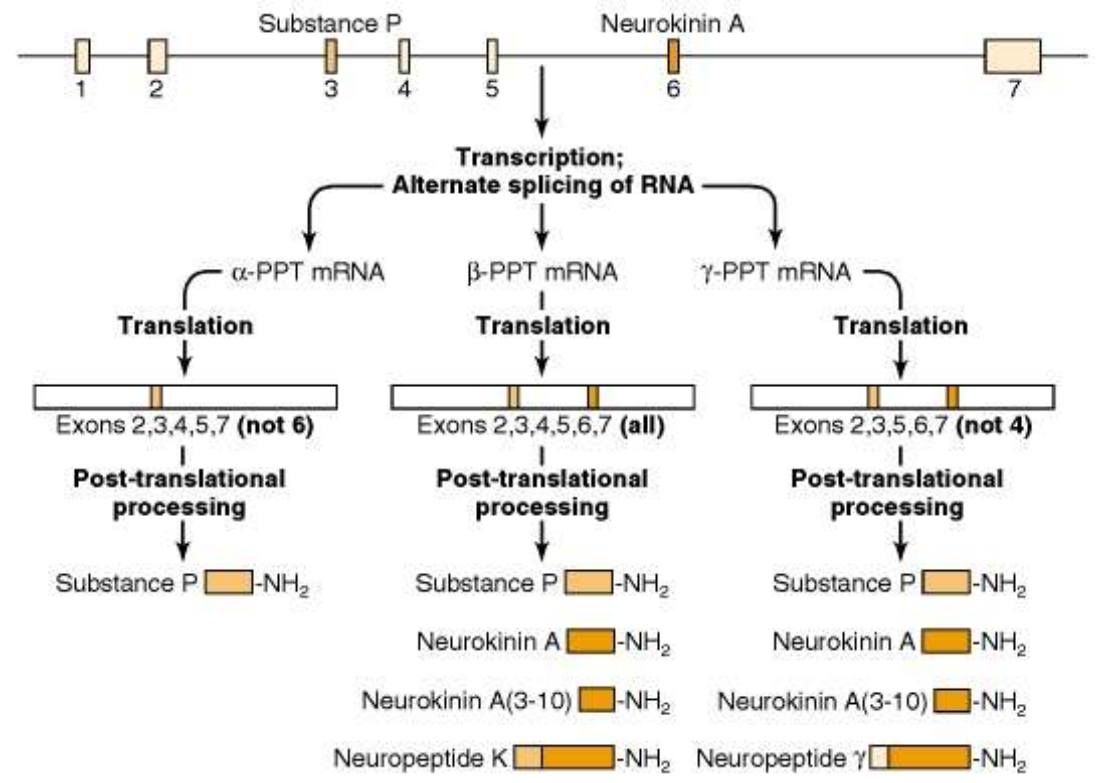
Stages of action

- 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



Diversity: alternative splicing

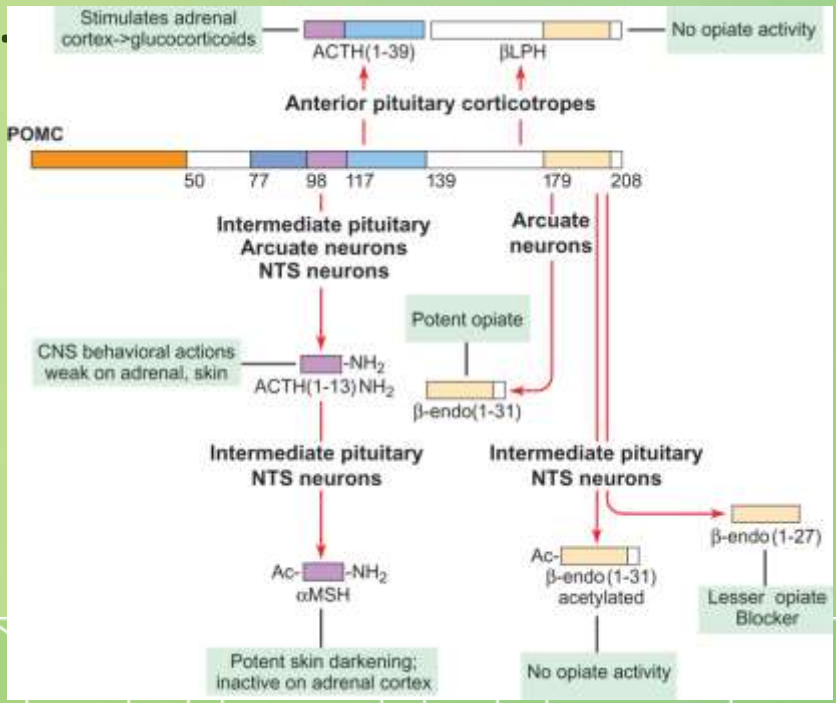
- **Alternative splicing of mRNA leads to translation of distinct precursors, and subsequent processing leads to unique mature peptides.**
  - Example is the substance P mRNA that normally also includes mRNA encoding substance K.



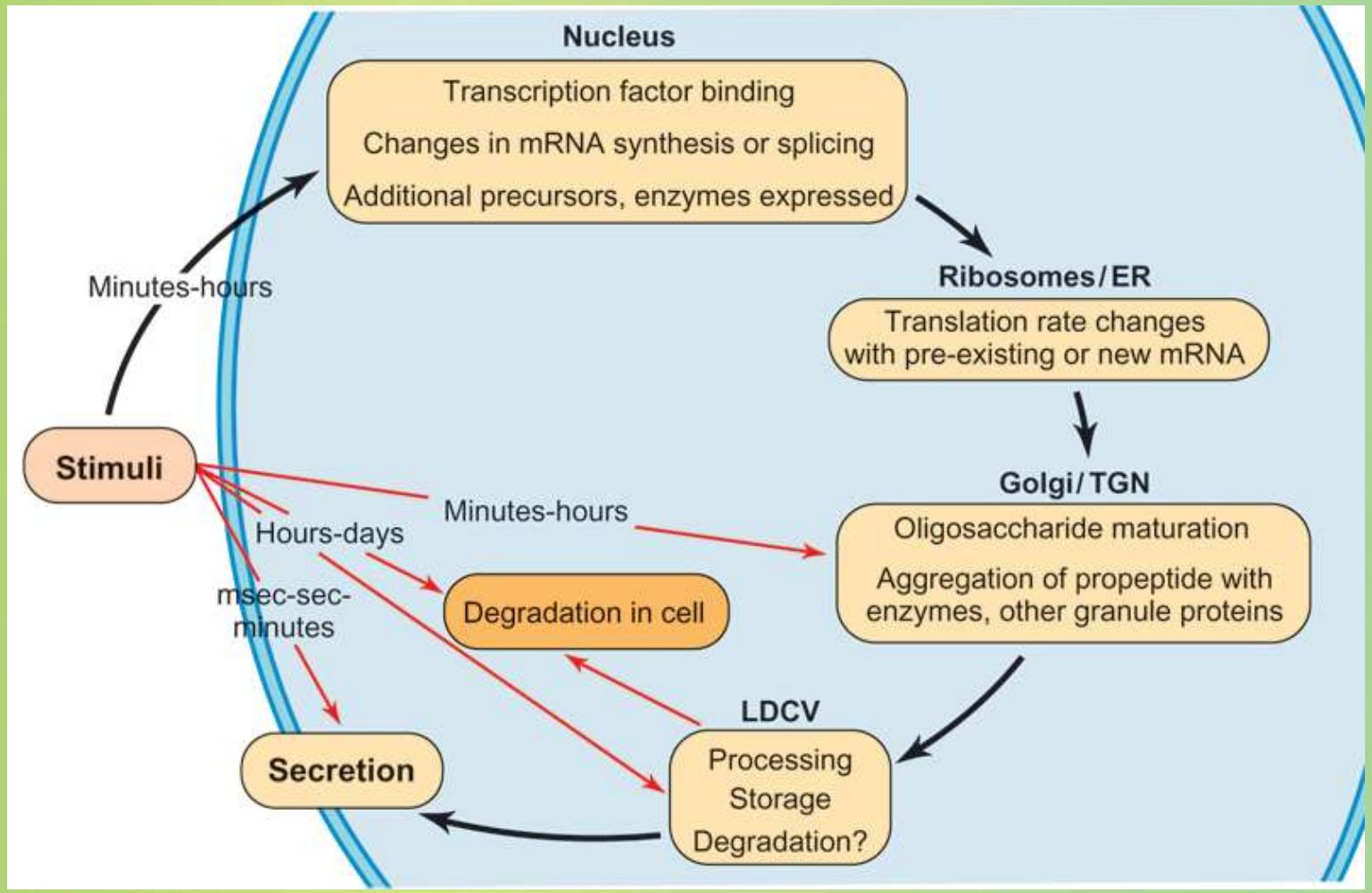
Diversity: proteolytic, differential, sequential processing

- **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

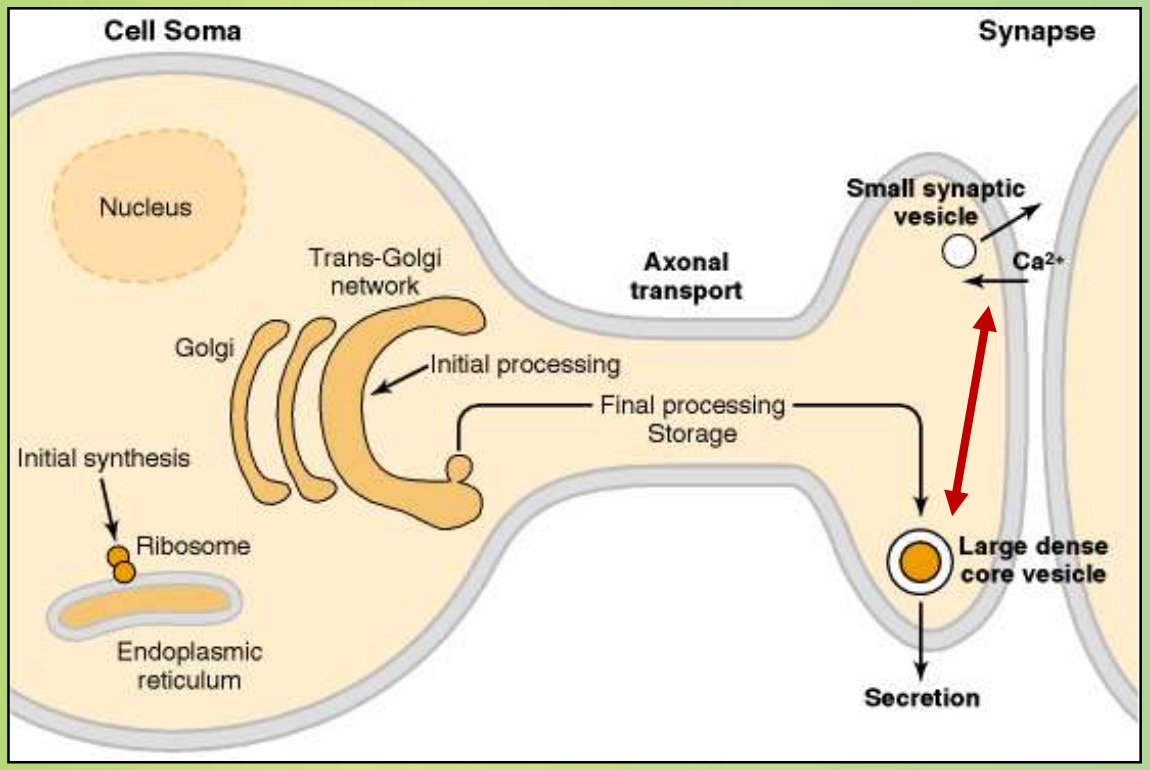
Processing of the pro-opiomelanocortin (*POMC)* precursor proceeds in an ordered, stepwise fashion. Some of the reactions are tissue specific. *ACTH,* adrenocorticotropic hormone; *CLIP,* corticotropin-like intermediate lobe peptide; *JP,* joining peptide; *LPH,* lipotropin; *MSH,* melanocyte-stimulating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



The levels of regulation of neuropeptide expression



Role of Ca^{2+} ions



- Vesicles are located further away from the presynaptic membrane and away from 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 neurotrasmitters.

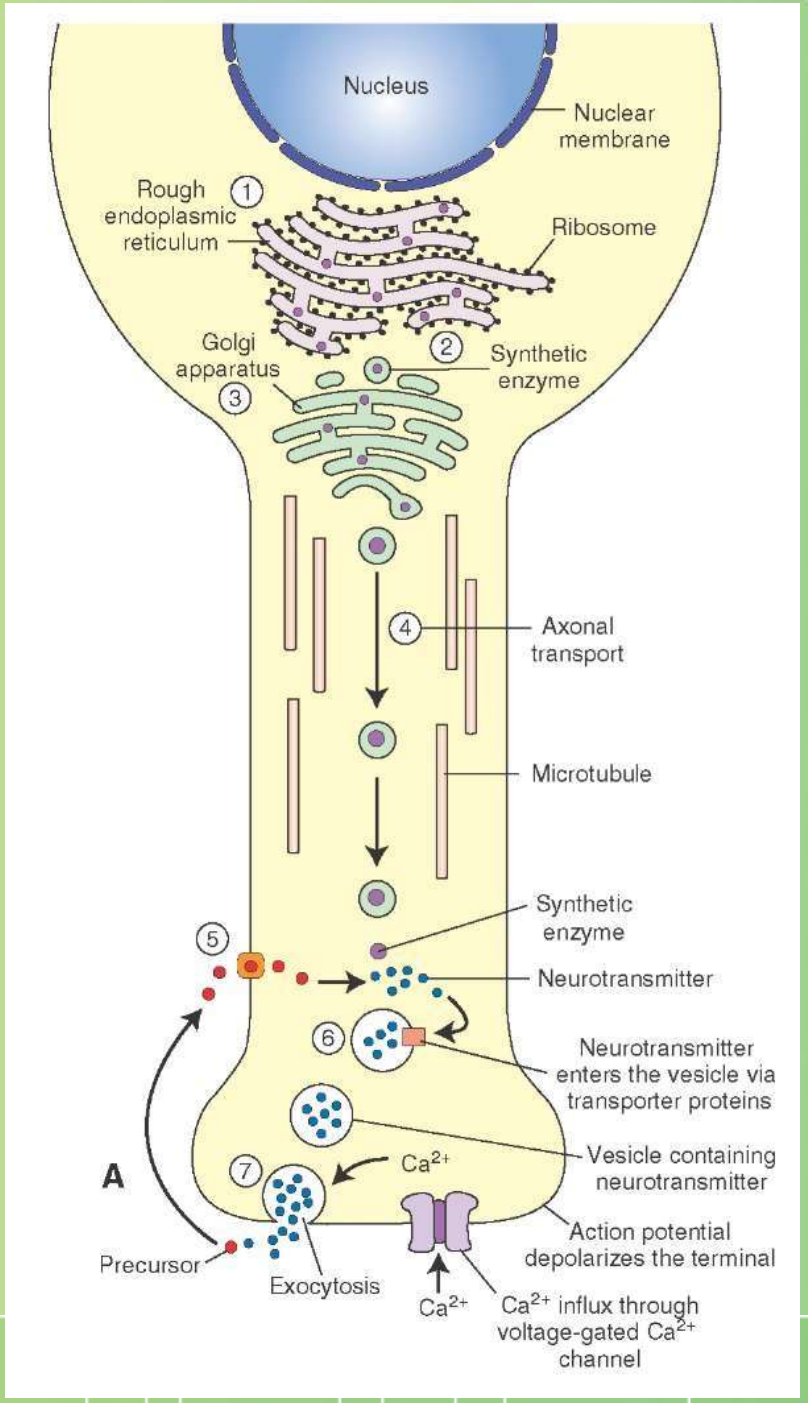
SMALL-MOLECULE NEUROTRANSMITTERS

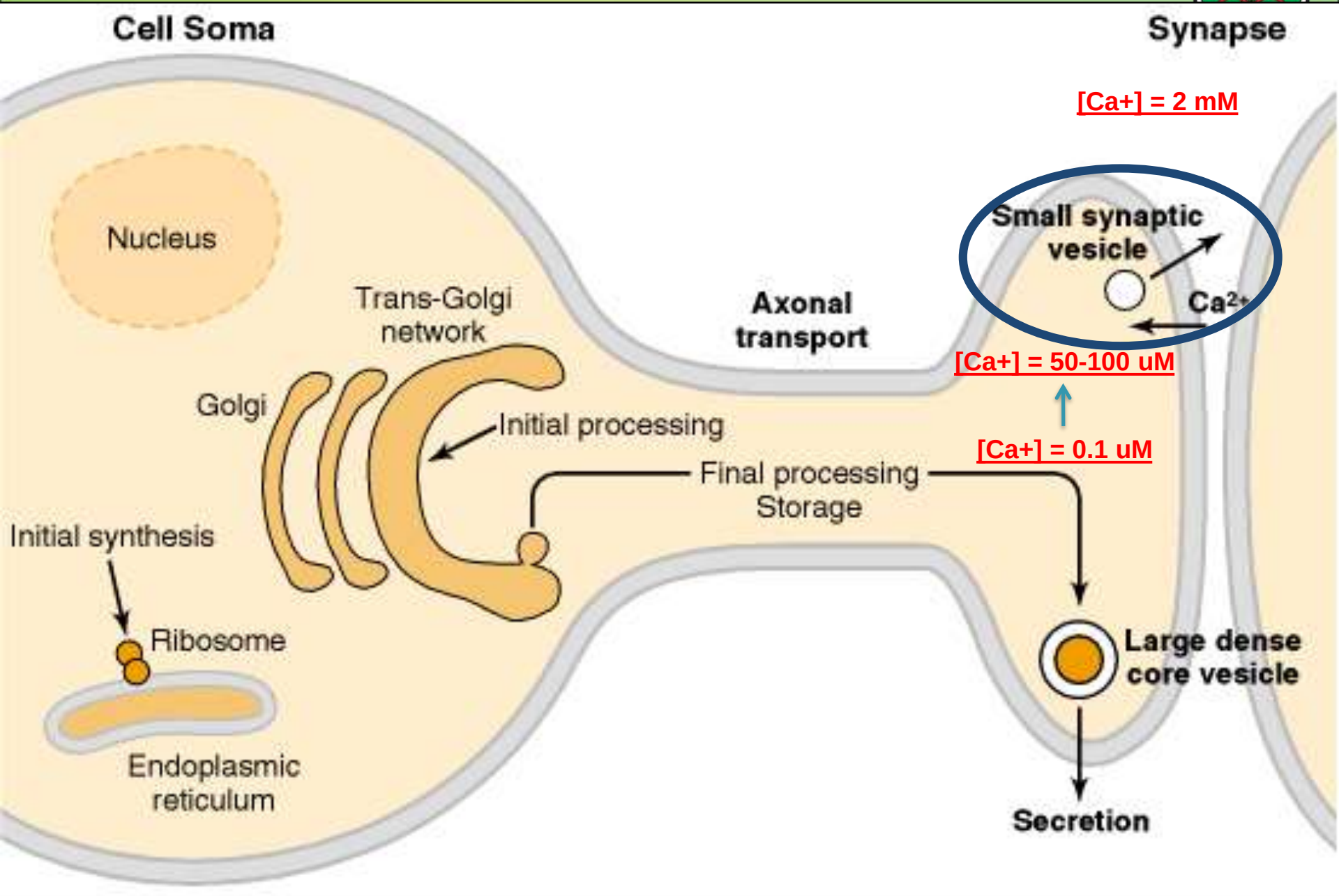
Types of small-molecule neurotransmitters

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

Stages of action

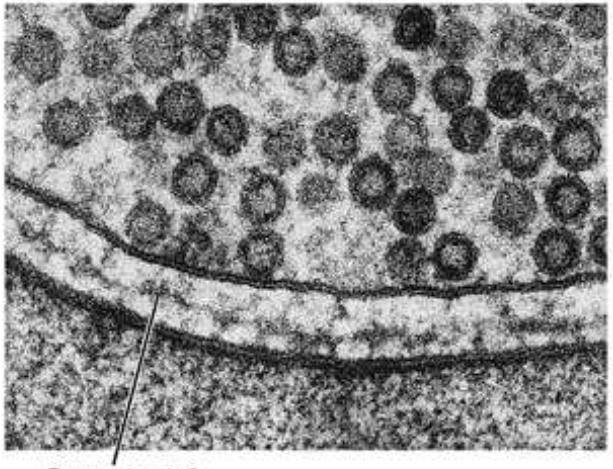
- Synthesis of enzymes
  - Cytosol
  - ER-Golgi apparatus (packaging into large-dense core vesicles)
- Transport of enzymes (axonal transport)
- Synthesis in pre-synaptic terminal
- Packaging in small synaptic vesicles
- Release is stimulated by brief pulses each time an action potential triggers the influx 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

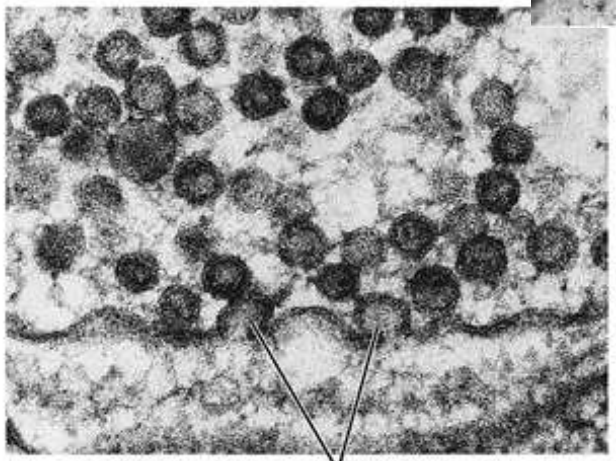
Presynaptic membrane (thin section)

A_2

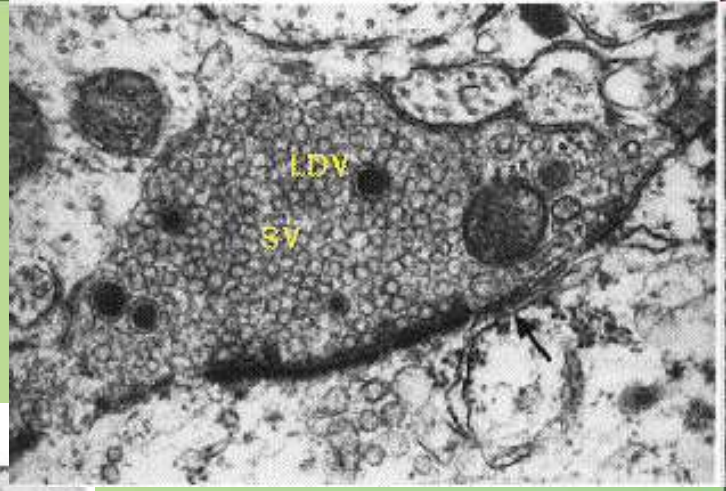


Synaptic cleft

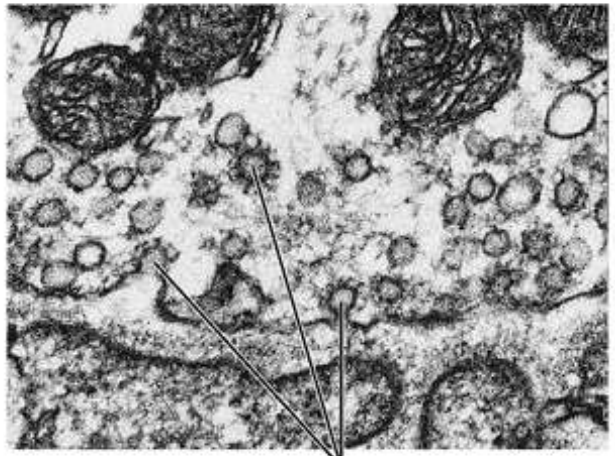
B_2



Vesicle fusions



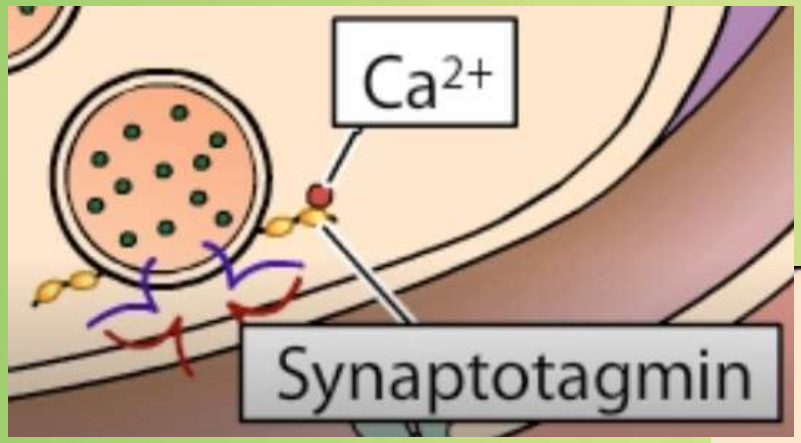
C_2

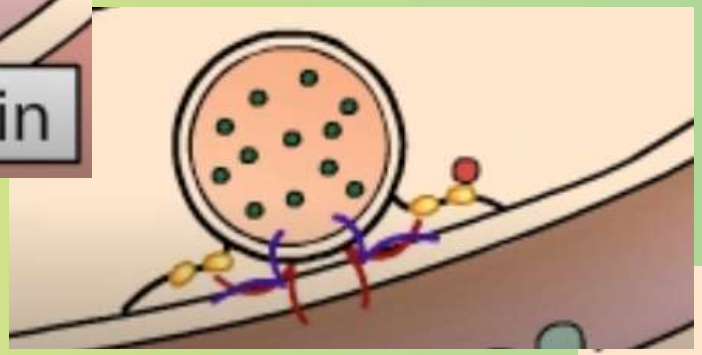


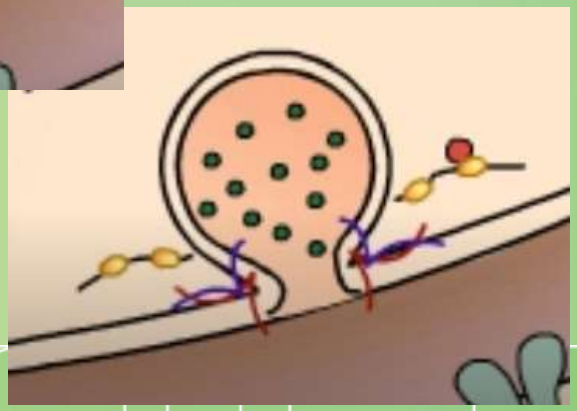
Coated vesicles

Proteins and exocytosis

The influx of Ca2+ ions them to interact with synaptotagmin leading to fusion of the vesicular and presynaptic membranes.







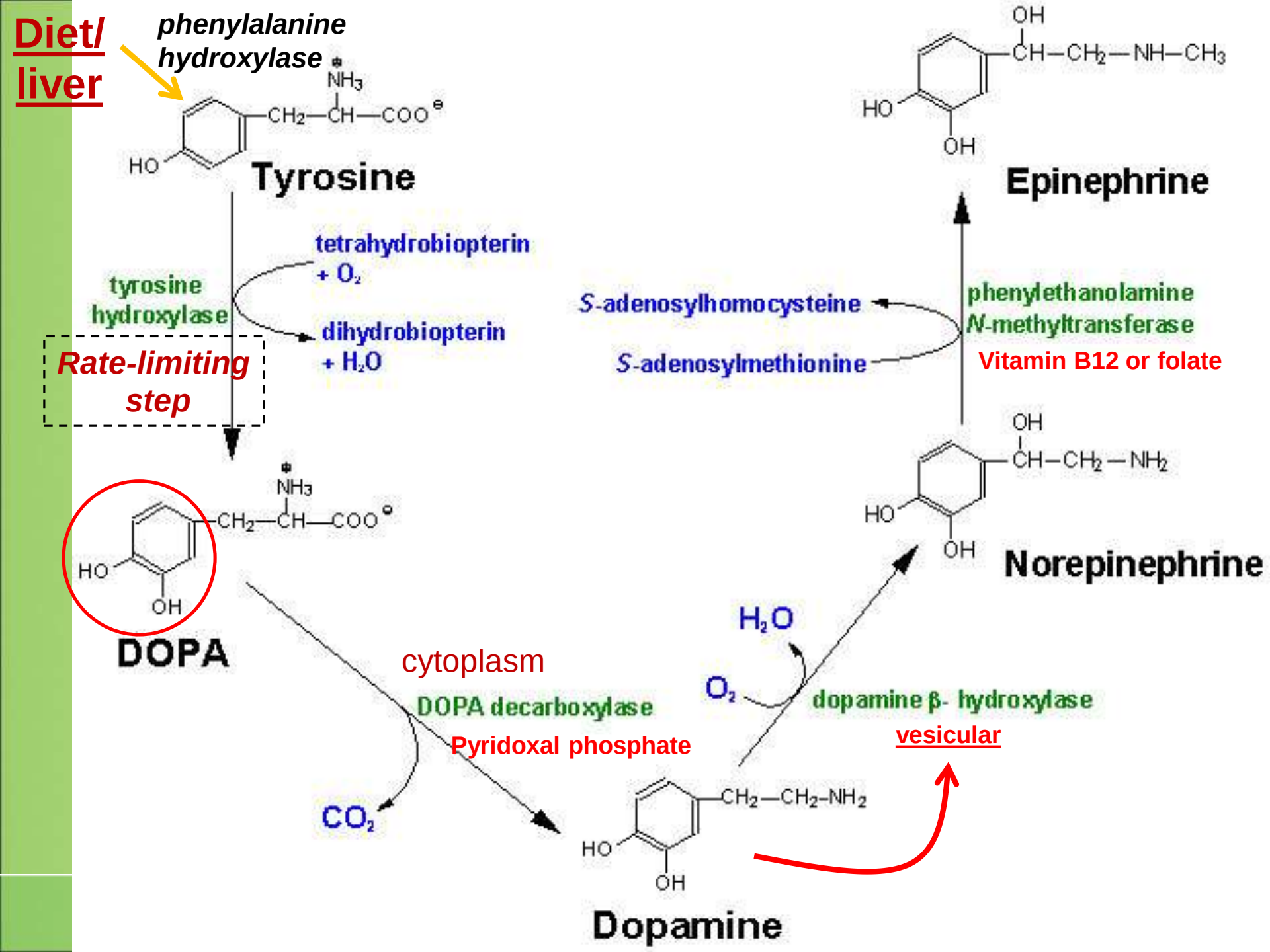
http://www.sumanasinc.com/webcontent/animations/content/synaptictransmission.html

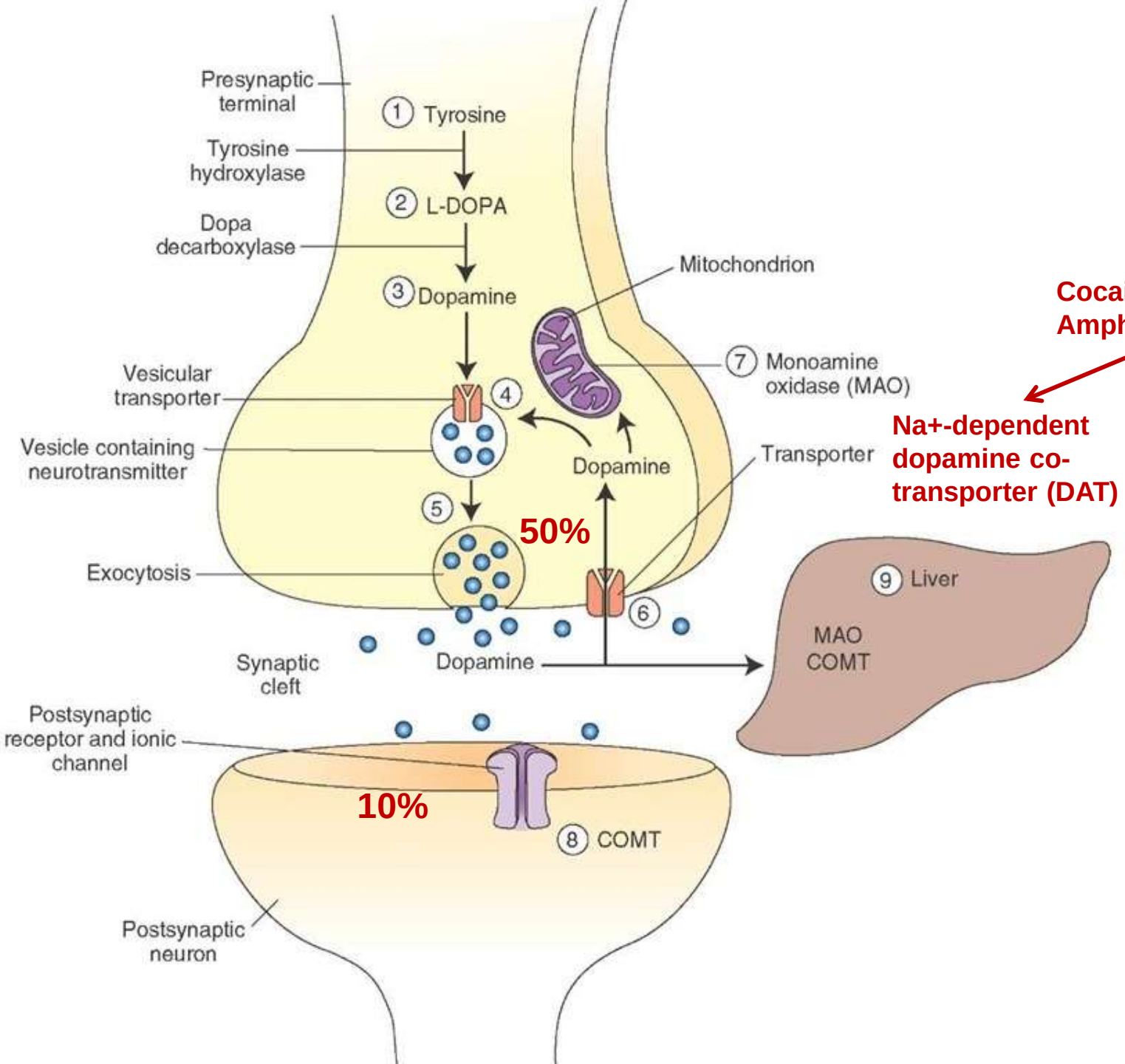
TYROSINE-DERIVED NEUROTRANSMITTERS

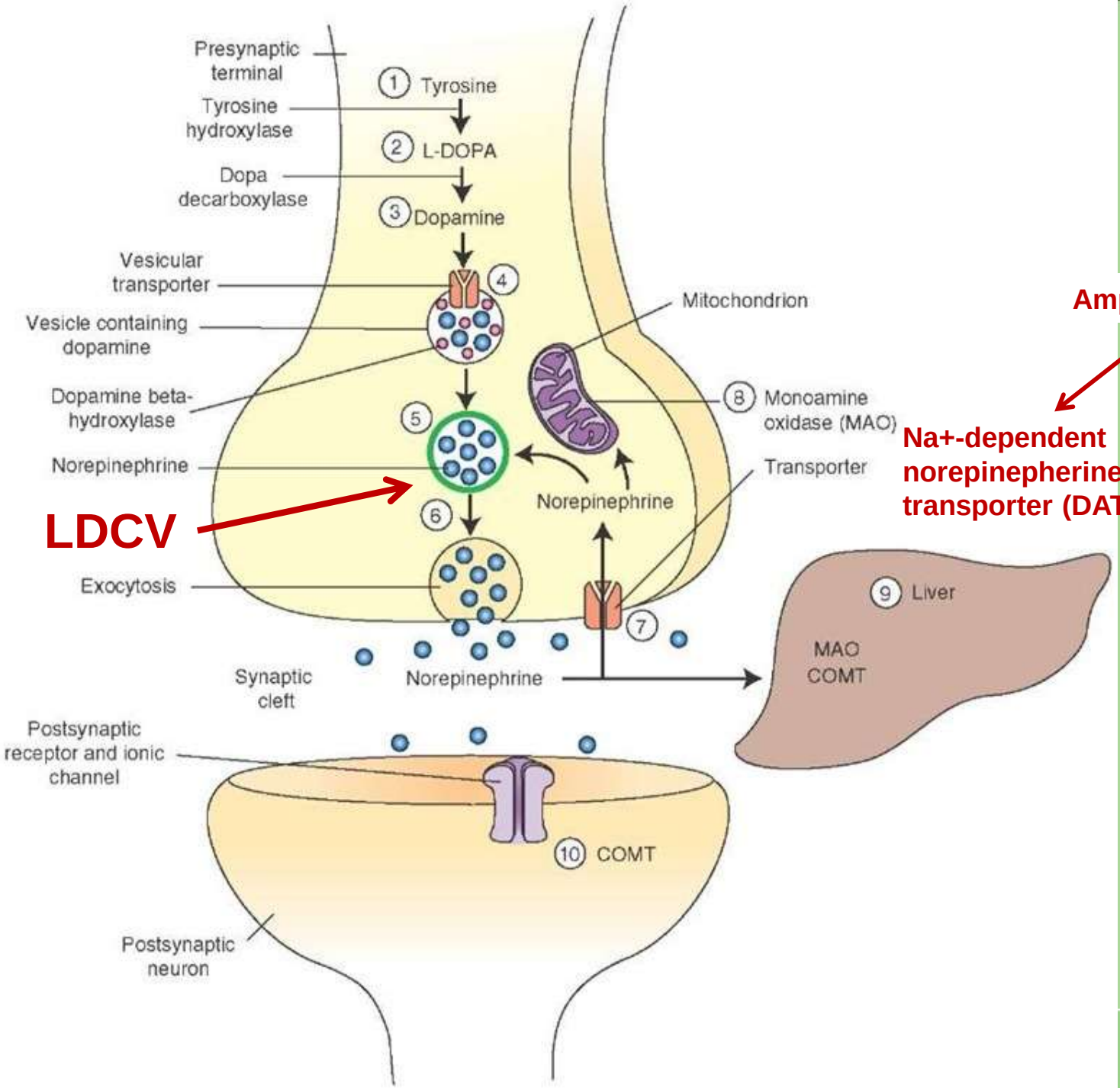
Dopamine, norepinephrine, and epinephrine

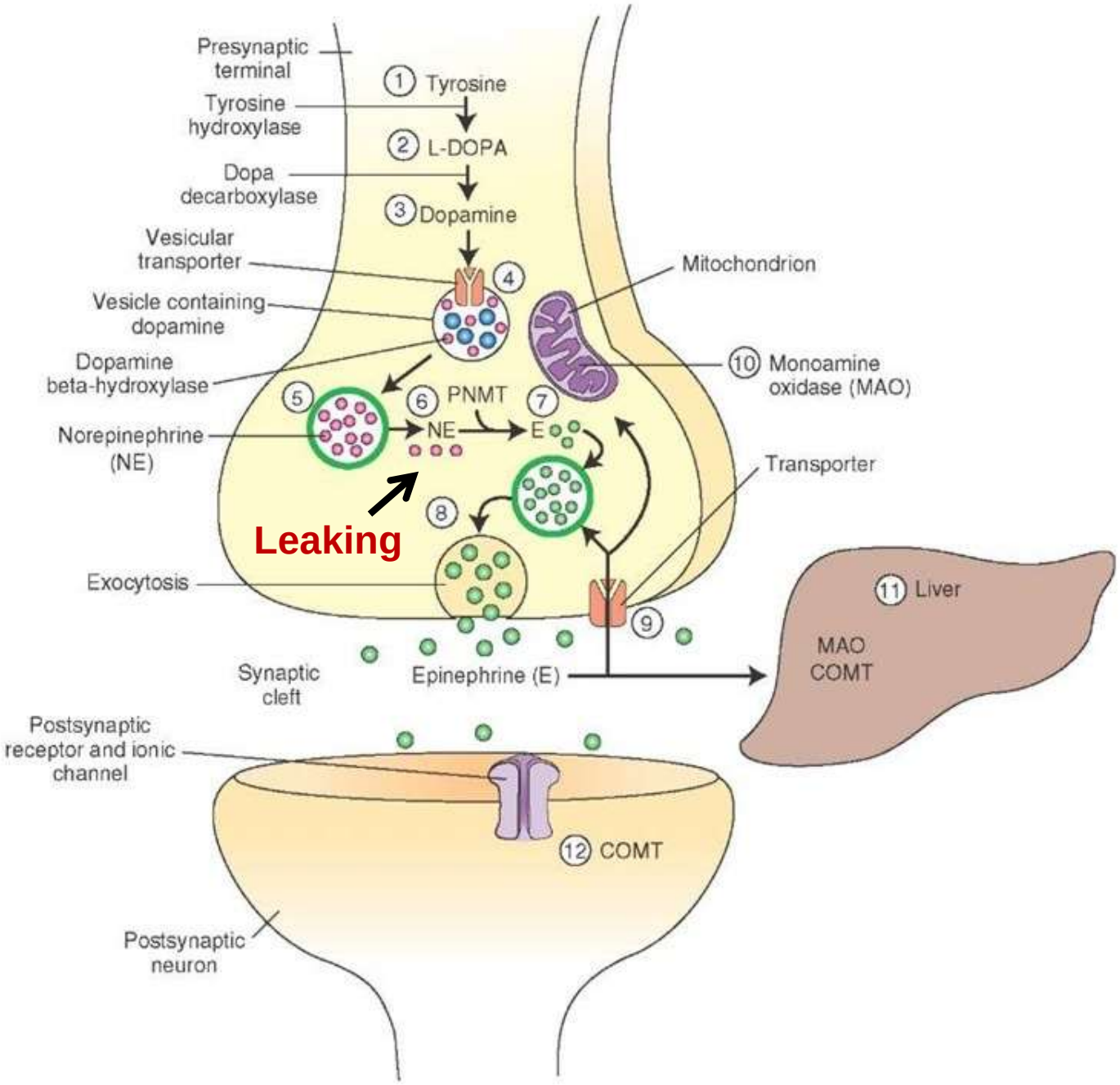
Notes

- **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

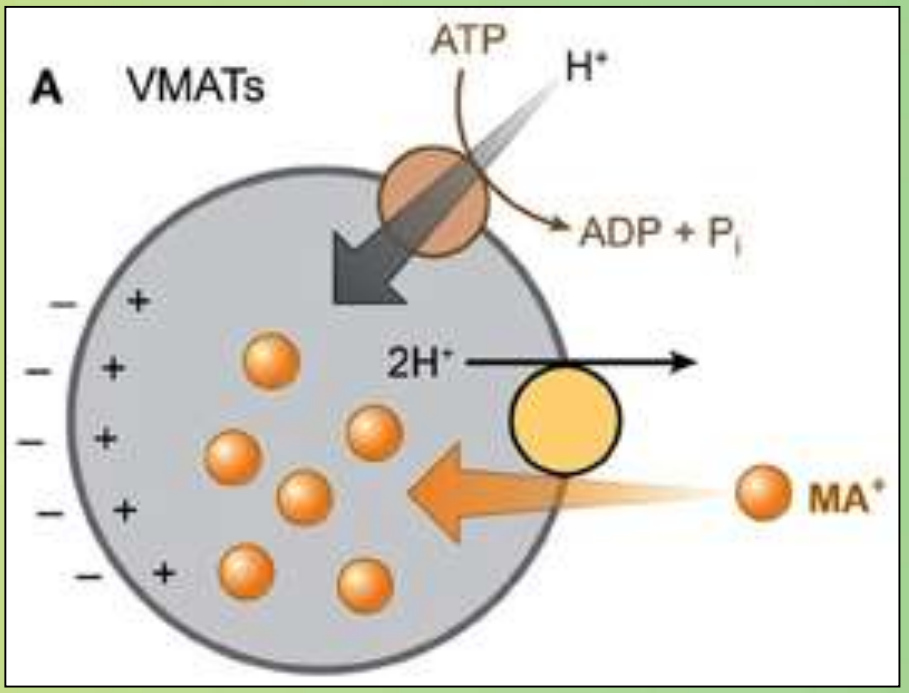

Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Mitochondrion
7 Monoamine oxidase (MAO)
Vesicular transporter
4
Vesicle containing neurotransmitter
Dopamine
Transporter
5
50%
Exocytosis
6
9 Liver
MAO
COMT
Synaptic cleft
Dopamine
Postsynaptic receptor and ionic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


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

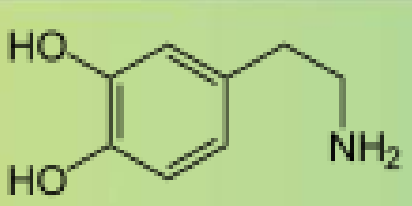

Presynaptic terminal
1 Tyrosine
Tyrosine hydroxylase
2 L-DOPA
Dopa decarboxylase
3 Dopamine
Vesicular transporter
4
Mitochondrion
Vesicle containing dopamine
Dopamine beta-hydroxylase
10 Monoamine oxidase (MAO)
5
6 PNMT
7
Norepinephrine (NE)
NE
E
Transporter
Leaking
8
Exocytosis
11 Liver
MAO
COMT
9
Synaptic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

Packaging into vesicles

- The catecholamines (dopamine and 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 VMAT (vesicular monoamine transporter).



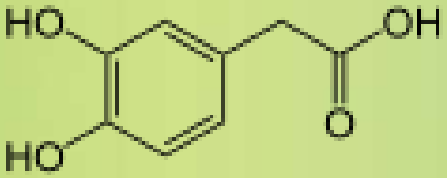
COMT and MAO



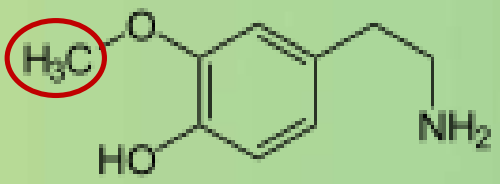
Dopamine (DA)

Monoamine oxidase (MAO), Aldehyde dehydrogenase

Catechol-O-methyl-transferase (COMT)



3,4-Dihydroxyphenyl-acetic acid (DOPAC)

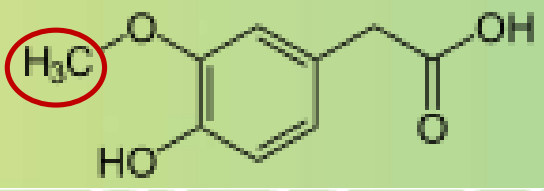


3-Methoxytyramine (3-MT)

Inactivation is dependent on SAM, vitamin B12 and folate

Catechol-O-methyl-transferase (COMT)

Monoamin oxidase (MAO), Aldehyde dehydrogenase



Homovanillic acid (HVA)

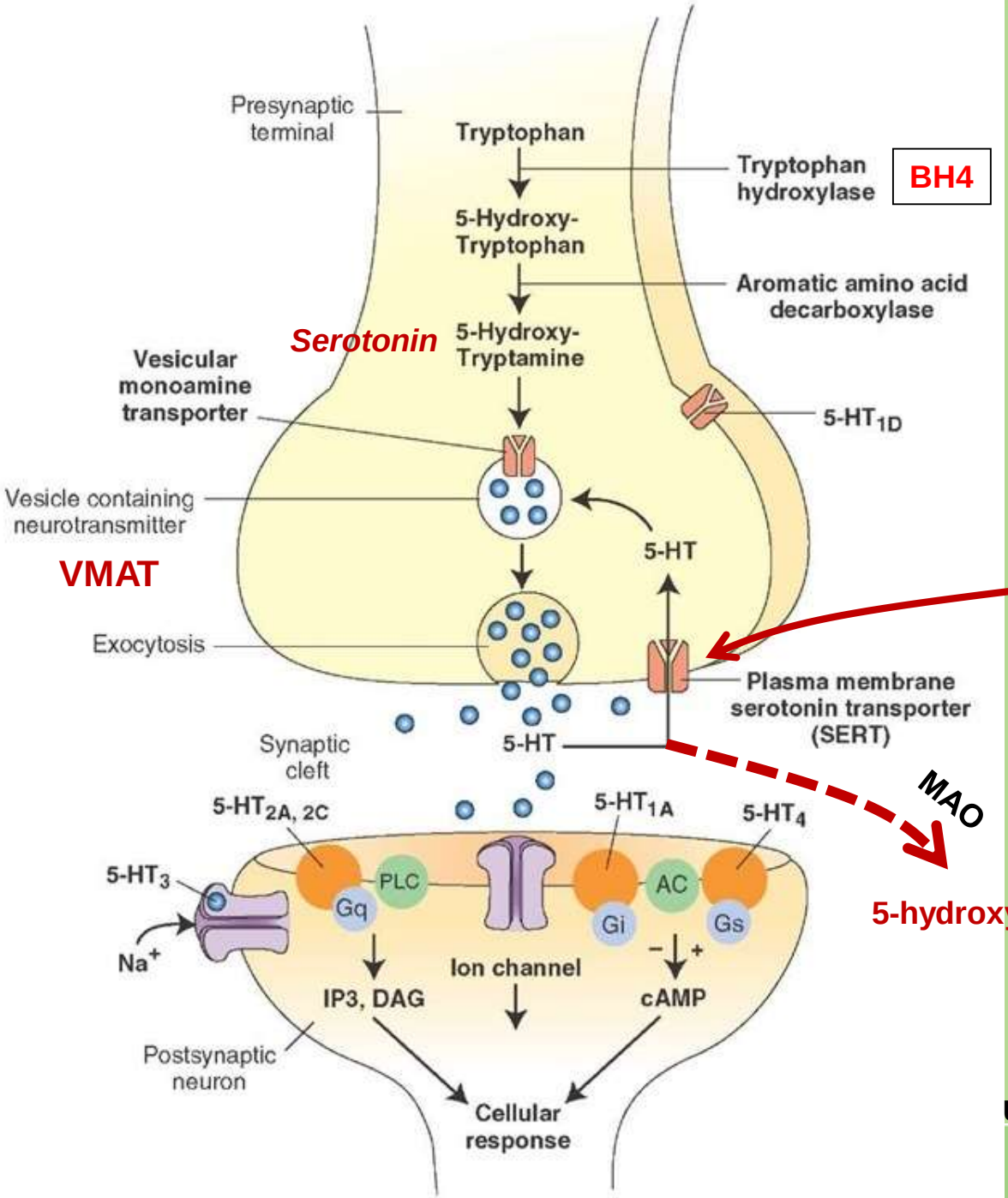
Parkinson's disease

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



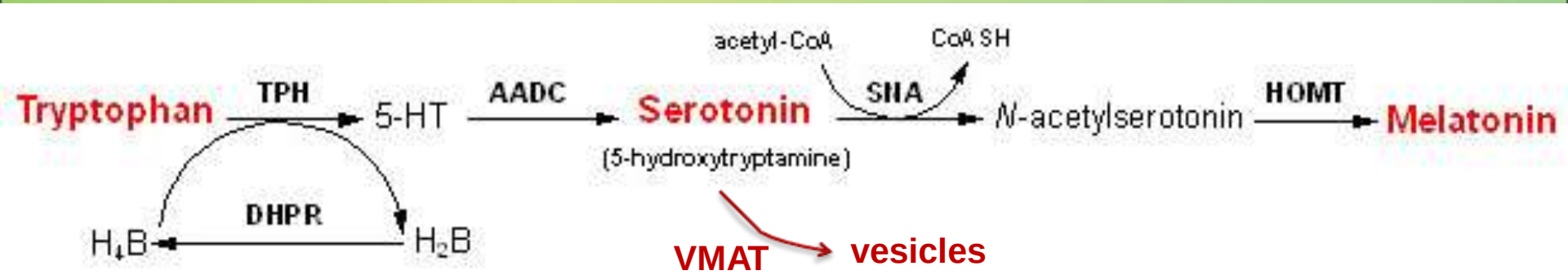
Antidepressants , called selective serotonin re-uptake inhibitors (SSRIs) like Prozac® inhibit the reuptake process resulting in prolonged serotonin presence in the synaptic cleft.

5-hydroxyindoleacetic acid

urine

Melatonin

- **Serotonin 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) rythyms**
  - **Dark-light cycle**



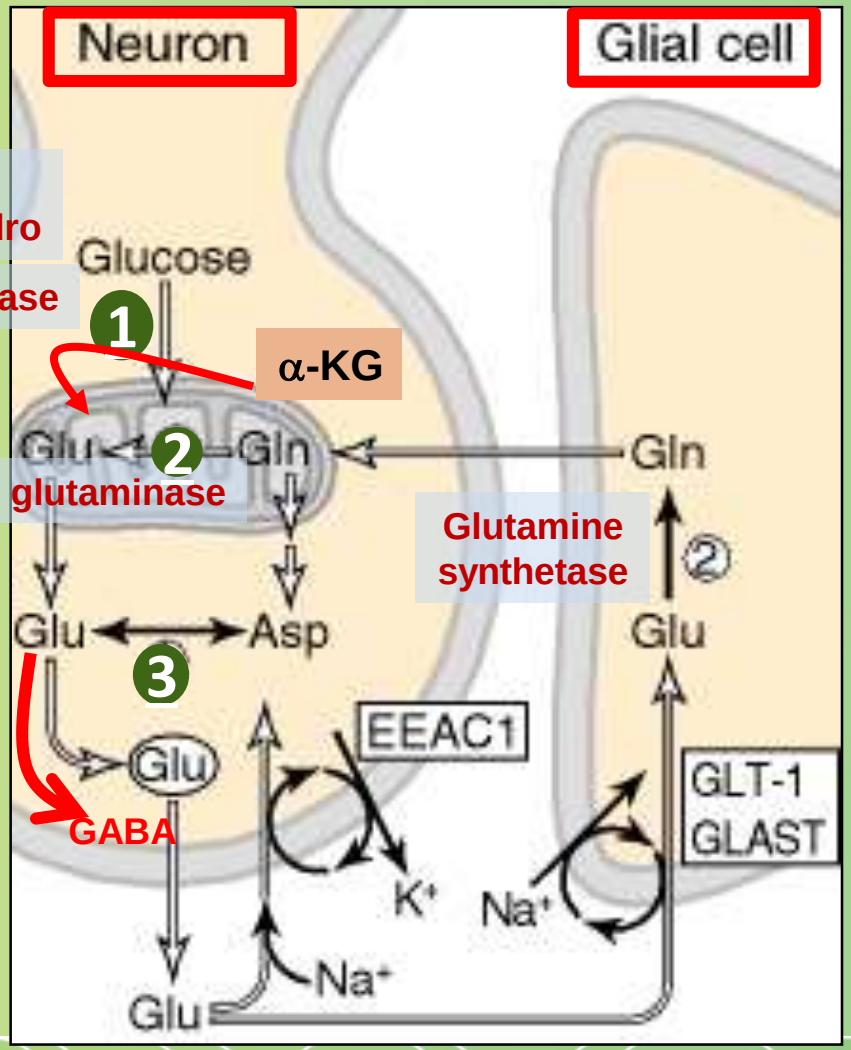
GLUTAMATE AND ASPARTATE

Glutamate and aspartate

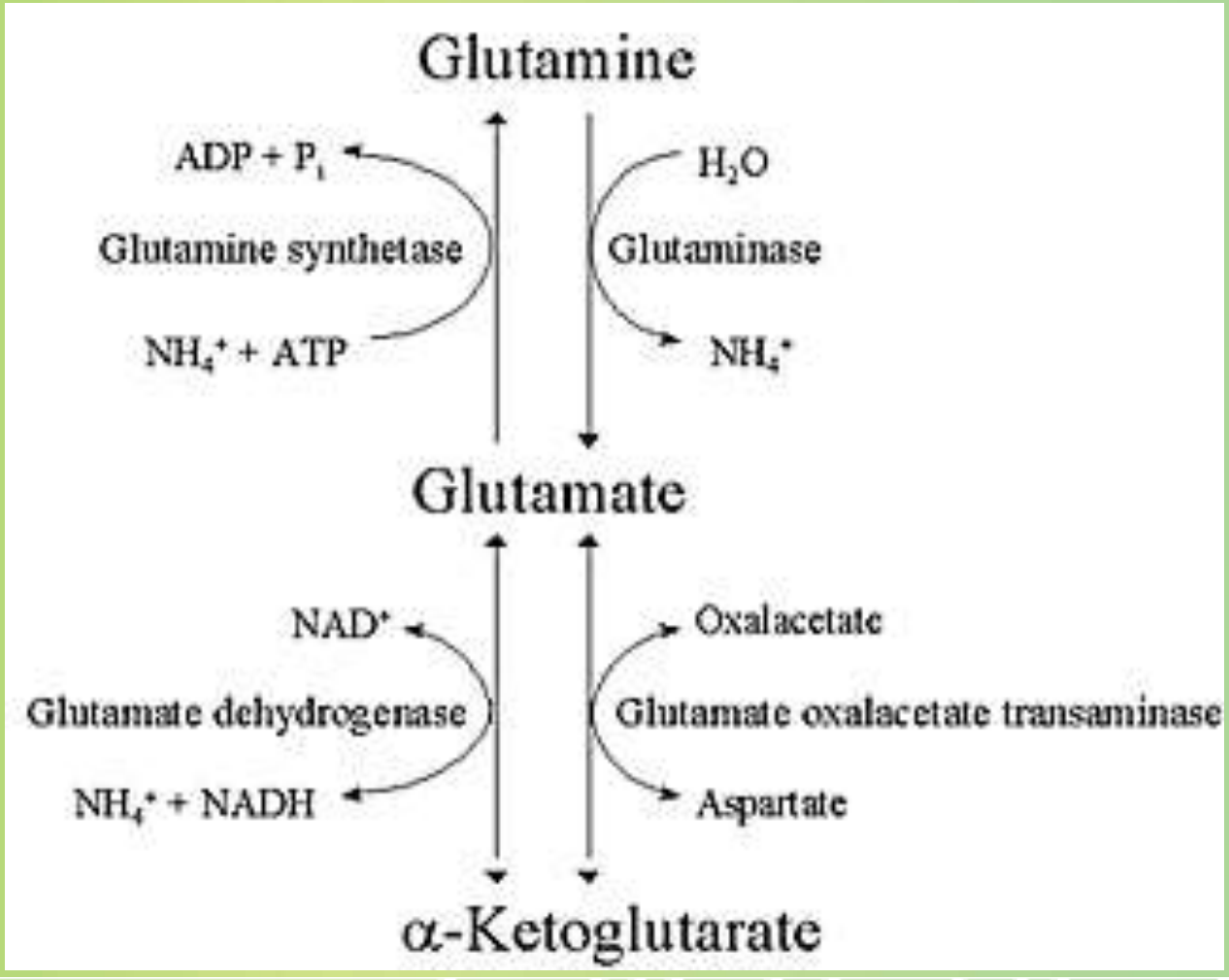
- Nonessential amino acids
- Do not cross BBB
  - must be synthesized in neurons
- Main synthetic compartments
  - neurons
  - glial cells
- Both are excitatory neurotransmitters.

Synthesis of glutamate

- **Sources:**
  1. **Glycolysis → Krebs cycle → dehydrogenation of α-ketoglutarate**
  2. **Glutamine (deamination)**
  3. **Aspartate (transamination)**
- **Removal**
  - **excitatory amino acid carrier-1 *(EAAC1)***
  - **glutamate transporter-1 (GLT-1) and glutamate—aspartate transporter (GLAST)**

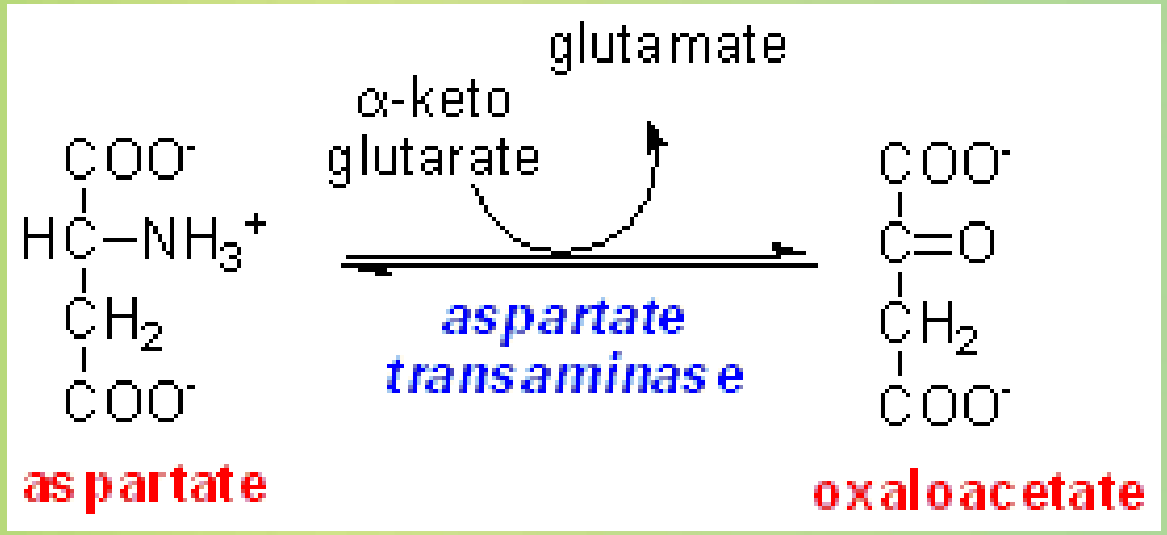


Sources of glutamate *(supplementary)*



Aspartate

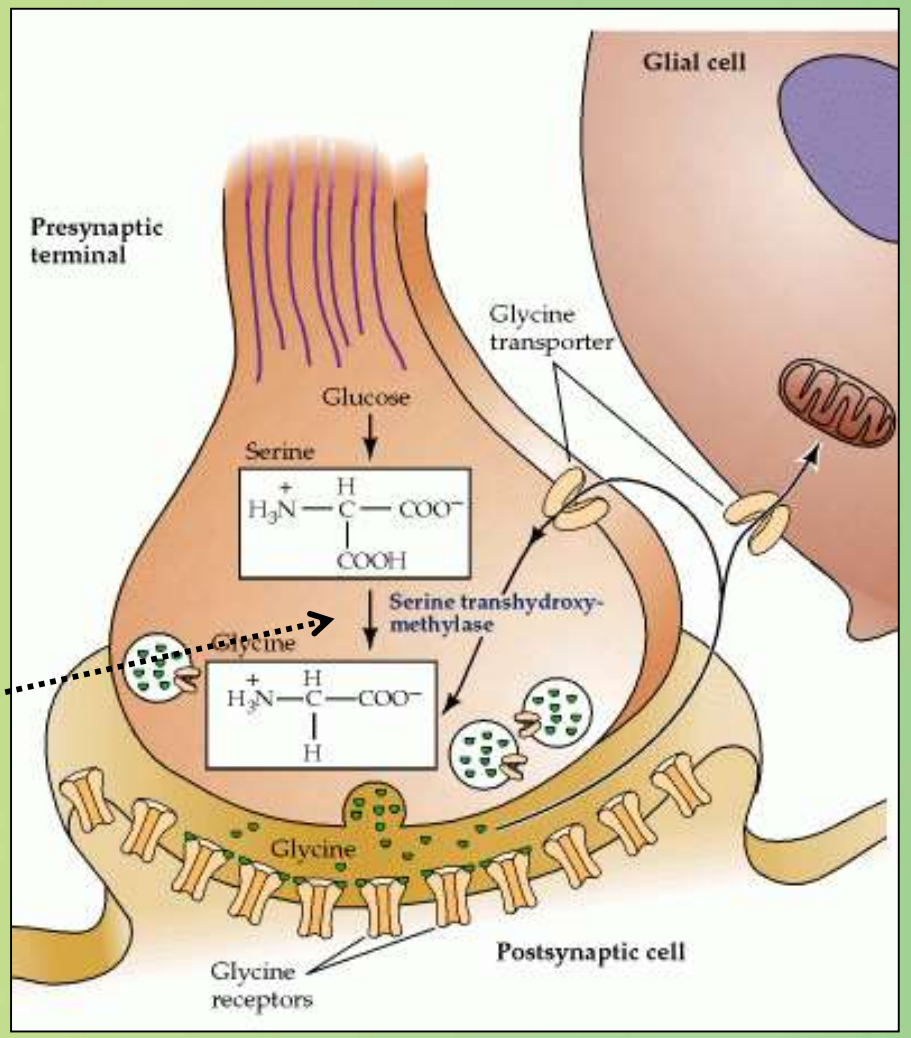
- A vesicular uptake mechanism for aspartate has not yet been demonstrated, somewhat weakening the case for considering aspartate to be a neurotransmitter
- Precursor: oxaloacetate (transamination)



Glycine

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

Folic acid

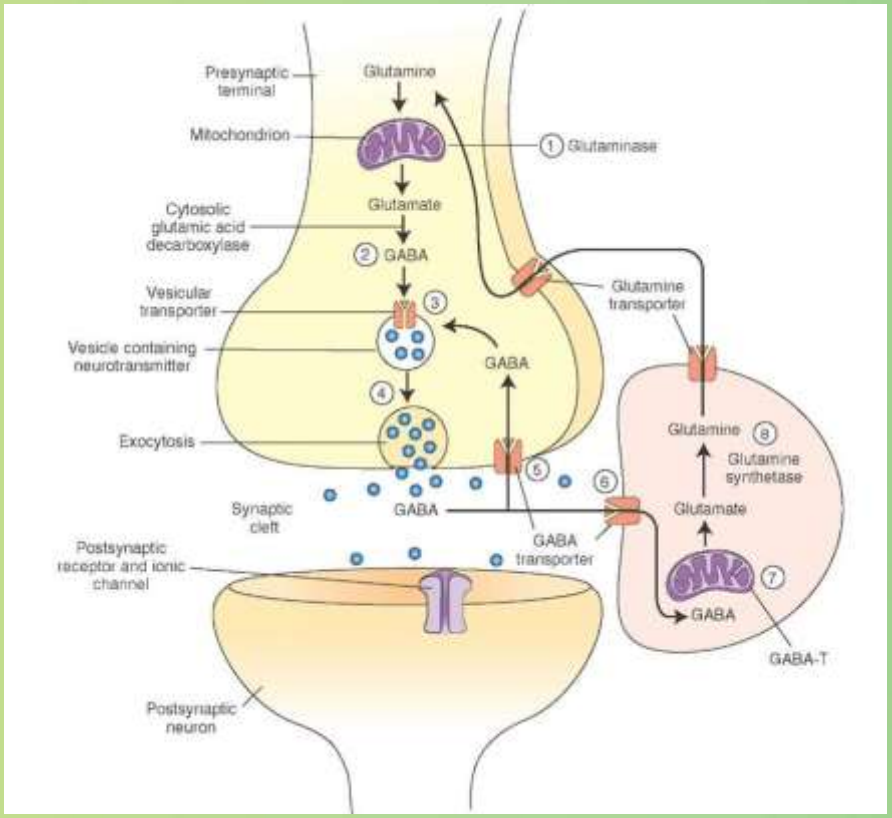


GABA

- 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.

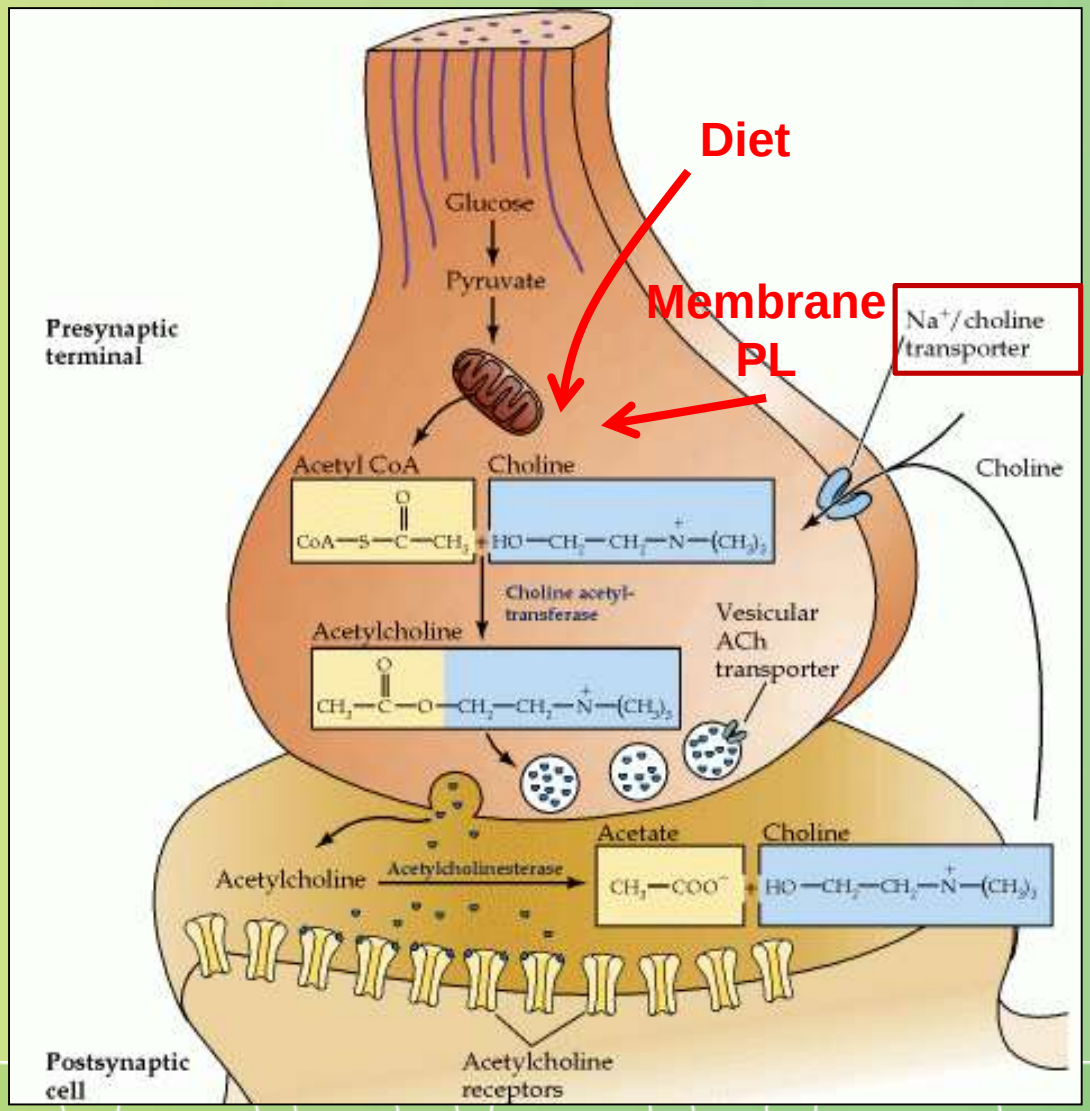
GABA shunt

- 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 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.



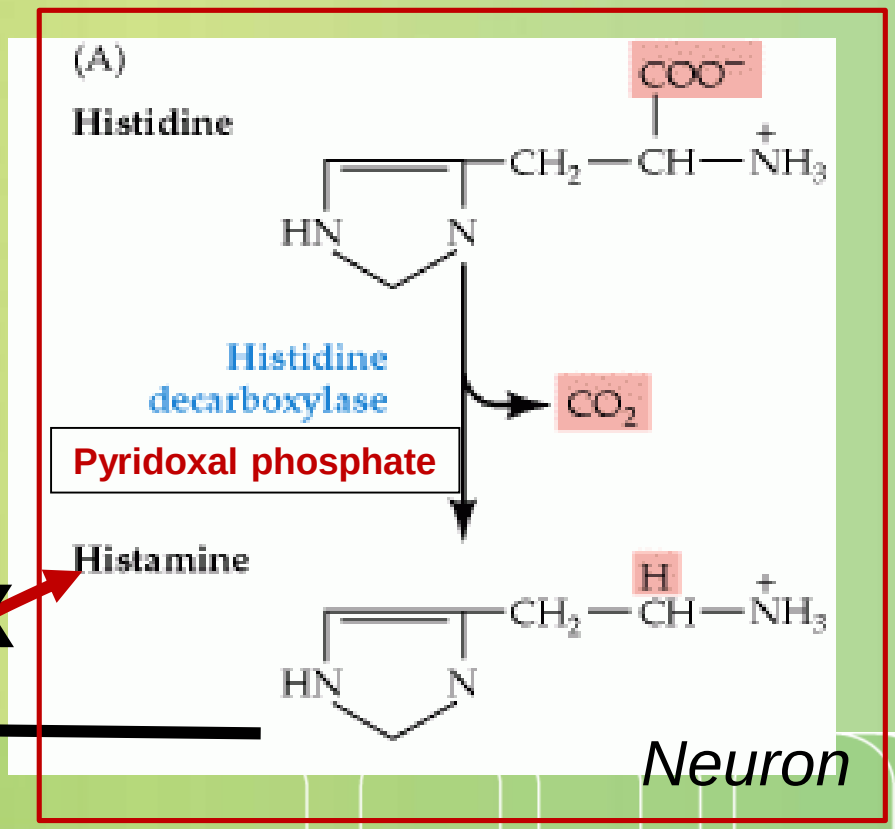
Synthesis of acetylcholine

- 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 and diamine oxidase (histaminase).



No recycling

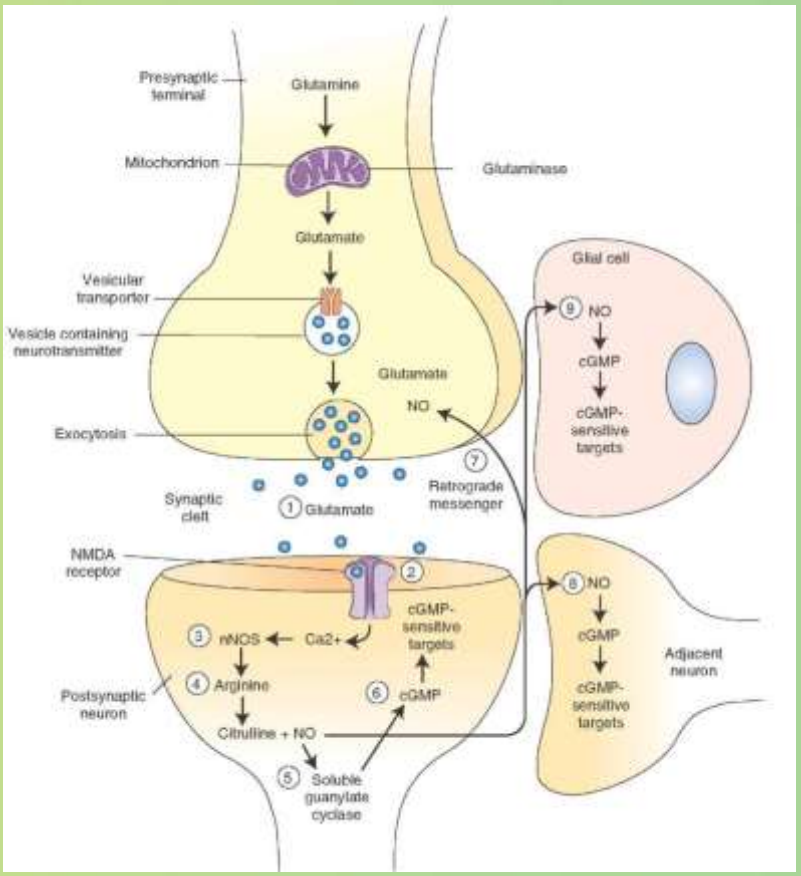
X

Astrocytes (MAO)

Histamine is packaged into vesicles by VMAT

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 froms 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.



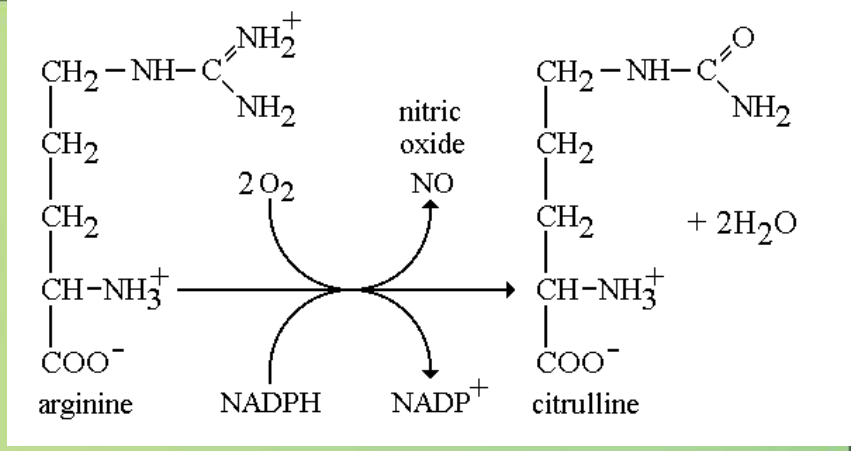
Half-life: 2-4 seconds

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

Note the differences between neuropeptides and neurotransmitters

- Activity (slow vs. fast), response (slow vs. fast), and duration of action (long vs. short)
- Receptor targets (multiple vs. single)
- Gene expression (yes vs. no)
- Synthesis, transport, and packaging
- Concentration for action (low vs. high)
- Speed of release (slow vs. fast)
- Concentration of [Ca^{+2}] for release
- Site of synthesis, modification, action
- Fate