Biochemical Neuron
BCH 575

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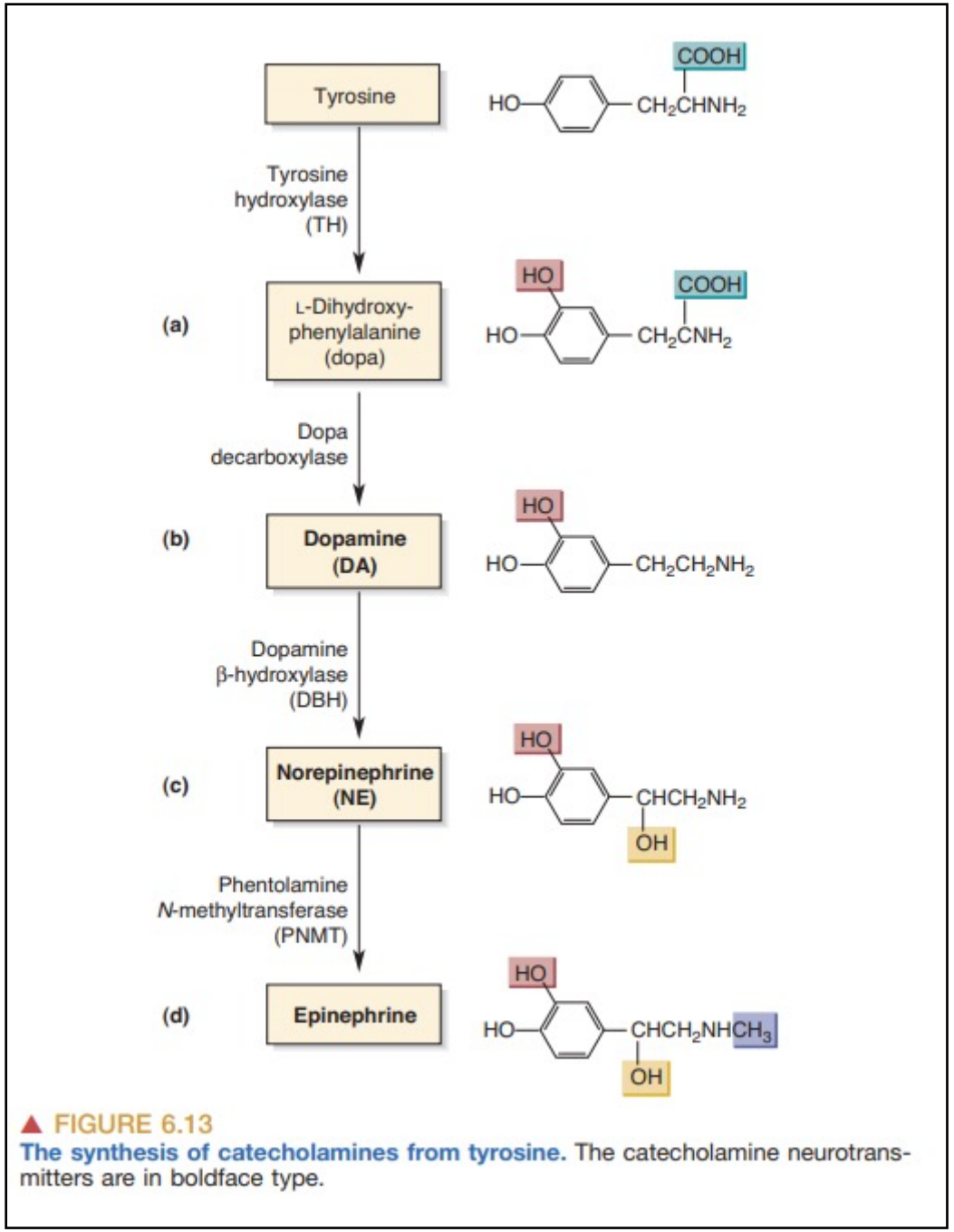
Neurotransmitters-2-

Catecholaminergic synthesis

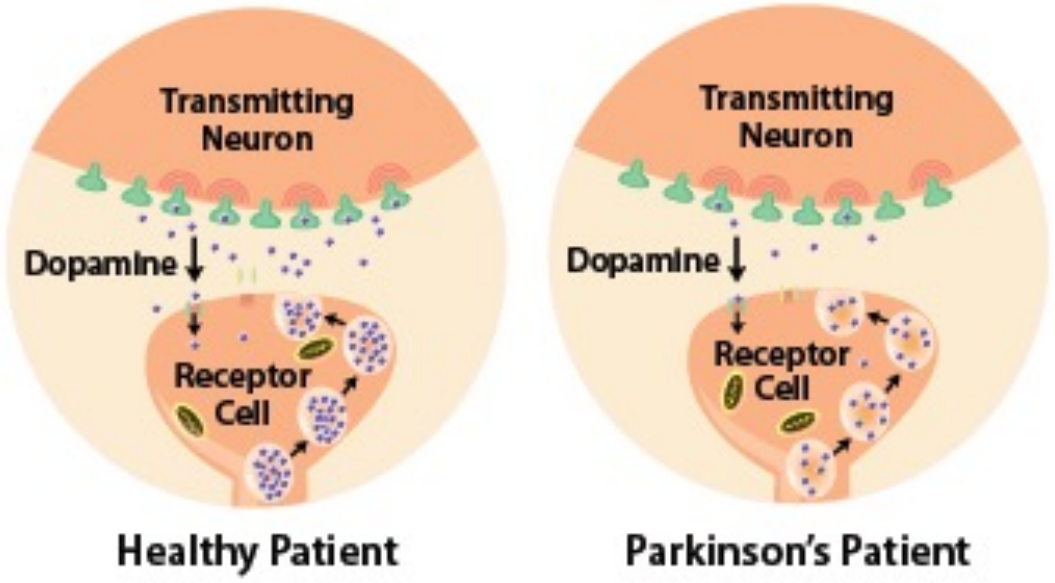


▲ FIGURE 6.13
The synthesis of catecholamines from tyrosine. The catecholamine neurotransmitters are in boldface type.

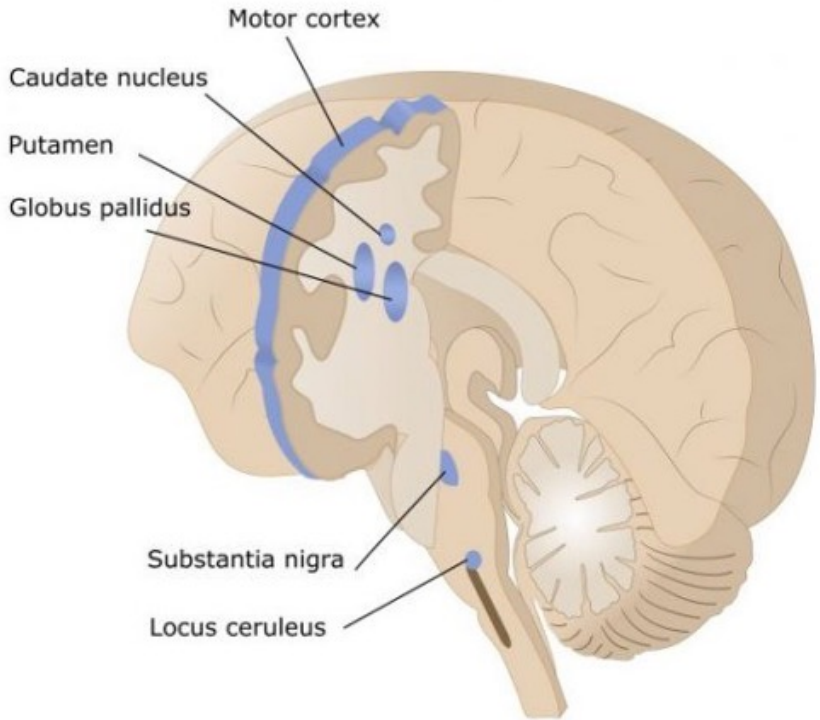
Parkinson’s disease and dopamine:

The amount of DA synthesized depends primarily on the amount of dopa available. In Parkinson’s disease (movement disorder), dopaminergic neurons in the brain slowly degenerate and eventually die.

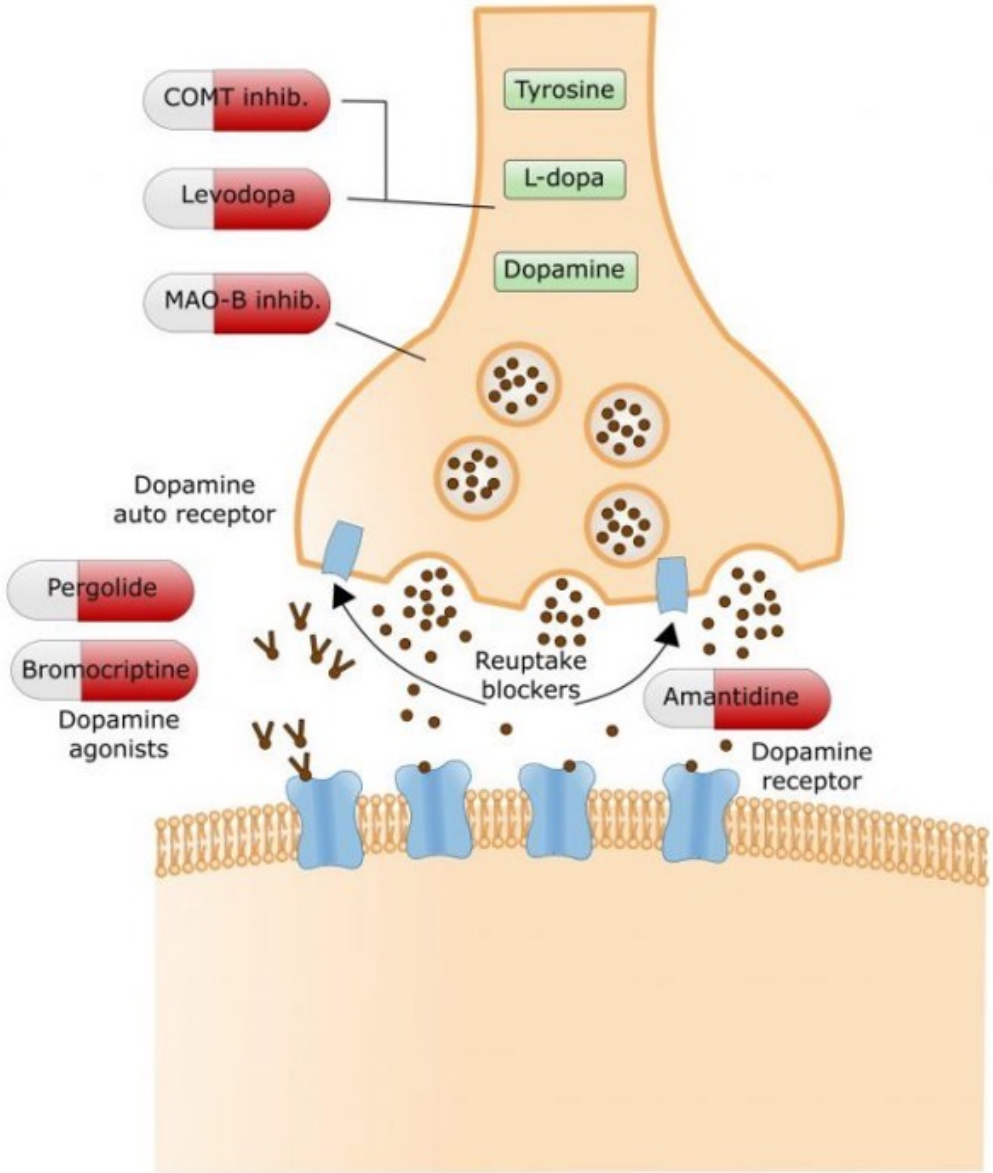
One strategy for treating Parkinson’s disease is the administration of dopa, which causes an increase in DA synthesis in the surviving neurons, increasing the amount of DA available for release.



Main brain regions affected by Parkinson's Disease



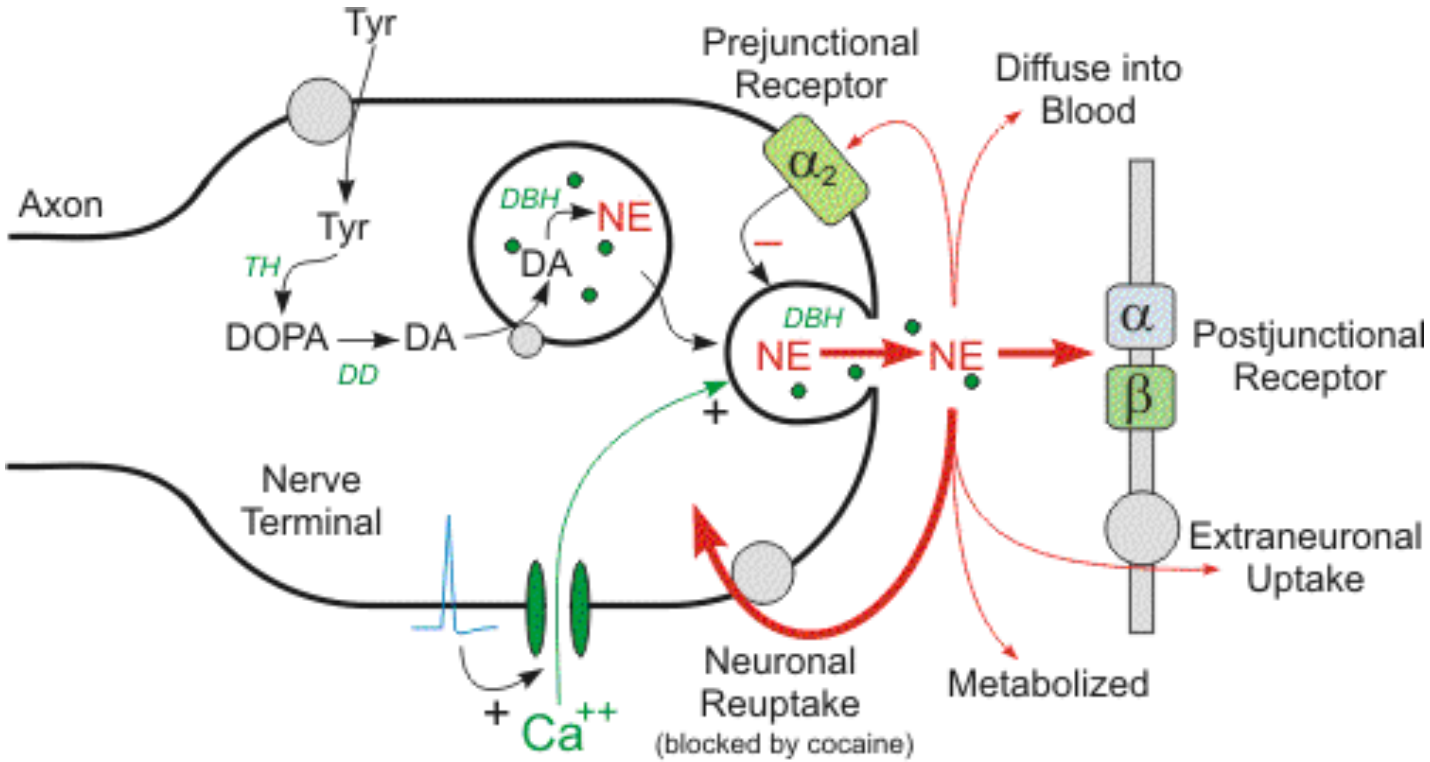
Common drugs for Parkinson treatment



Norepinephrine (NE)

Neurons that use norepinephrine(NE) as a neurotransmitter contain, in addition to TH and dopa decarboxylase, the enzyme dopamine -hydroxylase (DBH) , which converts DA to NE

Interestingly, DBH is not found in the cytosol but instead is located within the synaptic vesicles. Thus, in noradrenergic axon terminals, DA is transported from the cytosol to the synaptic vesicles, and there it is made into NE.



Tyr = tyrosine; TH = tyrosine hydroxylase; DD = DOPA decarboxylase; DA = dopamine; DBH = dopamine β-hydroxylase; NE = norepinephrine

Epinephrine

Adrenergic neurons contain the enzyme phentolamine N - methyltransferase (PNMT), which converts NE to epinephrine (adrenaline).

Curiously, PNMT is in the cytosol of adrenergic axon terminals.

Thus, NE must first be synthesized in the vesicles and released into the cytosol for conversion into epinephrine, and then the epinephrine must again be transported into vesicles for release.

In addition to serving as a neurotransmitter in the brain, epinephrine acts as a hormone when it is released by the adrenal gland into the bloodstream.

circulating epinephrine acts at receptors throughout the body.

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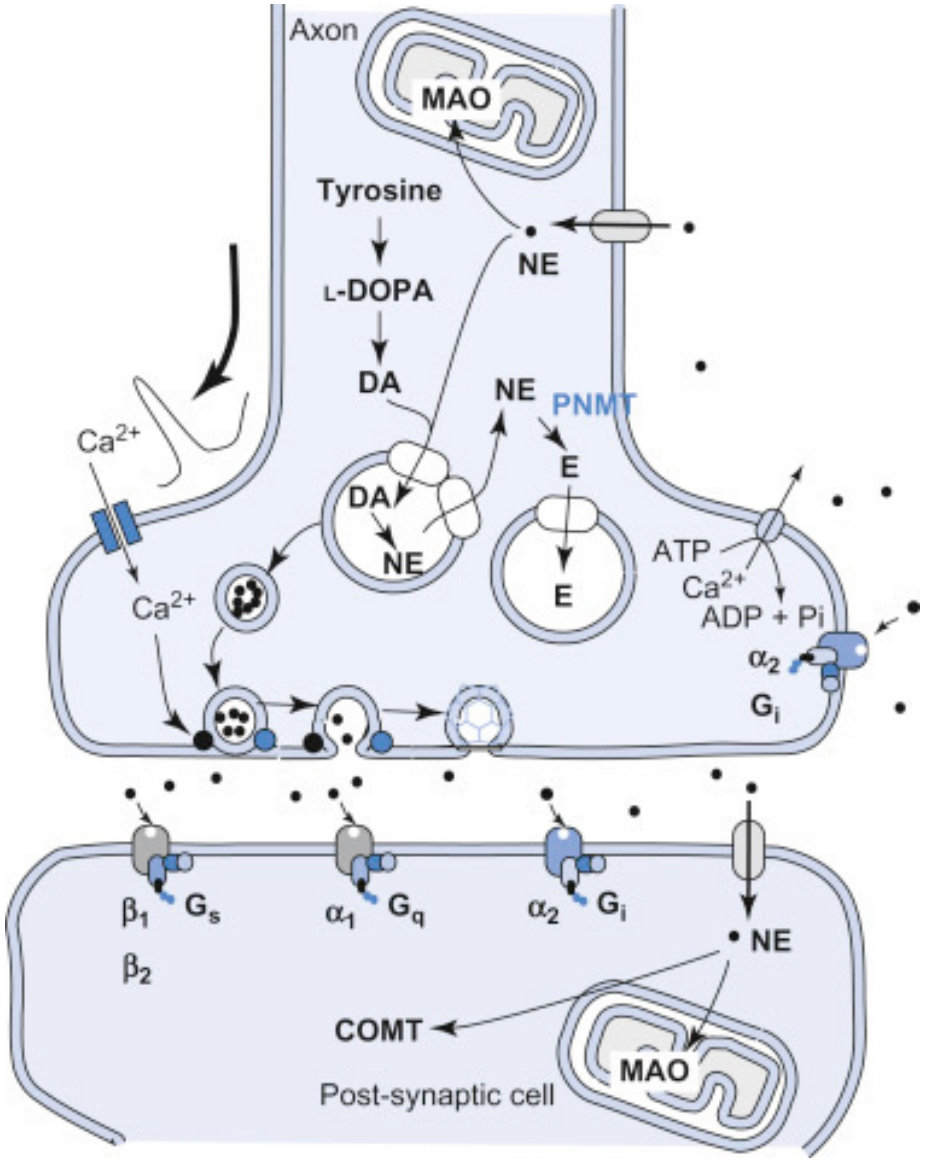


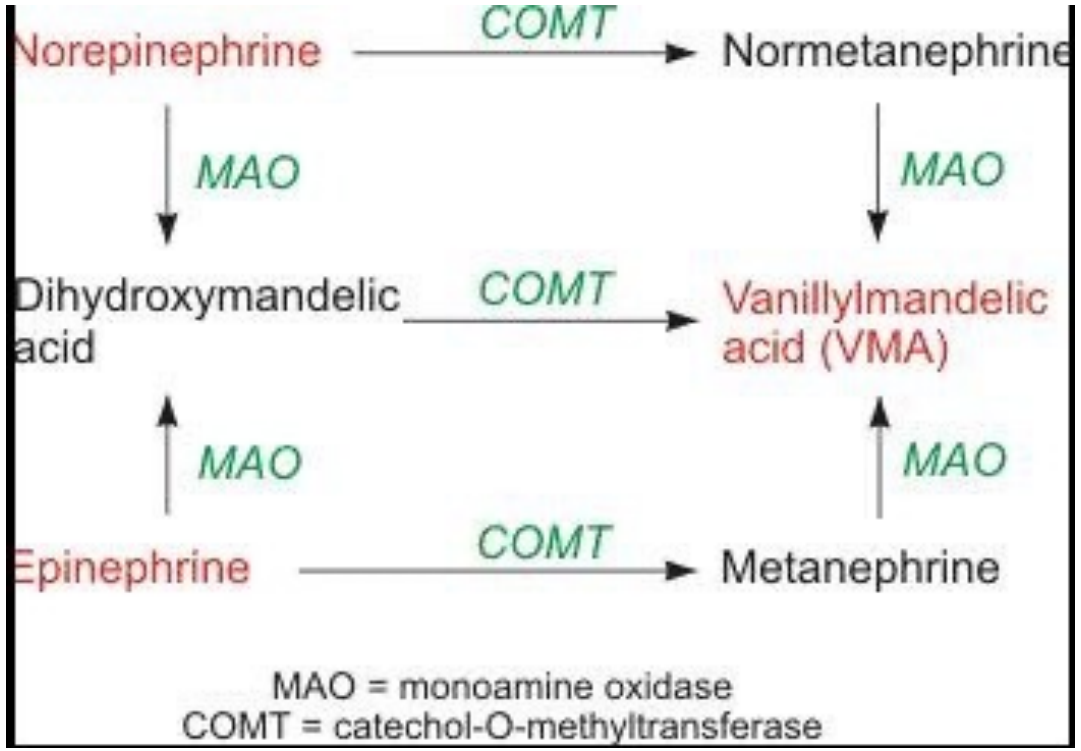
Catecholamine degradation:

- The catecholamine systems have no fast extracellular degradative enzyme analogous to AChE.
- Instead, the actions of catecholamines in the synaptic cleft are terminated by selective uptake of the neurotransmitters back into the axon terminal via Na - dependent transporters.
- This step is sensitive to a number of different drugs.

Catecholamine degradation:

For example, **amphetamine and cocaine** block catecholamine uptake and therefore prolong the actions of the neurotransmitter in the cleft.

- Once inside the axon terminal, the catecholamines may be reloaded into synaptic vesicles for reuse, or they may be enzymatically destroyed by the action of monoamine oxidase (MAO) , an enzyme found on the outer membrane of mitochondria

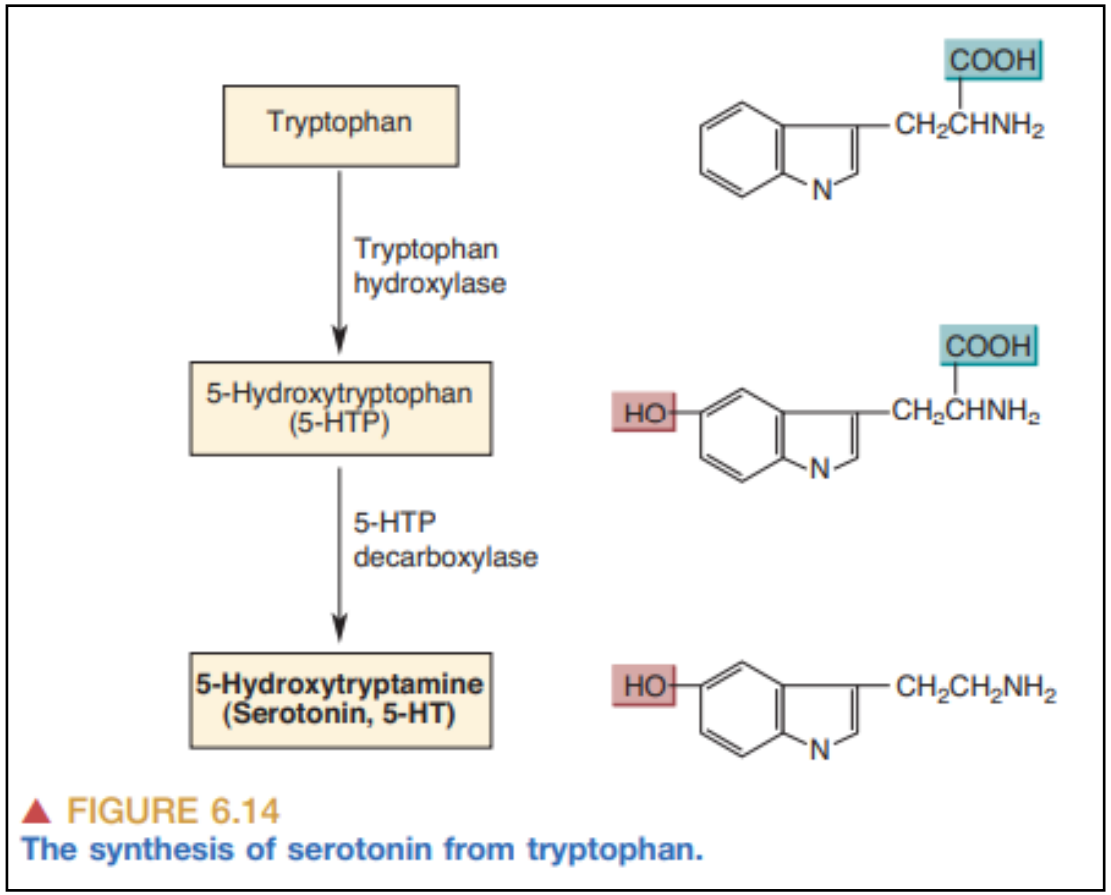


3. Serotonergic Neurons

- The amine neurotransmitter serotonin, also known as 5-hydroxytryptamine (5-HT)
- serotonin is derived from the amino acid tryptophan.
- Serotonergic neurons are relatively few in number, however, they appear to play an important role in the brain systems that regulate mood, emotional behavior, and sleep.

Serotonin synthesis

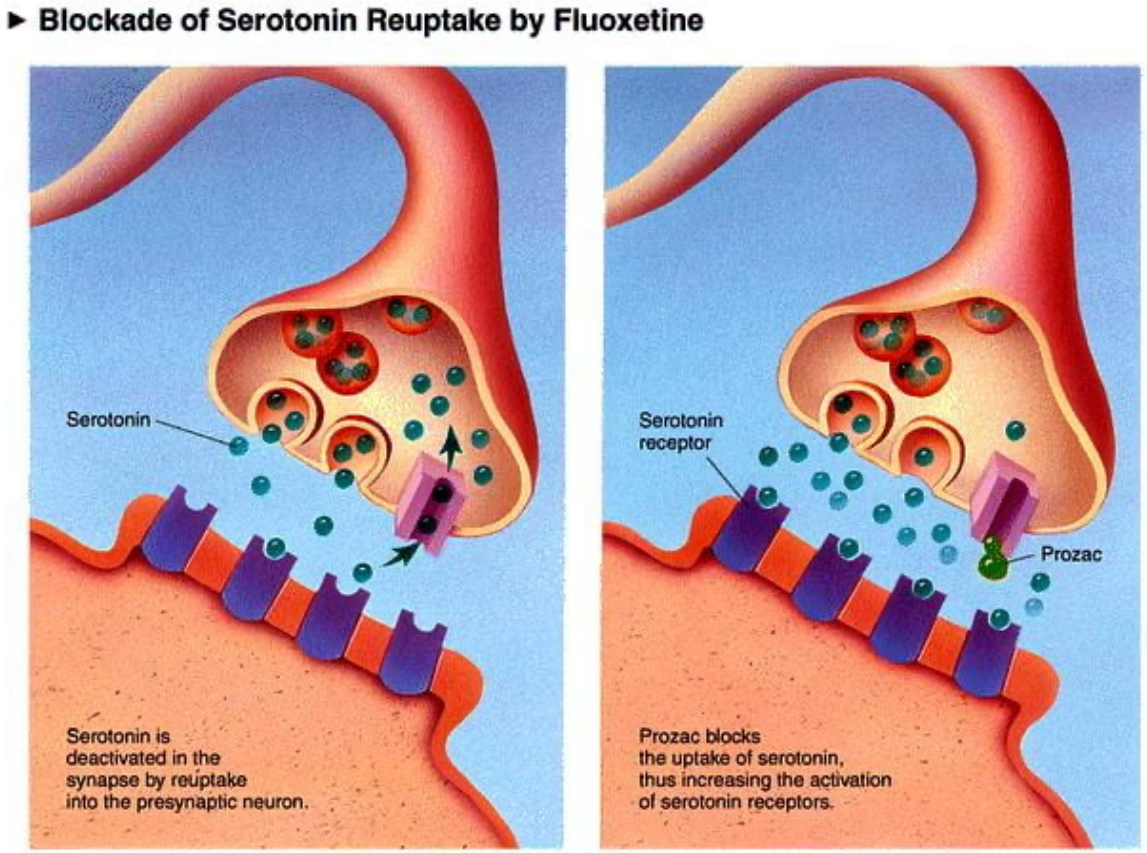
- The synthesis of serotonin occurs in two steps, just like the synthesis of DA.
- Tryptophan is converted first into an intermediary called 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase.
- The 5-HTP is then converted to 5-HT by the enzyme 5-HTP decarboxylase.



▲ FIGURE 6.14
The synthesis of serotonin from tryptophan.

- Serotonin synthesis appears to be limited by the availability of tryptophan in the extracellular fluid bathing neurons.
- The source of brain tryptophan is the blood, and the source of blood tryptophan is the diet (grains, meat, dairy products, and chocolate are particularly rich in tryptophan).
- Following release from the axon terminal, 5-HT is removed from the synaptic cleft by the action of a specific transporter.

- The process of serotonin reuptake, like catecholamine reuptake, is sensitive to several different drugs.
- For example, antidepressant and antianxiety drugs, including fluoxetine are selective inhibitors of serotonin reuptake.
- Once it is back in the cytosol of the serotonergic axon terminal, the transmitter is either reloaded into synaptic vesicles or degraded by monoamine oxidase.



4. Amino Acidergic Neurons

- The amino acids glutamate (Glu), glycine (Gly), and gamma-aminobutyric acid (GABA) serve as neurotransmitters at most CNS synapses.
- only GABA is unique to those neurons that use it as a neurotransmitter; the others are among the 20 amino acids that make up proteins.
- Glutamate and glycine are synthesized from glucose and other precursors by the action of enzymes that exist in all cells.

Differences among neurons in the synthesis of these amino acids are quantitative rather than qualitative.

- For example:

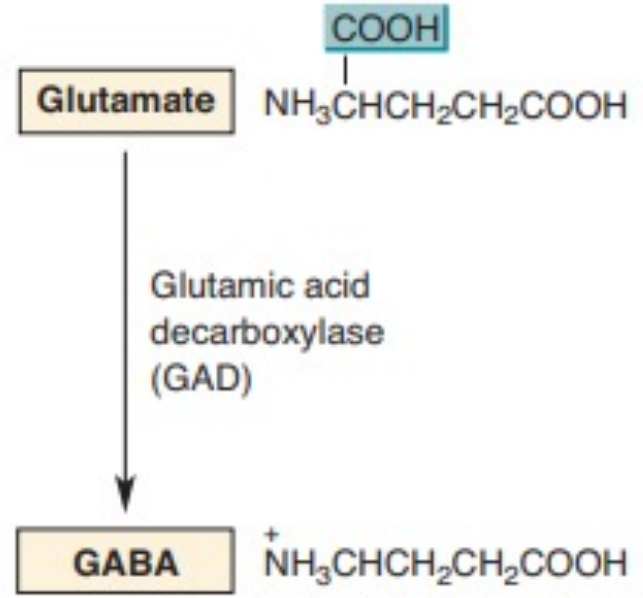
The average glutamate concentration in the cytosol of glutamatergic axon terminals has been estimated to be about 20 mM, two or three times higher than that in nonglutamatergic cells.

The more important distinction between glutamatergic and nonglutamatergic neurons, however, is the transporter that loads the synaptic vesicles. In glutamatergic axon terminals, but not in other types, glutamate transporters concentrate glutamate until it reaches a value of about 50 mM in the synaptic vesicles.

GABA Neurotransmission

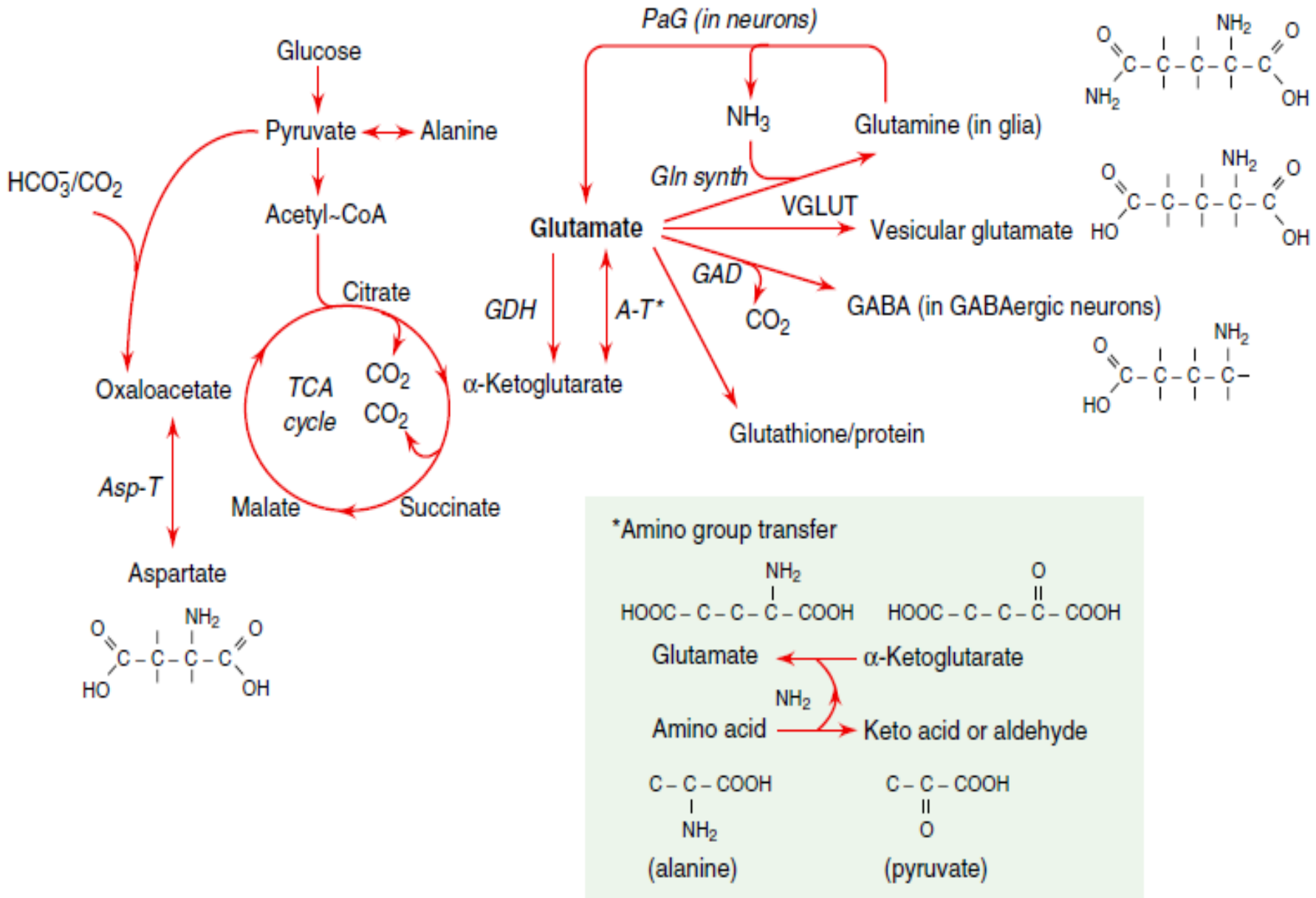
GABA

- Because GABA is not one of the 20 amino acids used to construct proteins, it is synthesized in large quantities only by the neurons that use it as a neurotransmitter.
- The precursor for GABA is glutamate, and the key synthesizing enzyme is glutamic acid decarboxylase (GAD).
- GAD, therefore, is a good marker for GABAergic neurons.
- Immunocytochemical studies have shown that GABAergic neurons are distributed widely in the brain. GABAergic neurons are the major source of synaptic inhibition in the nervous system.

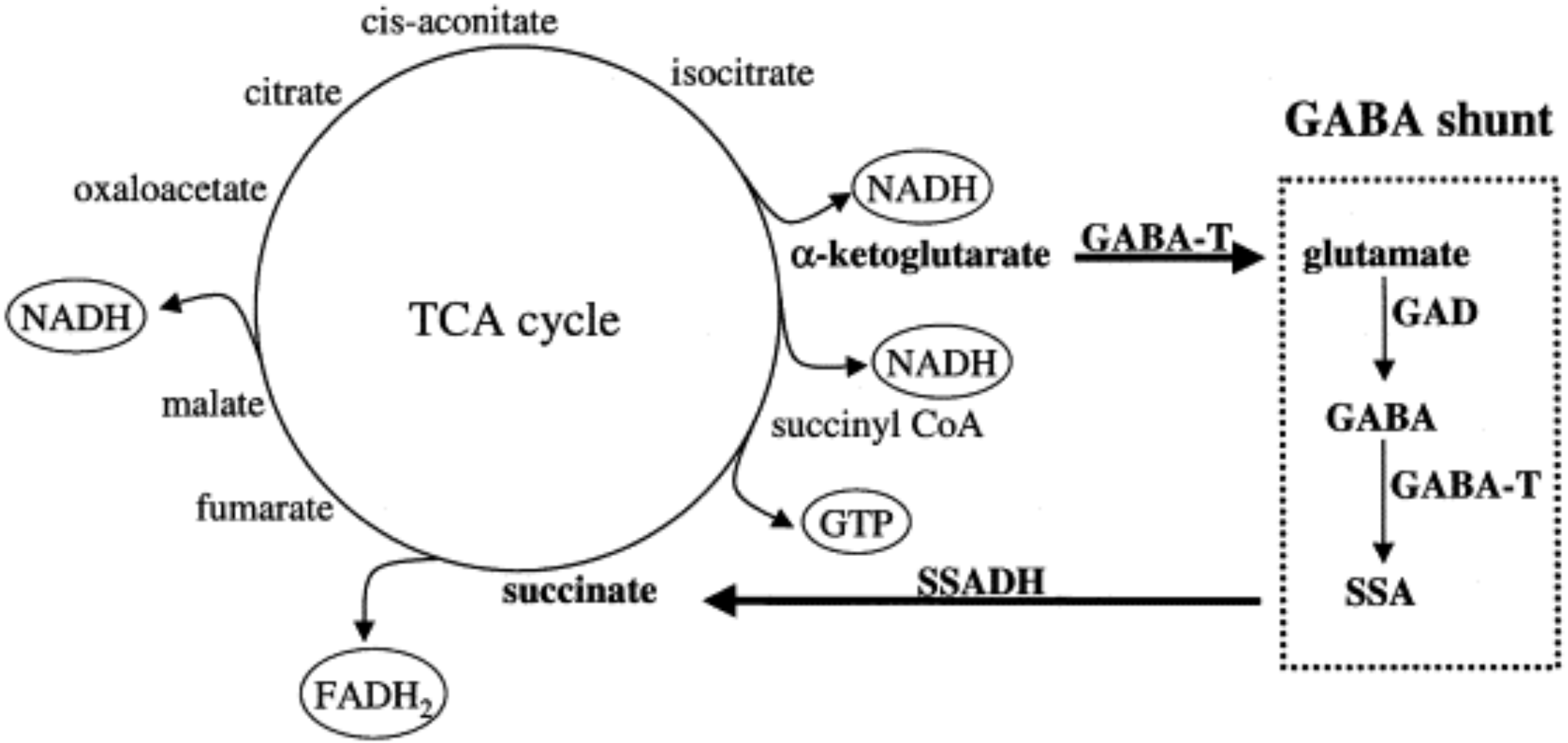


▲ FIGURE 6.16
The synthesis of GABA from glutamate.

- GABA cannot cross the blood-brain barrier and as such must be synthesized within neurons in the CNS. The synthesis of GABA in the brain occurs via a metabolic pathway referred to as the **GABA shunt.**


Glucose
Pyruvate
Alanine
HCO_3^-/CO_2
Acetyl~CoA
Citrate
Oxaloacetate
TCA cycle
CO_2
CO_2
Asp-T
Malate
Succinate
Aspartate
PaG (in neurons)
NH_3
Glutamine (in glia)
Gln synth
Glutamate
VGLUT
Vesicular glutamate
GAD
CO_2
GABA (in GABAergic neurons)
GDH
A-T*
α-Ketoglutarate
Glutathione/protein
*Amino group transfer
Glutamate
α-Ketoglutarate
Amino acid
Keto acid or aldehyde
(alanine)
(pyruvate)

GABA synthesis and degradation



This metabolic pathway traces the synthesis and degradation of the neurotransmitter pool of GABA. GAD, glutamic acid decarboxylase; GABA-T, GABA transaminase; SSADH, succinic semialdehyde dehydrogenase.

GABA synthesis and Degradation

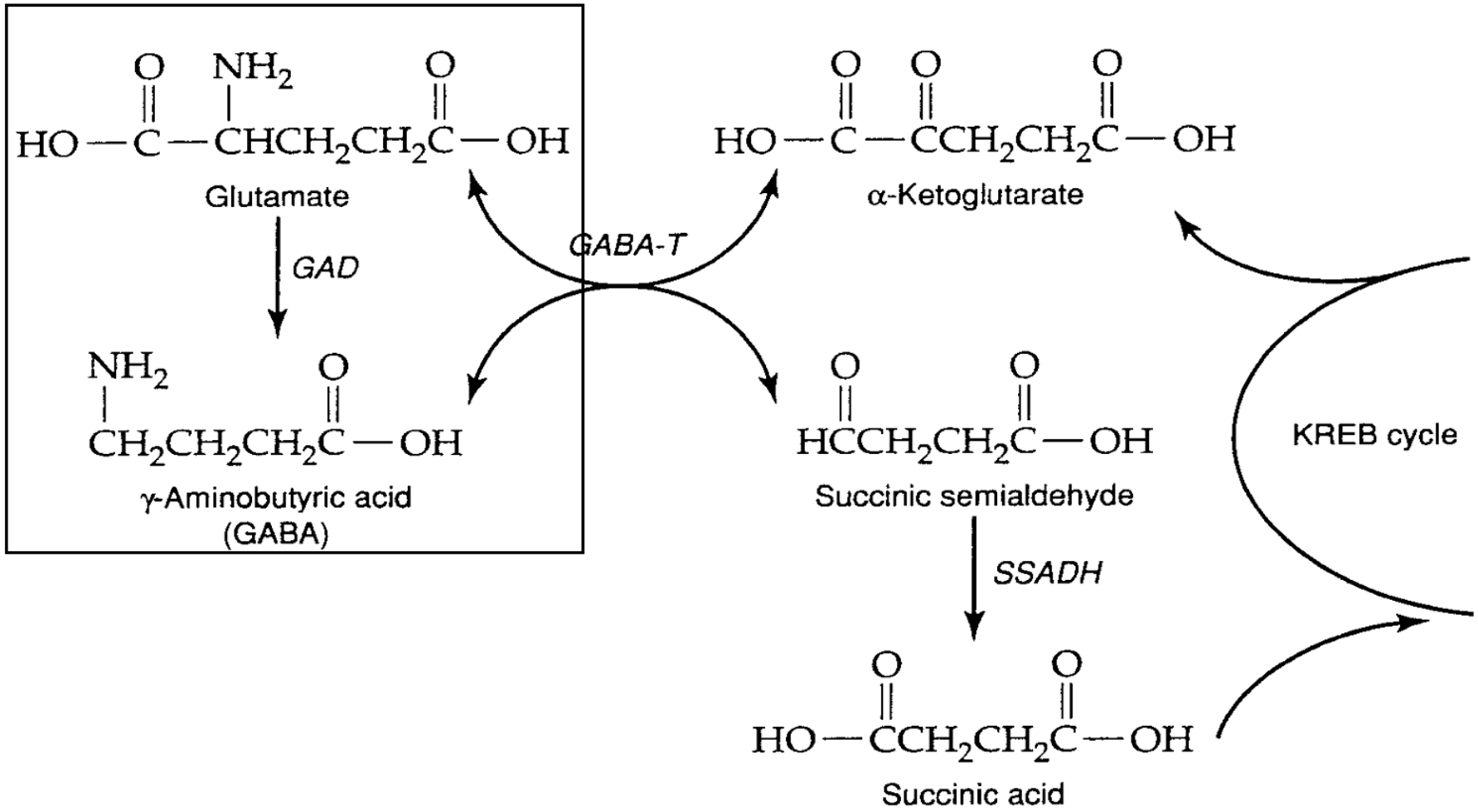
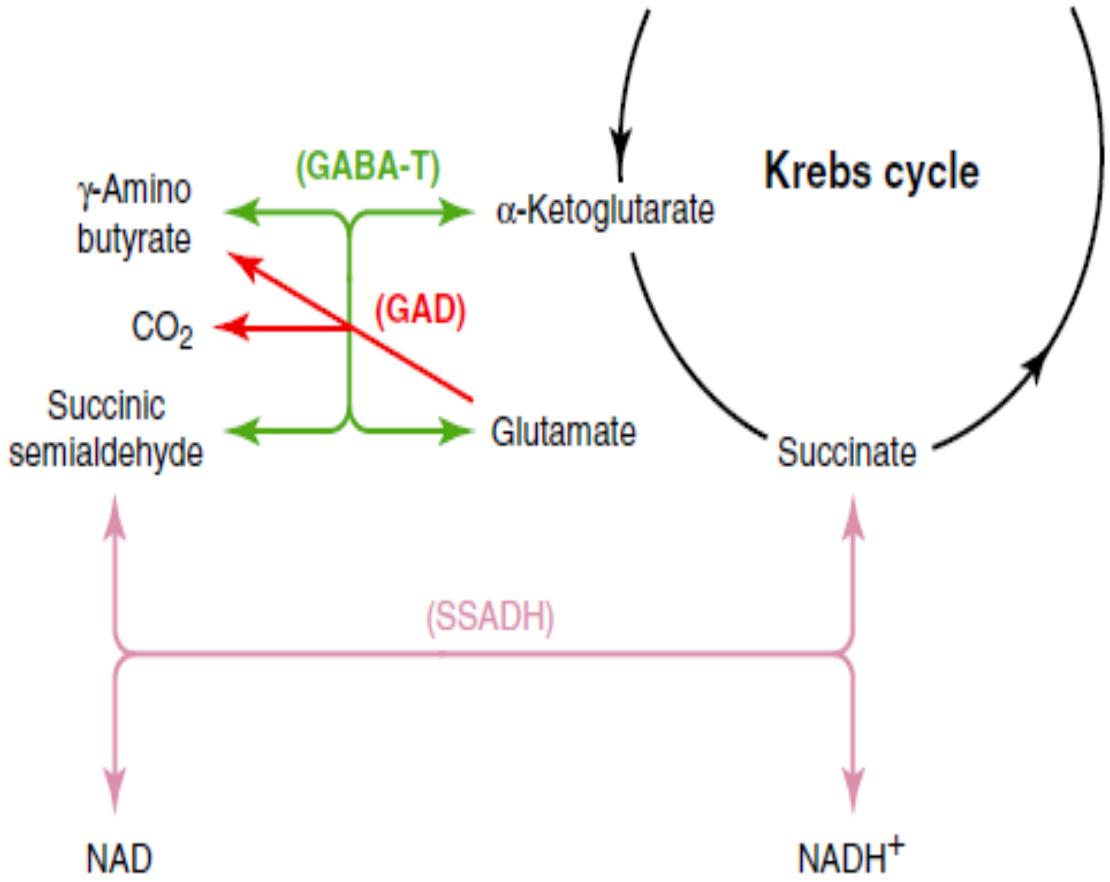
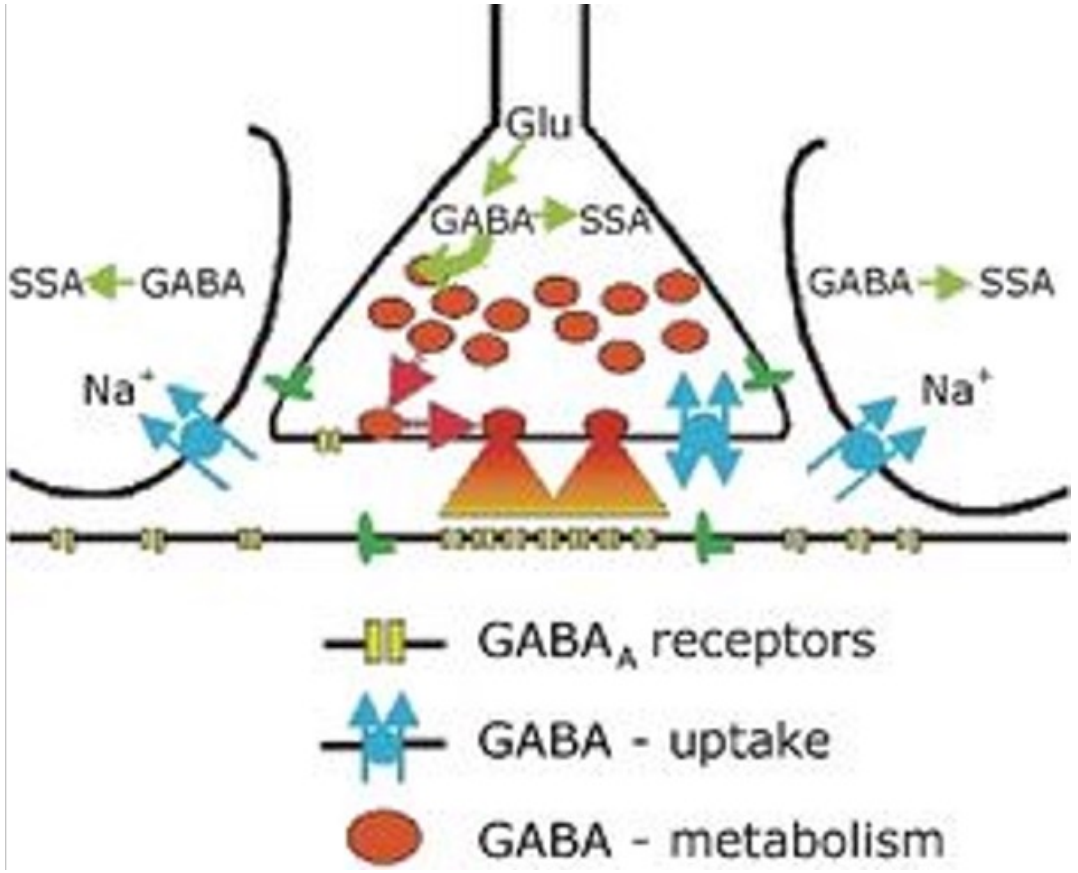


Figure 7–8. The GABA shunt. This metabolic pathway traces the synthesis and degradation of the neurotransmitter pool of GABA. GAD, glutamic acid decarboxylase; GABA-T, GABA transaminase; SSADH, succinic semialdehyde dehydrogenase.

- GABA-T removes GABA by converting it to succinic semialdehyde (SSA) via transamination with α-ketoglutarate which re-forms glutamate (green arrows). SSA is oxidized by NAD and SSA dehydrogenase (lavender arrows) to NADH+ and succinate, which re-enters the tricarboxylic cycle.



- GABA release into the synaptic cleft is stimulated by depolarization of presynaptic neurons.
- GABA diffuses across the cleft to the target receptors on the postsynaptic surface.
- The action of GABA at the synapse is terminated by reuptake into both presynaptic nerve terminals and surrounding glial cells.



Summary of GABA synthesis, release, reuptake, degradation

1. GABA is formed by removal of carboxyl group of glutamate, by the enzyme GAD.
2. GABA is packaged into synaptic vesicles by VIAAT(vesicular inhibitory amino acid transporter) and released by depolarization
3. GABA may be taken up by nerve terminal by GAT proteins for repackaging into synaptic vesicles
4. GABA may be taken up by glial cells, where it undergoes reconversion to glutamate (amine group is transferred to α-ketoglutarate, generating glutamate and succinic semialdehyde)
5. Glutamate is transported back into nerve terminal, where it serves as precursor for new GABA synthesis

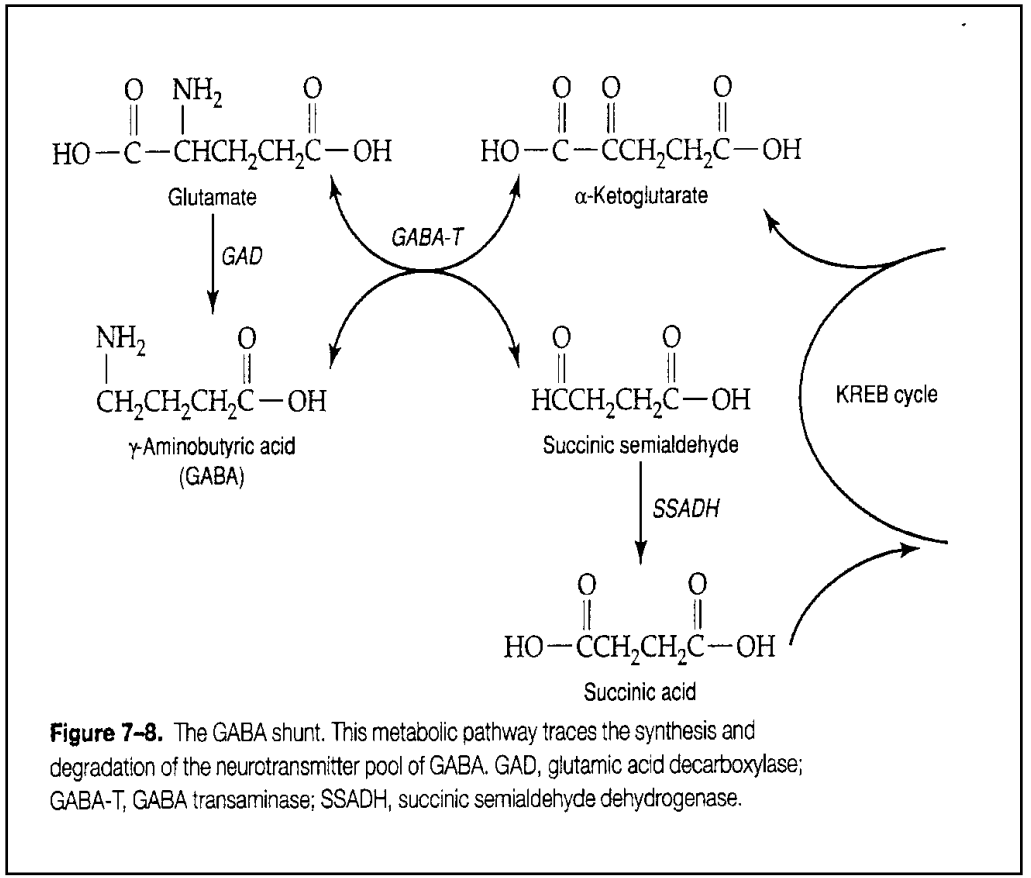
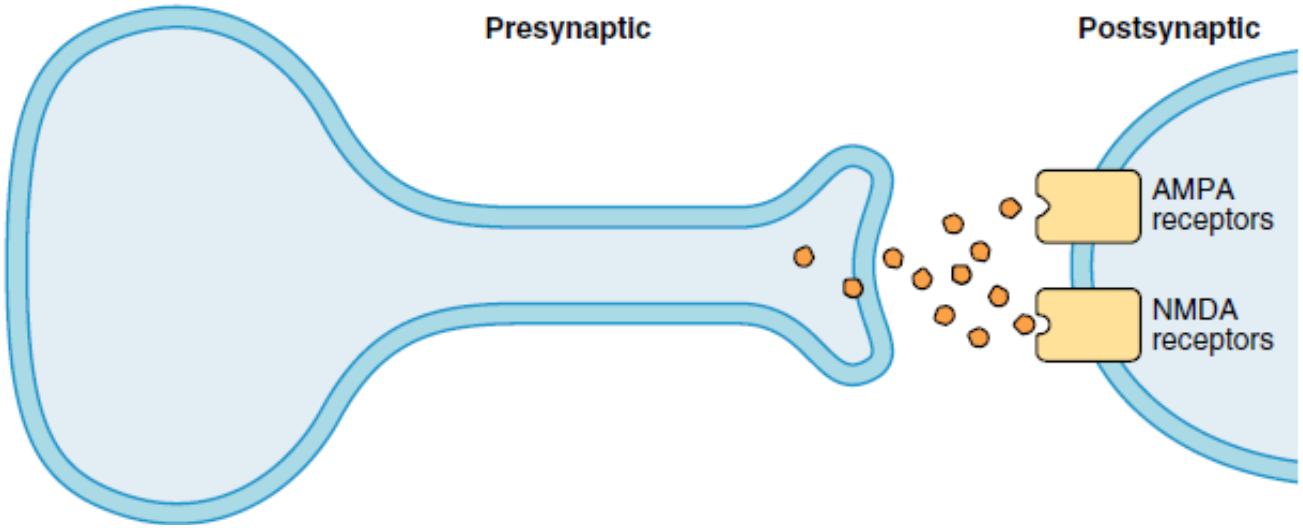


Figure 7–8. The GABA shunt. This metabolic pathway traces the synthesis and degradation of the neurotransmitter pool of GABA. GAD, glutamic acid decarboxylase; GABA-T, GABA transaminase; SSADH, succinic semialdehyde dehydrogenase.

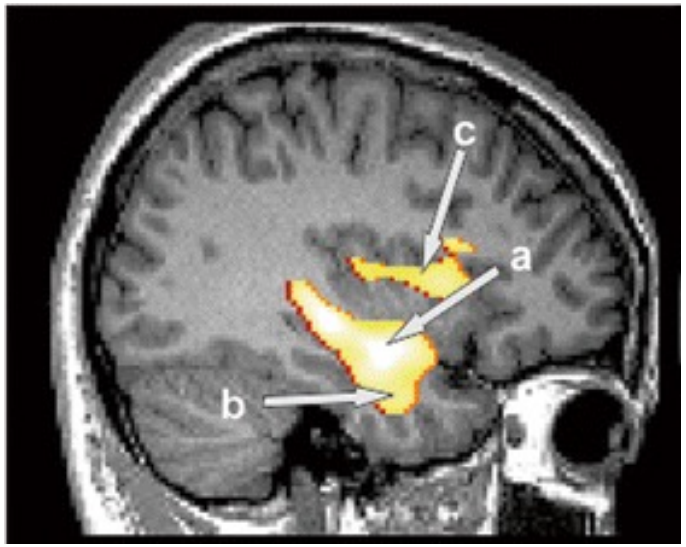
Glutamate, a major excitatory neurotransmitter in the brain



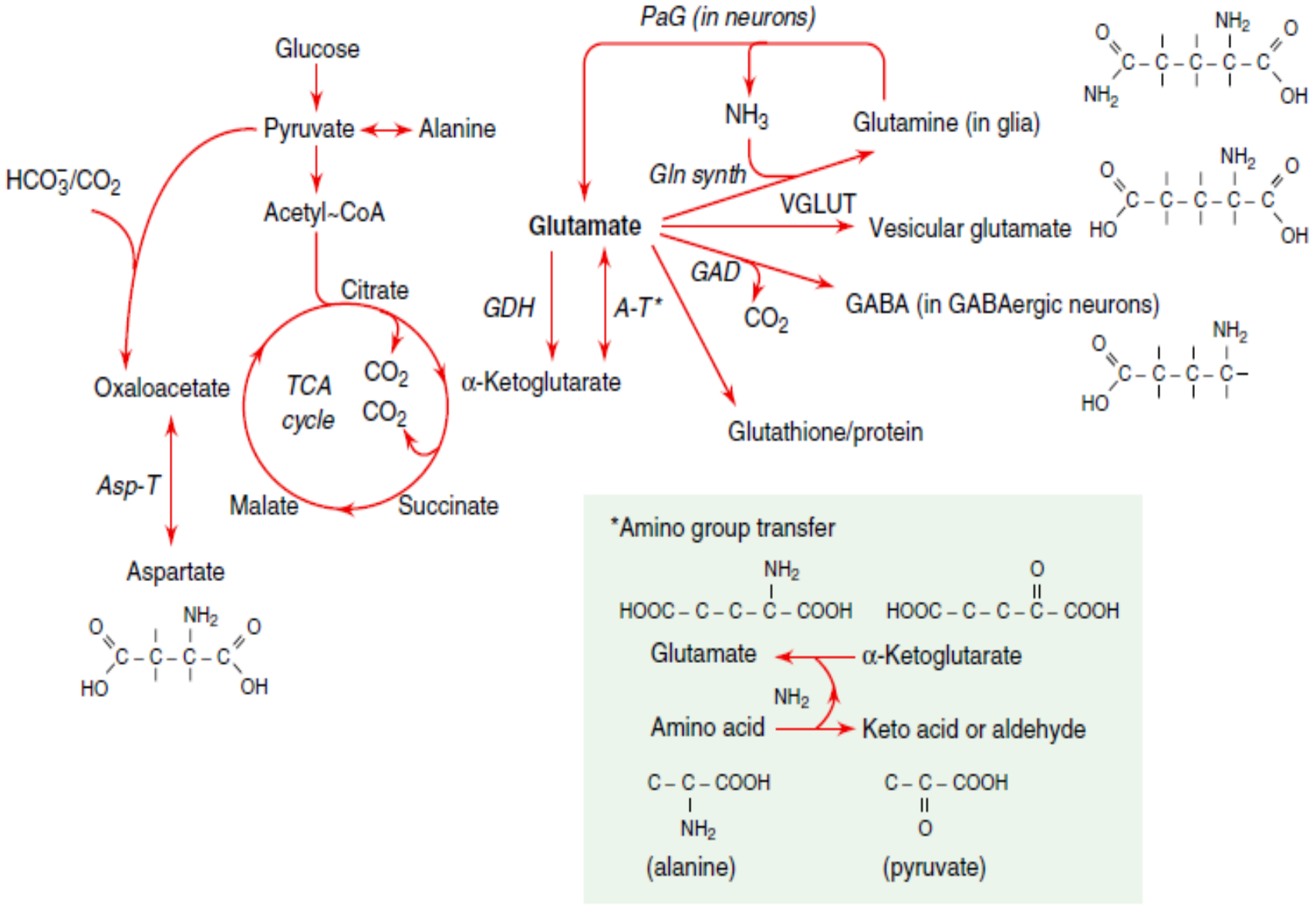
Glutamate

- Glutamate mediates most of the fast excitatory neurotransmission in the CNS, and it excites every neuron.
- Glutamate is the principal mediator of sensory information, motor coordination, emotions, and cognition, including memory formation and memory retrieval.
- As many as 90% of the neurons of the brain use glutamate as their neurotransmitter, and approximately 80–90% of the synapses in the brain are glutamatergic.

- Repolarization of membranes that are depolarized during glutamatergic activity may account for as much as 80% of the energy expenditure of the brain.
- The very high consumption of glucose and oxygen by the brain therefore largely fuels glutamatergic activity.
- The concentration of glutamate in brain gray matter structures varies between 10 and 15 µmol /gram of tissue, higher than in virtually all other tissues of the body. In white matter, the glutamate concentration is 4–6 µmol/g.
- Glutamate is a nonessential amino acid that does not cross the blood-brain barrier and therefore must be synthesized in neurons from local precursors.

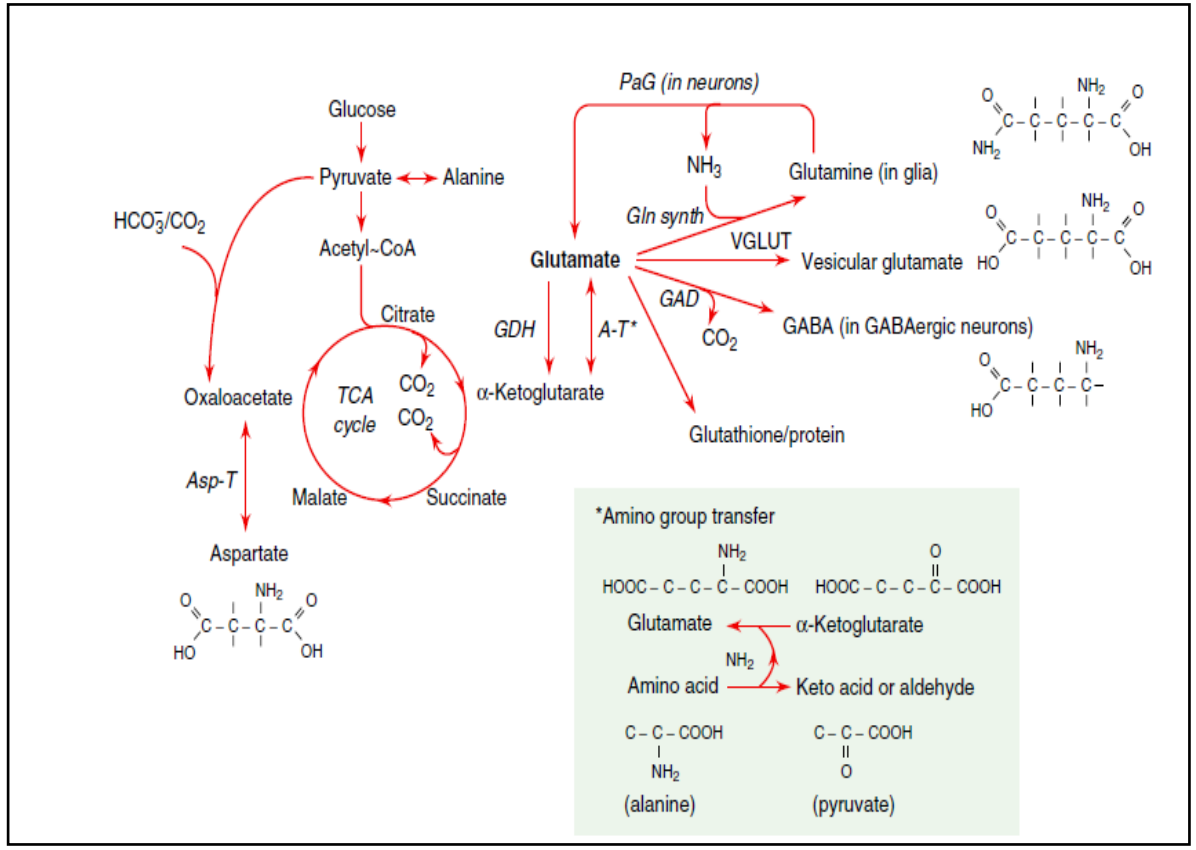


Glutamate metabolism



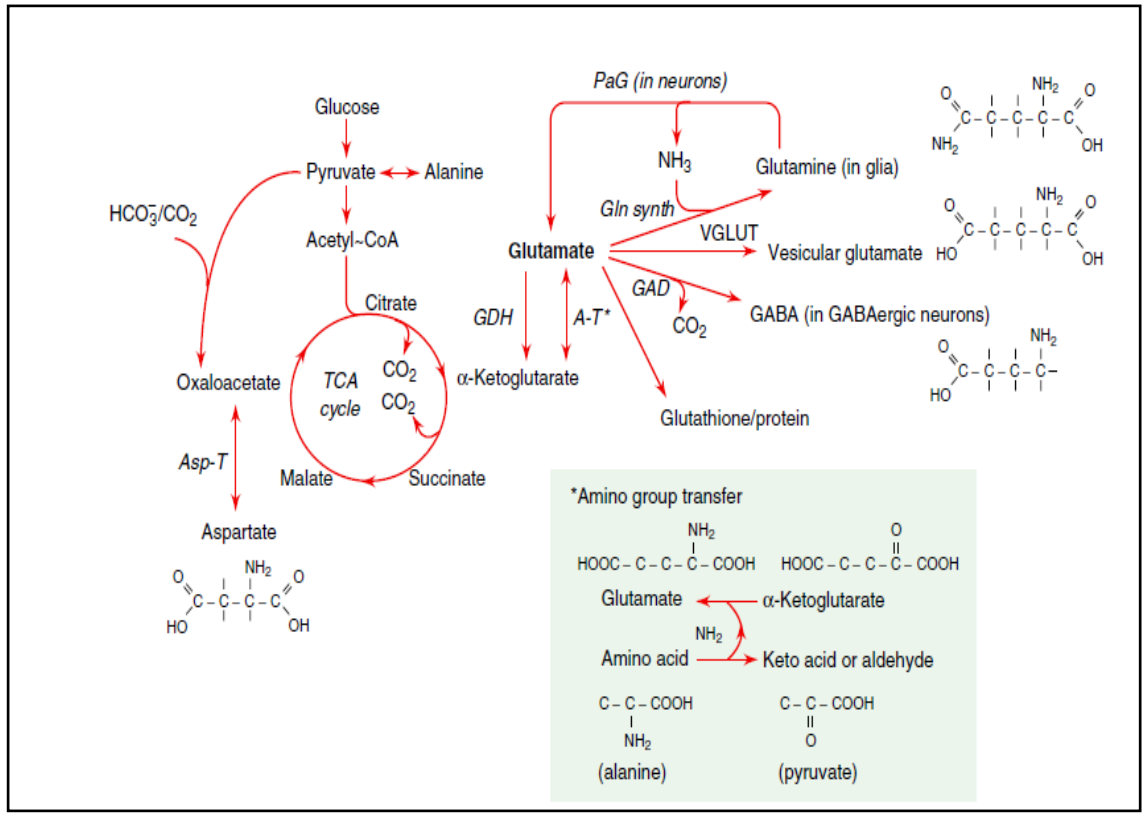
- The formation of glutamate from α-ketoglutarate, a TCA cycle intermediate. α-Ketoglutarate, which is formed from glucose, constitutes the carbon backbone of glutamate.
- The amino group derives from another amino acid, which after the donation of its amino group becomes a keto acid or an aldehyde . This amino donor may be aspartate, alanine or some other amino acid.
- Glutamate takes part in many biochemical reactions: in glial cells, it is converted to glutamine, and in GABAergic neurons, it is converted into GABA.
- Transmitter glutamate is defined by its accumulation into synaptic vesicles. The glutamine cycle is shown, with the conversion of glutamate into glutamine in glia and the conversion of glutamine into glutamate in neurons.

Glutamate metabolism



- *PaG: periaqueductal gray*
- *VGLUTs :vesicular glutamate transporters*
- *GAD: glutamic acid decarboxylase*

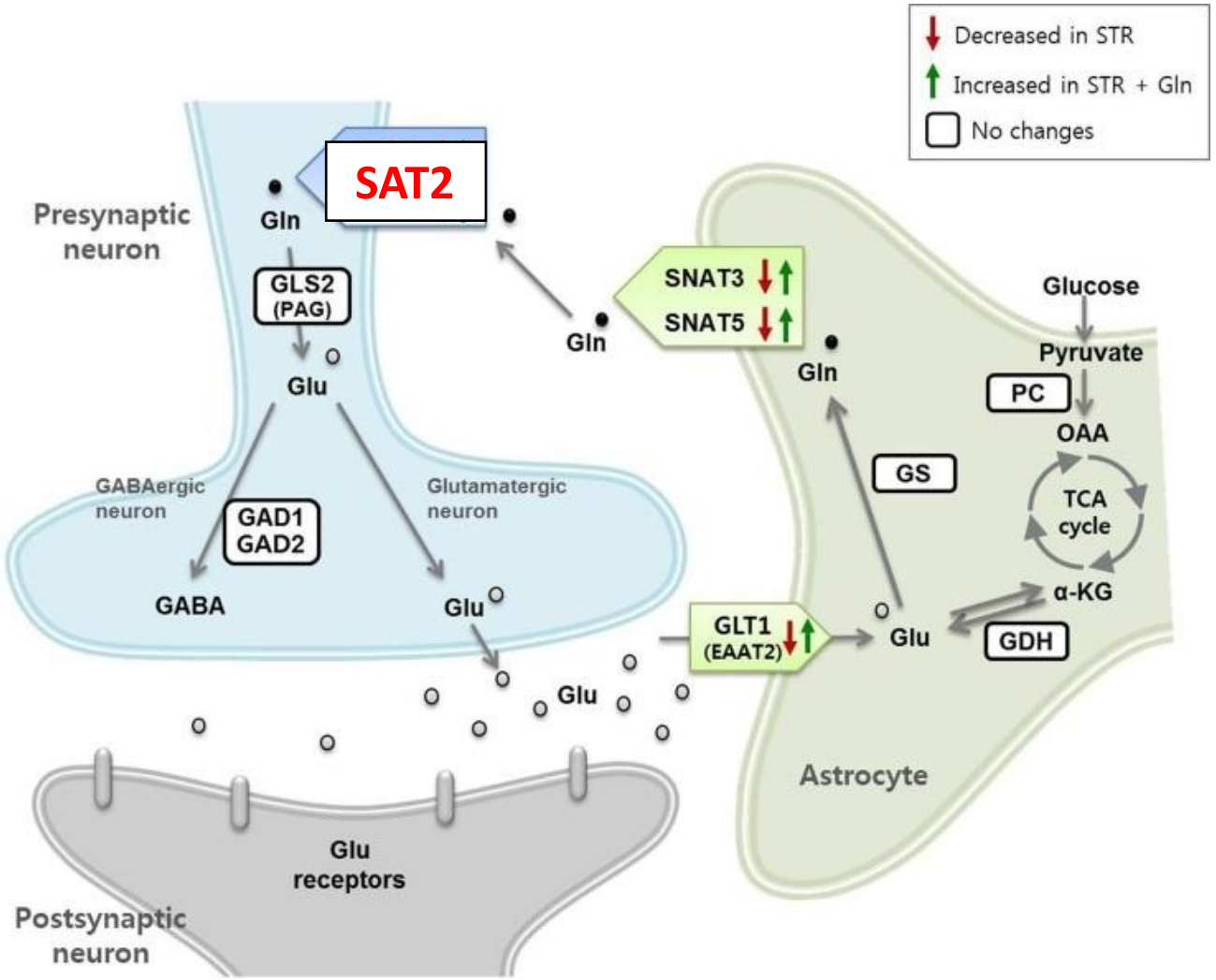
the pyruvate carboxylation (anaplerosis) which replenishes the TCA cycle (glial or neuronal) with malate or oxaloacetate to compensate for the loss of glutamate, glutamine, or (in GABAergic neurons) GABA.



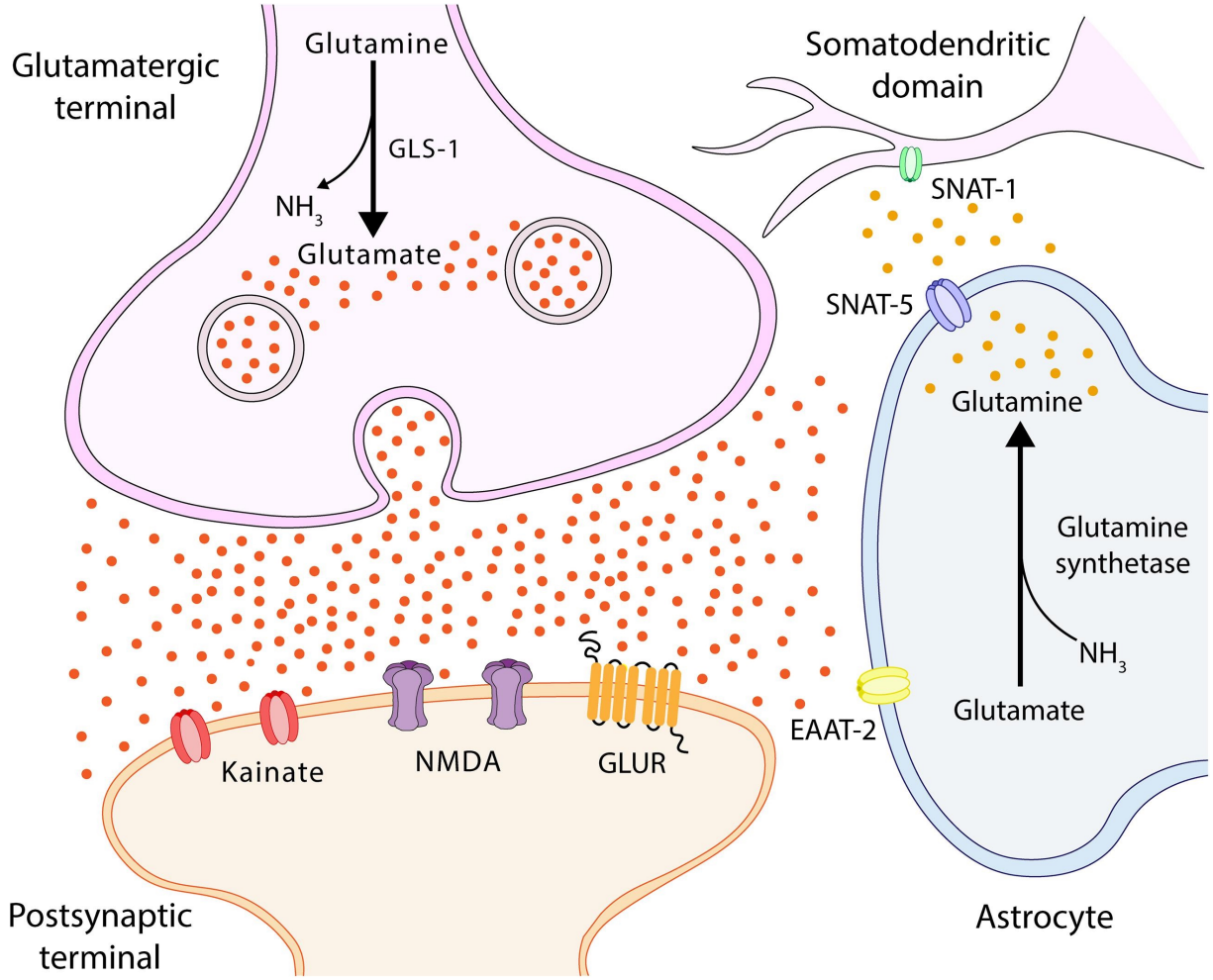
Asp-T, aspartate aminotransferase; *A-T*, aminotransferase reaction (Asp-T or other); *GABA*, γ-aminobutyric acid;*GAD*, glutamic acid decarboxylase; *GDH*, glutamate dehydrogenase; *Gln synth*, glutamine synthetase; *PaG*, phosphate-activated glutaminase, *TCA* cycle, tricarboxylic acid cycle; *VGLUT*, vesicular glutamate transporter

Glutamate –Glutamine cycle

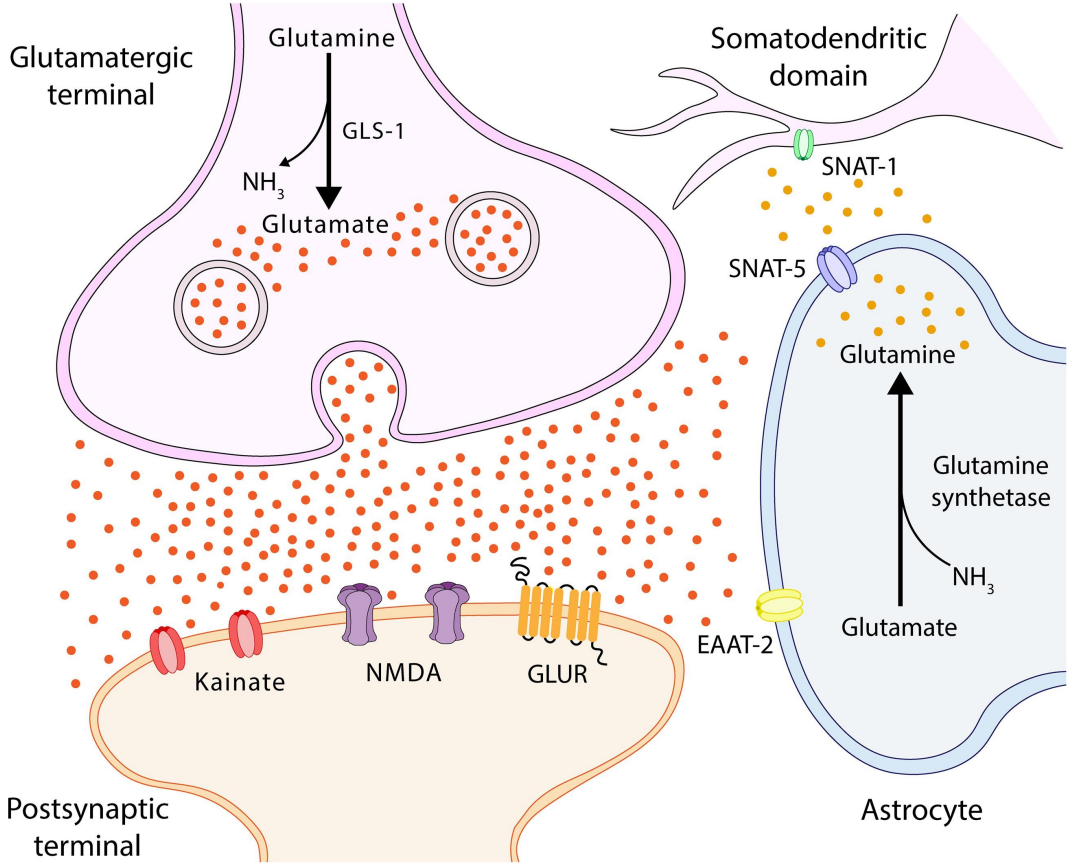
- The most prevalent precursor for glutamate synthesis is glutamine, which is taken up into presynaptic terminals by the system A transporter 2 (SAT2) and is then metabolized to glutamate by the mitochondrial enzyme glutaminase.
- Glucose metabolized by neurons also can be used to synthesize glutamate by transamination of 2- oxoglutarate, an intermediate of the tricarboxylic acid (Krebs) cycle.



- Glutamate synthesized in the presynaptic cytoplasm is packaged into synaptic vesicles by vesicular glutamate transporters (VGLUTs). Once released, glutamate is removed from the synaptic cleft by the excitatory amino acid transporters (EAATs). EAATs are a family of five different Na+-dependent glutamate co-transporters.

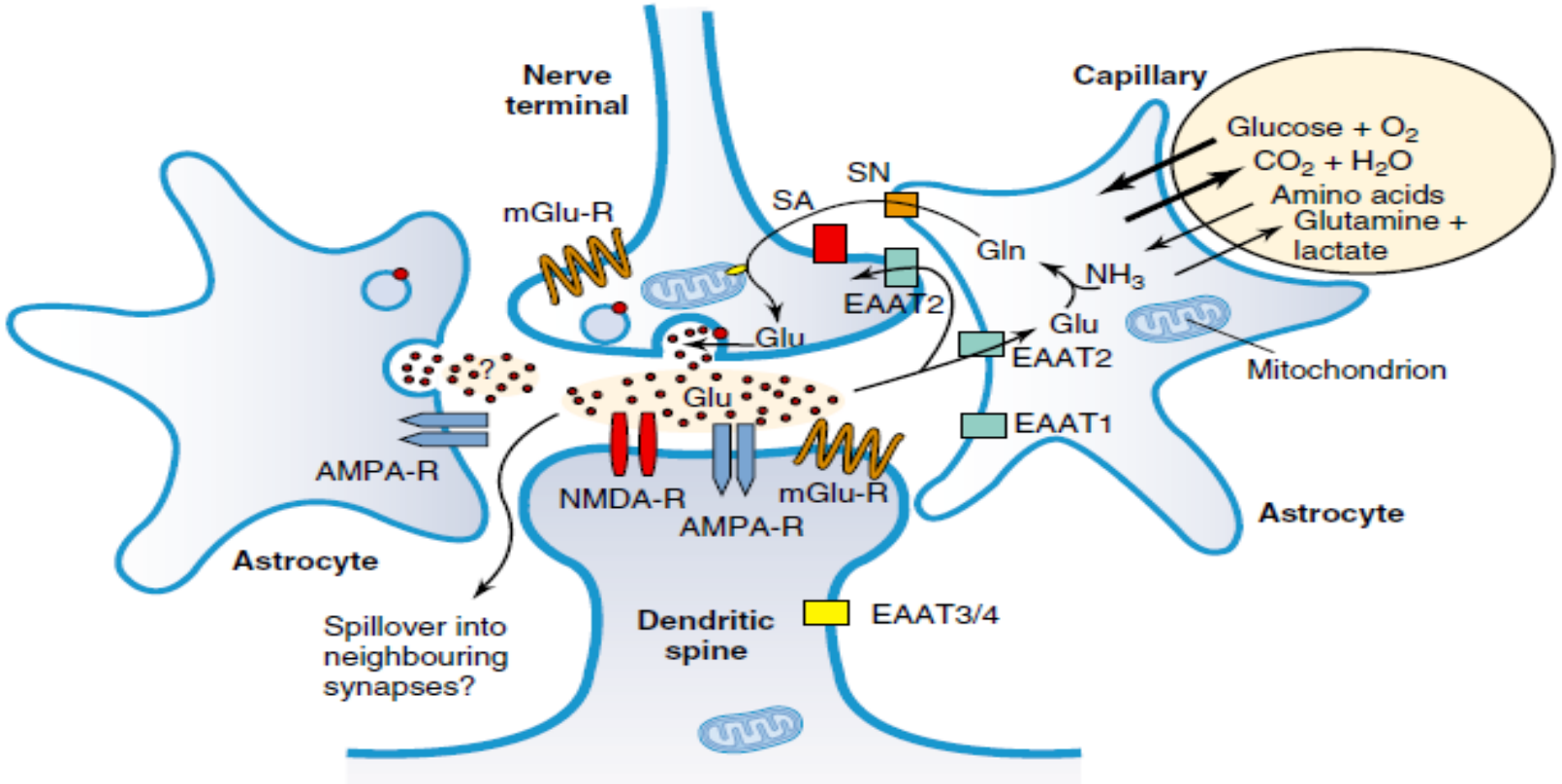


- Some EAATs are present in glial cells and others in presynaptic terminals. Glutamate transported into glial cells via EAATs is converted into glutamine by the enzyme glutamine synthetase.
- Glutamine is then transported out of the glial cells by a different transporter, the system N transporter 1 (SN1), and transported into nerve terminals via SAT2. This overall sequence of events is referred to as the **glutamate–glutamine cycle .** This cycle allows glial cells and presynaptic terminals to cooperate both to maintain an adequate supply of glutamate for synaptic transmission and to rapidly terminate postsynaptic glutamate action.



A glutamatergic, axodendritic synapse consisting of a presynaptic nerve terminal and a postsynaptic dendritic spine.

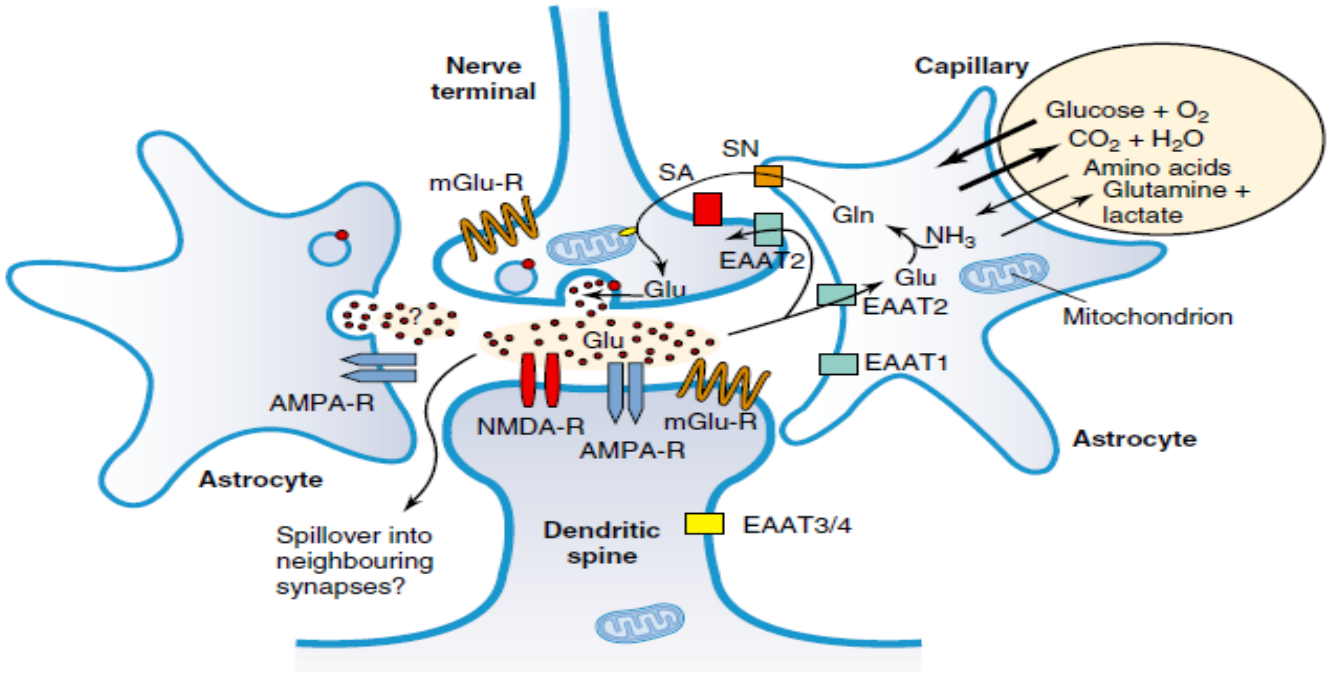
The nerve terminal contains synaptic vesicles with glutamate transporters (*red dot*), mitochondria (*blue*) with glutaminase in nerve terminal (*yellow dot*), metabotropic glutamate receptors, and transporters for glutamate (EAAT2) and glutamine (SA transporters).



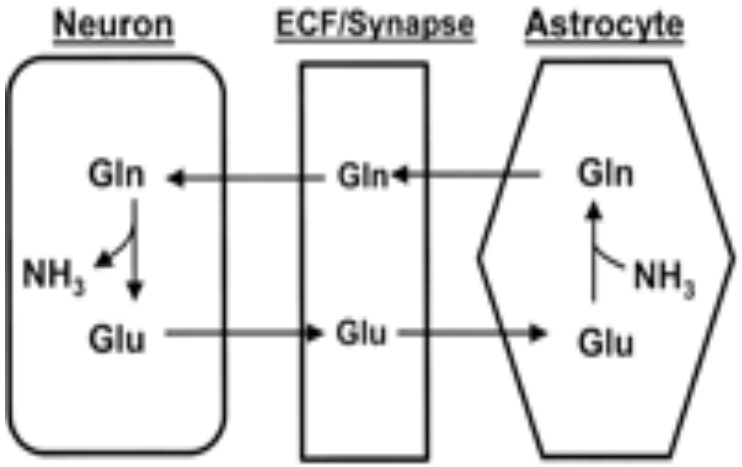
The postsynaptic dendritic spine contains glutamate receptors, both ionotropic (AMPA and NMDA type) and metabotropic, and glutamate transporters (EAAT3 and EAAT4), Surrounding the synapse are astrocytic processes with glutamate (EAAT1 and EAAT2) and glutamine transporters (SN transporters), glutamate receptors and even glutamate-filled vesicles.

Green rectangles in plasma membrane of the axon terminal represent EAAT2 and of the astrocyte represent EAAT1/EAAT2 glu transporters.

Glutamate that escapes out of the synapse without being cleared by transporters may spill over into neighboring synapses.



- Much of the glutamate that is released from nerve terminals is taken up from the extracellular fluid into astrocytic processes that surround synapses.
- In astrocytes glutamate reacts with ammonia to form glutamine through the activity of glutamine synthetase, a cytosolic, ATP-dependent enzyme that astrocytes and oligodendrocytes express but neurons do not.
- This reaction is important even for the detoxification of free ammonia because accumulation of ammonia would interfere with synaptic function. Glutamine, which does not have neurotransmitter properties, is exported to the extracellular fluid and taken up by neurons.

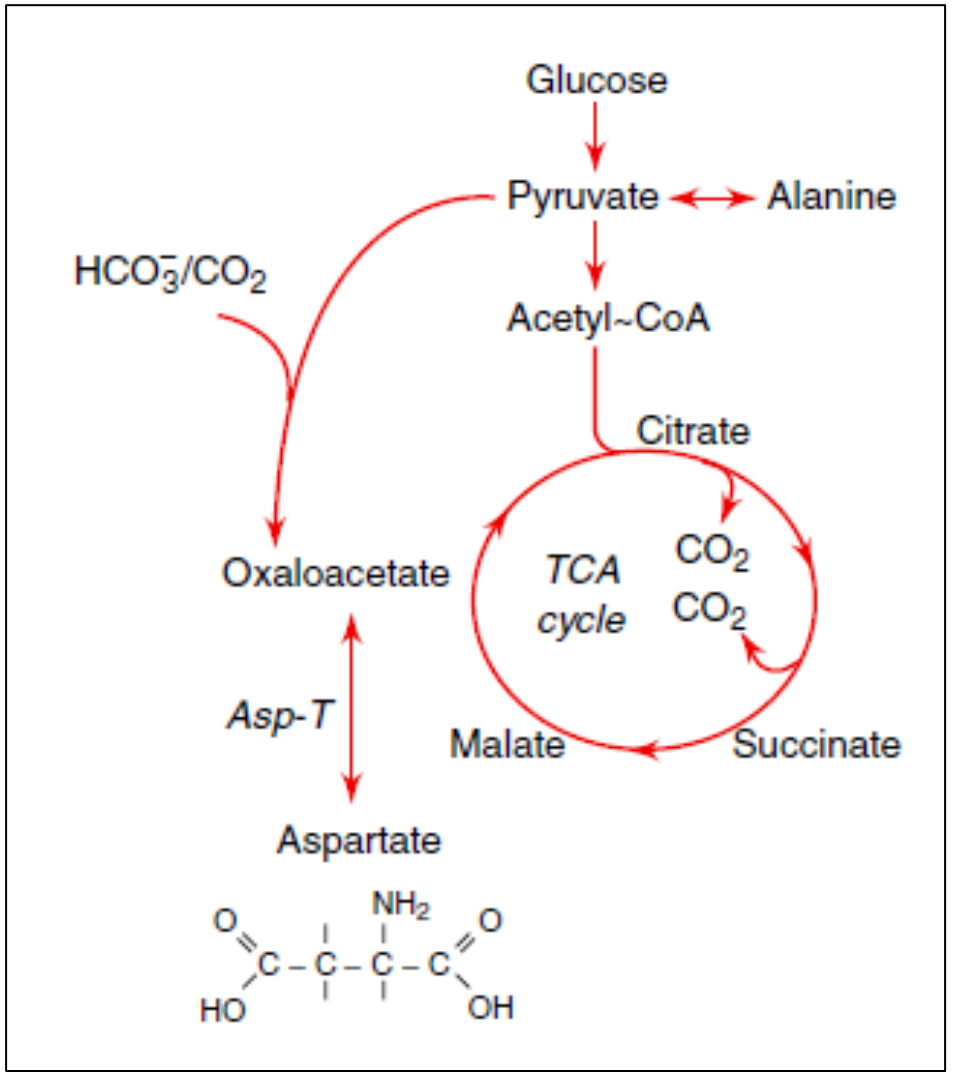


The release of glutamate from nerve endings leads to the loss of α-ketoglutarate from the tricarboxylic acid cycle.

The glutamine cycle counteracts an important metabolic challenge inherent in glutamatergic neurotransmission:

- It helps prevent loss of α-ketoglutarate from the neuronal TCA cycle. When neurons release glutamate and astrocytes take it up, the neuronal TCA cycle loses α-ketoglutarate. If this loss of α-ketoglutarate from the TCA cycle is not countered, the downstream product oxaloacetate will not be available for the formation of citrate (and the energy metabolism stops).
- The return of glutamine for transmitter glutamate reduces the loss of neuronal α-ketoglutarate but probably does not prevent it completely, partly because astrocytes metabolize some transmitter glutamate through their own TCA cycle as an energy substrate.

Two additional mechanisms exist that maintain the level of glutamate and TCA cycle intermediates in the nerve terminal: one is the reuptake of glutamate from the extracellular fluid back into the nerve terminal, and **the other is the reaction of pyruvate (derived from glycolysis) with CO2 to form TCA cycle intermediate malate, the precursor of oxaloacetate.** This process is called pyruvate carboxylation. Pyruvate carboxylation in the nerve terminal may be catalyzed by the malic enzyme, a reversible enzyme that utilizes CO2 and NADPH to form malate from pyruvate

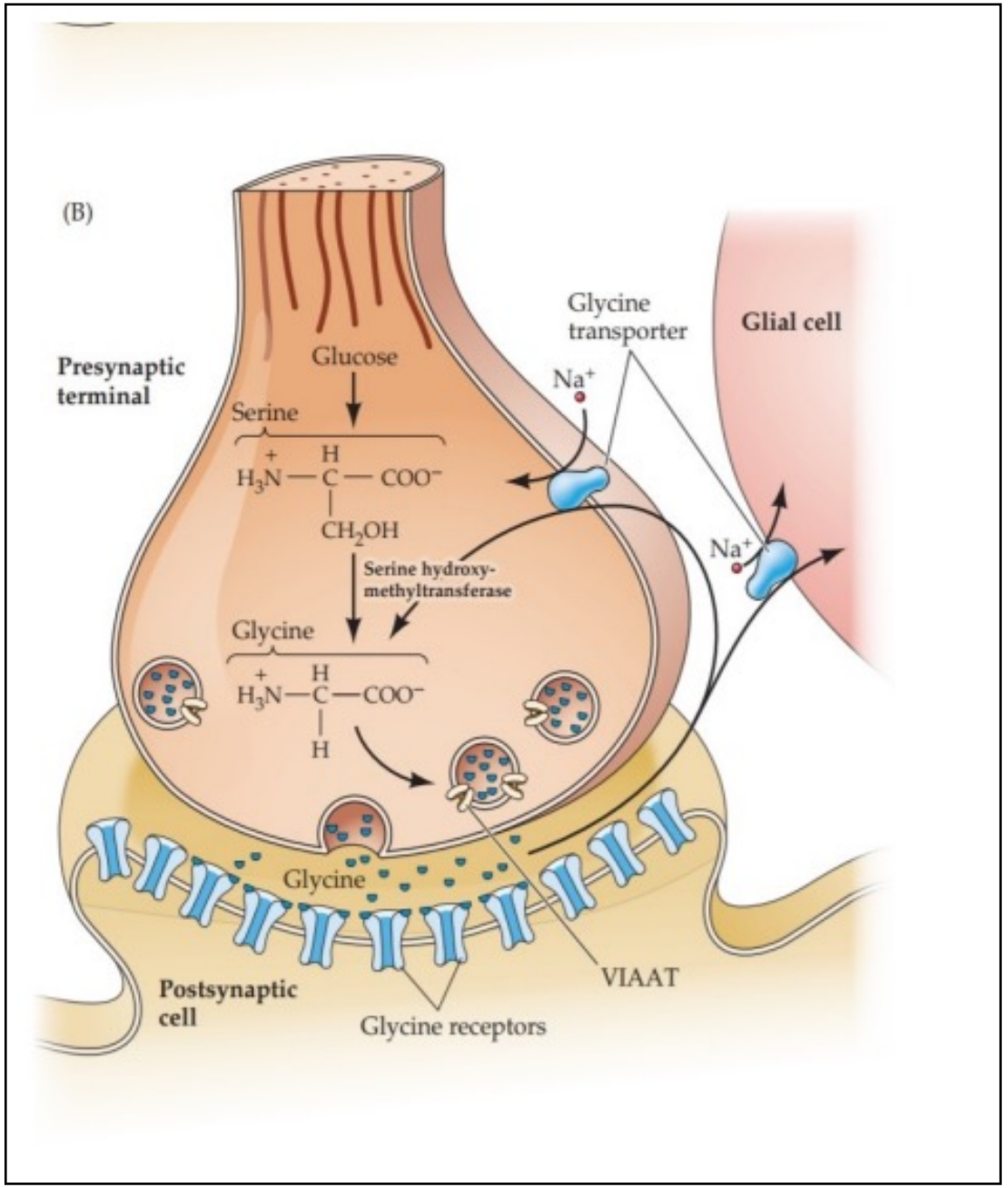


Glycine

Glycine is synthesized from serine by the mitochondrial isoform of serine hydroxymethyltransferase After synthesis glycine is transported into synaptic vesicles via the same vesicular inhibitory amino acid transporter that loads GABA into vesicles.

Once released from the presynaptic cell, glycine is rapidly removed from the synaptic cleft by glycine transporters in the plasma membrane.

Mutations in the genes coding for some of these transporters result in hyperglycinemia, a devastating neonatal disease characterized by lethargy, seizures, and mental retardation.

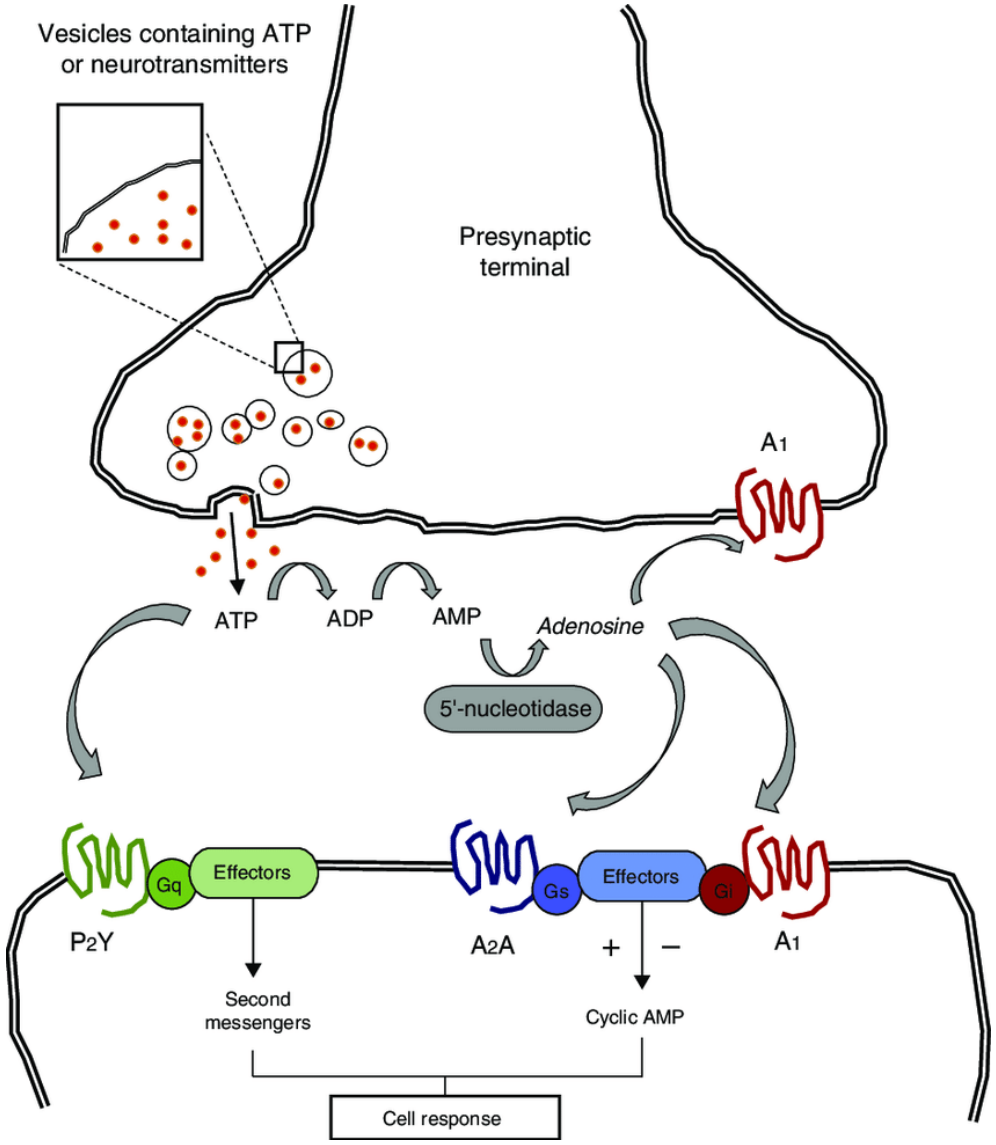


Adenosine triphosphate (ATP) as neurotransmitter

- ATP is concentrated in all synaptic vesicles in the CNS and PNS, and it is released into the cleft by presynaptic increase in a Ca+2-dependent manner, just as the classic transmitters .

- ATP is often packaged in vesicles along with another classic transmitter. For example, catecholamine (400mM) containing vesicles may have ATP (100mM), as co-transmitter.

- ATP also occurs as a co-transmitter with GABA, glutamate, ACh, DA, and peptide transmitters in various specialized types of neurons.
- ATP directly excites some neurons by gating cation channels.

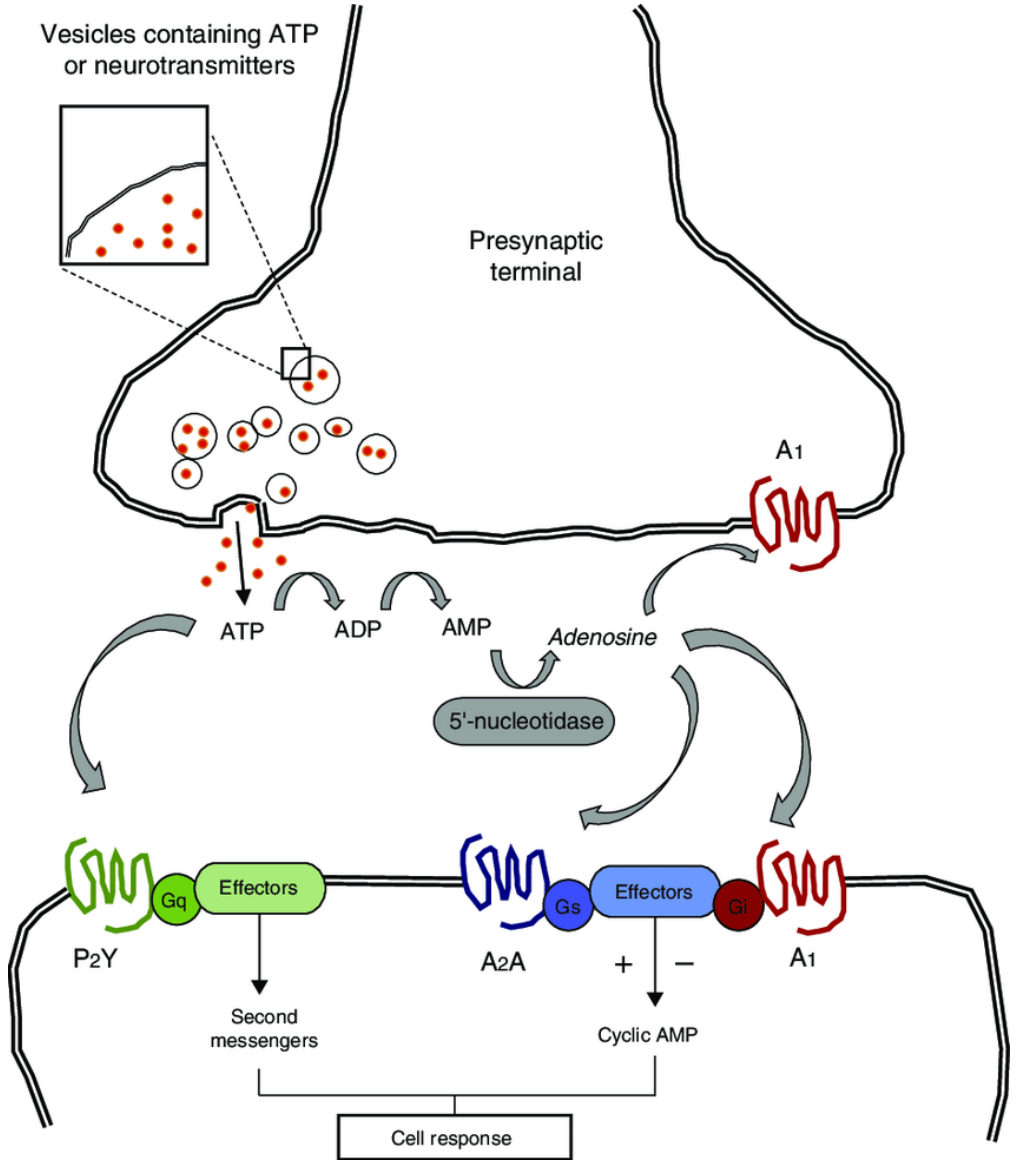


Adenosine triphosphate (ATP) as neurotransmitter

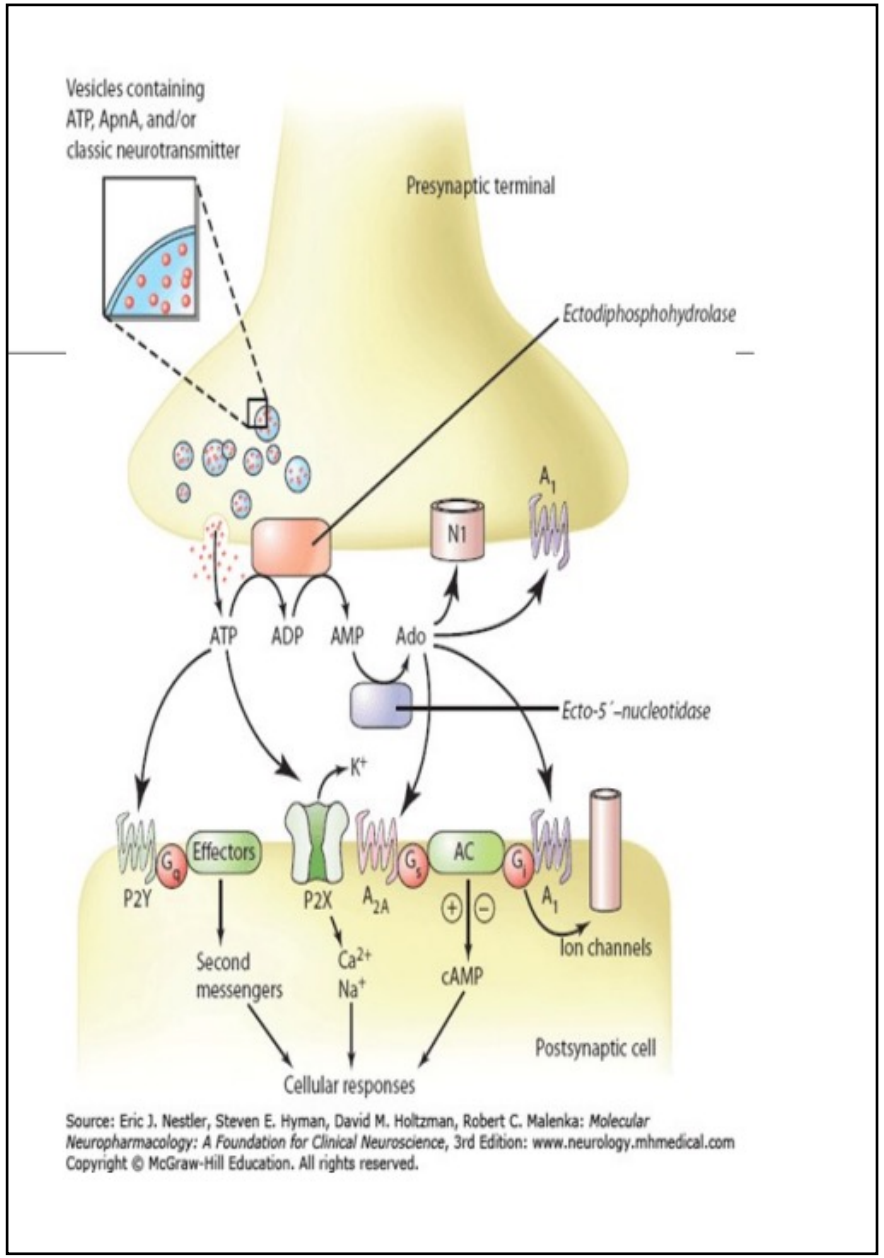
- Some of the neurotransmitter functions of ATP are similar to those of glutamate and ACh.
- ATP binds to purinergic receptors, some of which are transmitter-gated ion channels.

- There is also a large class of G-protein-coupled purinergic receptors.
- Following its release from synapses, ATP is degraded by extracellular enzymes, yielding adenosine.

- Adenosine itself does not meet the standard definition of a neurotransmitter because it is not packaged in vesicles, but it does activate several adenosine-selective receptors.



- A purinergic synapse Adenosine triphosphate (ATP) are colocalized with a classic neurotransmitter and are released into the synaptic cleft in a Ca2+-dependent fashion.
- After it is released, ATP can directly activate P2Y and P2X receptors (purinergic receptors).
- P2Y receptors are coupled to G proteins and activate second messenger systems. P2X receptors are ligand-gated channels that depolarize the postsynaptic membrane.
- ATP remaining in the synapse is rapidly converted into adenosine (Ado) by the actions of an ectodiphosphohydrolase and an ecto-5´-nucleotidase.
- Ado is able to activate presynaptic and postsynaptic G protein– coupled P1 receptors (A1 and A2) and regulate adenylyl cyclase (AC) and the cAMP pathway, and in turn can be recycled into the presynaptic cell by means of a Na+-dependent transporter (N1).



Source: Eric J. Nestler, Steven E. Hyman, David M. Holtzman, Robert C. Malenka: *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience*, 3rd Edition: www.neurology.mhmedical.com

Unconventional Neurotransmitters: Endocannabinoids & Nitric oxide (NO)

- These chemical signals can be considered as neurotransmitters because of their roles in interneuronal signaling and because their release from neurons is regulated by Ca2+.
- However, they are unconventional in comparison with other neurotransmitters because they are not stored in synaptic vesicles and are not released from presynaptic terminals via exocytotic mechanisms.
- In fact, these unconventional neurotransmitters need not be released from presynaptic terminals at all and are often associated with retrograde signaling (that is, from postsynaptic cells back to presynaptic terminals).

Endocannabinoids

Endocannabinoids (endogenous cannabinoids) are small lipid molecules, released from postsynaptic neurons and act on the presynaptic terminal (Retrograde messengers).

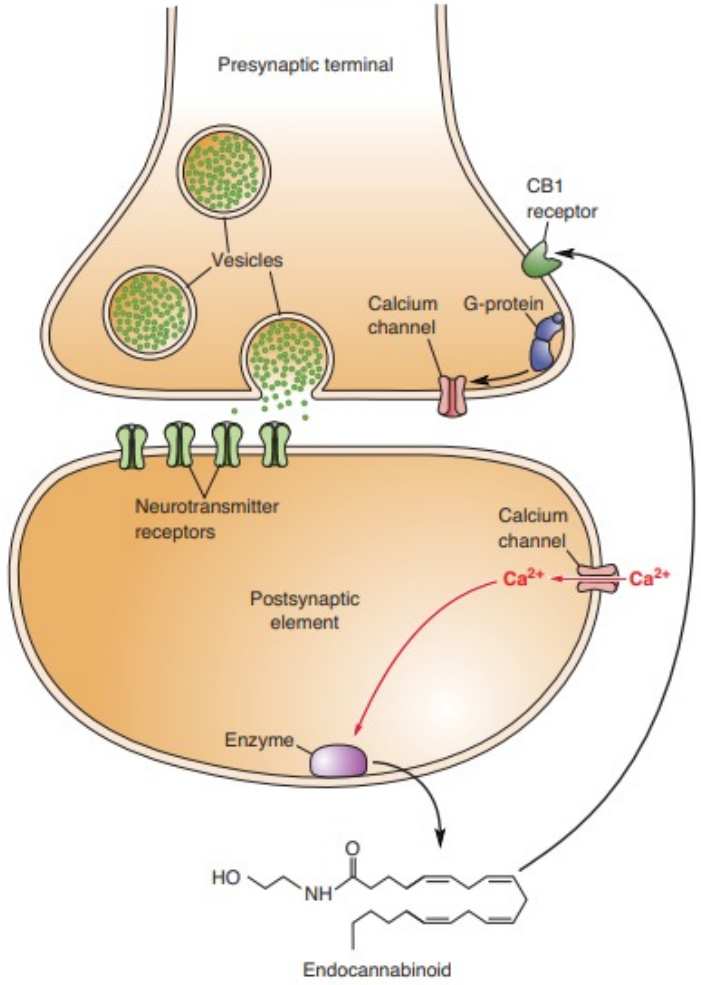
1. They are not packaged in vesicles like most other neurotransmitters; instead, they are manufactured rapidly and on demand.

2. They are small and membrane permeable; once synthesized, they can diffuse rapidly across the membrane of their cell of origin to contact neighboring cells.

3. They bind selectively to the CB1 type of cannabinoid receptor.

- These are G protein-coupled receptors located on certain presynaptic terminals.
- Their main effect is often to reduce the opening of presynaptic calcium channels.
- With its calcium channels inhibited, the ability of the presynaptic terminal to release its neurotransmitter (usually GABA or glutamate) is impaired.
- Thus, when a postsynaptic neuron is very active, it releases endocannabinoids which serve as a feedback system (suppress either the inhibitory or excitatory drive onto the neuron) to regulate the conventional forms of synaptic transmission, which of course go from “pre” to “post.”

▶ FIGURE 6.17
Retrograde signaling with endocannabinoids.



Nitric oxide (NO)

- NO (a gaseous molecule) is synthesized from the amino acid arginine by many cells of the body and has powerful biological effects, particularly in the regulation of blood flow.
- In the nervous system, NO may be another example of a retrograde messenger.
- NO is small and membrane permeable, similar to endocannabinoids, it can diffuse much more freely than most other transmitter molecules, even penetrating through one cell to affect another beyond it.
- Its influence may spread throughout a small region of local tissue, rather than being confined to the site of the cells that released it. NO is evanescent and breaks down very rapidly.

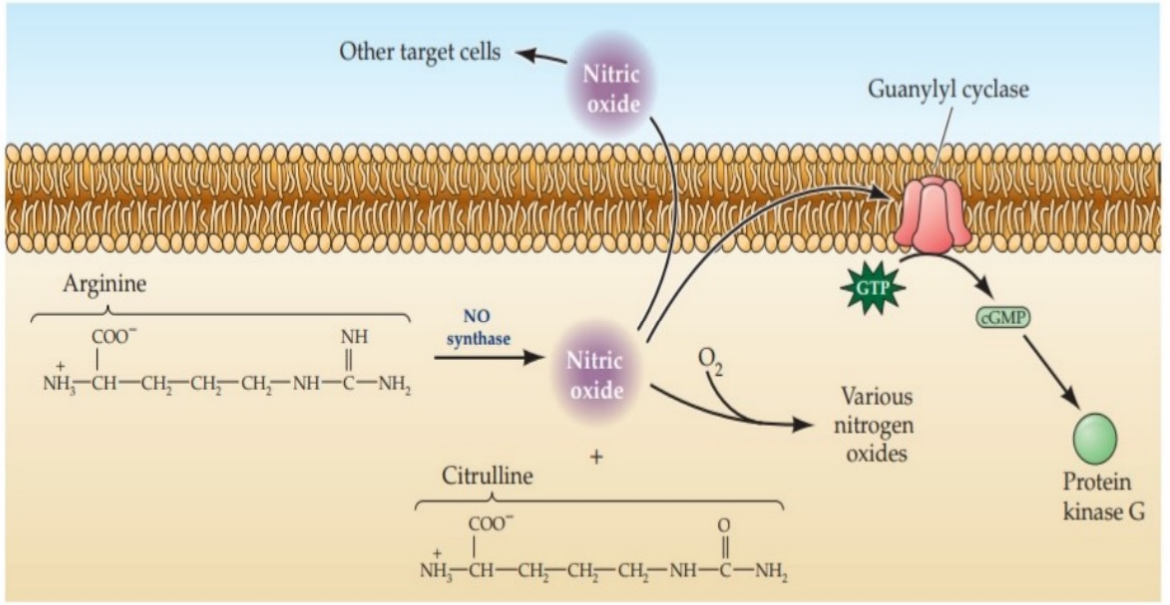


FIGURE 6.25 Synthesis, release, and termination of nitric oxide (NO).

Peptide Neurotransmitters

- The mechanisms responsible for the synthesis and packaging of peptide transmitters are fundamentally different from those used for the small-molecule neurotransmitters and are much like those used for the synthesis of proteins that are secreted from non-neuronal cells Peptide-secreting neurons generally synthesize polypeptides that are much larger than the final, “mature” peptide.
- Processing these polypeptides, which are called pre-propeptides (or pre-proproteins), takes place within the neuron’s cell body by a sequence of reactions that occur in several intracellular organelles.
- Pre-propeptides are synthesized in the rough endoplasmic reticulum, where the signal sequence is removed.
- The remaining polypeptide called a propeptide (or proprotein), then traverses the Golgi apparatus and is packaged into vesicles in the trans-Golgi network. In the vesicles, proteolytic cleavage, modification of the ends of the peptide, glycosylation, phosphorylation, and disulfide bond formation occurs A single propeptide can produce more than one neuropeptide

Figure 6.16 Proteolytic processing of pre-propeptides

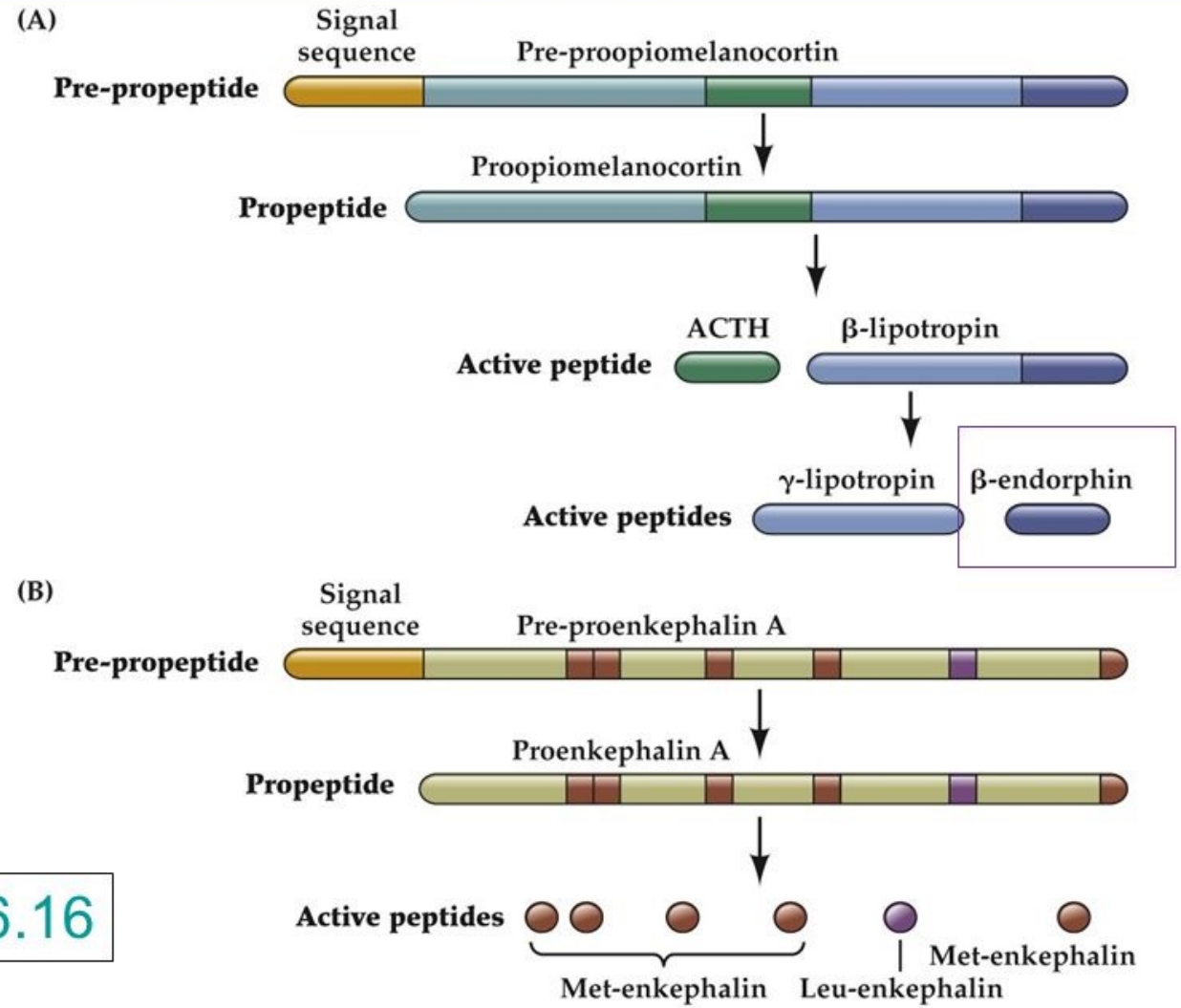
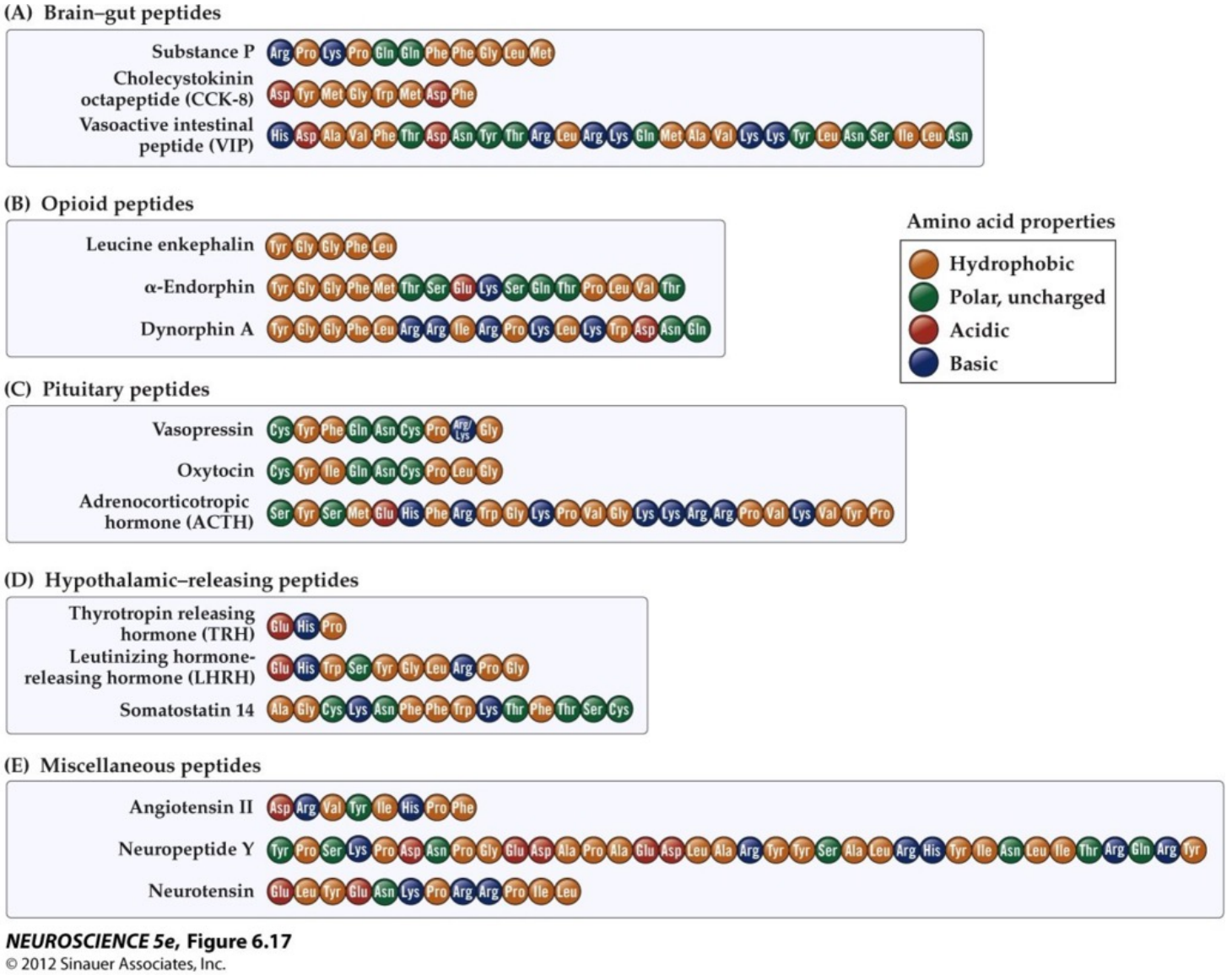


Fig. 6.16

NEUROSCIENCE 5e, Figure 6.16

FIGURE 6.21 Proteolytic processing of pre-propeptides. Shown here are pre-proopiomelanocortin (A) and pre-proenkephalin A (B). For each pre-propeptide, the signal sequence is indicated at the left; the locations of active peptide products are indicated by darker colors. The maturation of the pre-propeptides involves cleaving the signal sequence and other proteolytic processing. Such processing can result in several different neuroactive peptides such as ACTH, γ-lipotropin, and β-endorphin (A), or multiple copies of the same peptide, such as methionine-enkephalin (B).



***NEUROSCIENCE 5e*, Figure 6.17**

Figure 6.17: Amino acid sequences of neuropeptides. These neuropeptides vary in length, usually containing between 3 and 36 amino acids. The sequence of amino acids determines the biological activity of each peptide.