Biochemistry of neurotransmitters

Prof. Mamoun Ahram
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References

- This lecture
- Mark’s Basic Medical Biochemistry, 4th ed, pp. 908-918
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 (epinephrine, dopamine, histamine, serotonin)
  - Amino acids (GABA, glutamate, aspartate, glycine)
  - Acetylcholine
  - Purines (ATP)

- **Neuropeptides**

- **Gases** (nitric oxide, carbon monoxide)

*Two or more transmitters (usually a small-molecule 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.

- Peptides activate their receptors at low (nM to uM) concentrations compared to the concentrations required to activate receptors for small-molecule neurotransmitters. These properties allow the postsynaptic targets of peptides to be quite far removed from presynaptic terminals.

**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.

<table>
<thead>
<tr>
<th>Neuropeptide Families</th>
<th></th>
<th>Opiate Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tachykinins</strong>: substance P, bombesin, substance K</td>
<td></td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-Leu-OH</td>
</tr>
<tr>
<td><strong>Insulins</strong>: insulin, insulin-like growth factors</td>
<td></td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-Met-OH</td>
</tr>
<tr>
<td><strong>Somatostatins</strong>: somatostatin, pancreatic polypeptide</td>
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<tr>
<td><strong>Gastrins</strong>: gastrin, cholecystokinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Opioids</strong>: opiocortins, enkephalins, dynorphin</td>
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</table>

- 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

<table>
<thead>
<tr>
<th>Name</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu-enkephalin</td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-Leu-OH</td>
</tr>
<tr>
<td>Met-enkephalin</td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-Met-OH</td>
</tr>
<tr>
<td>Beta-endorphin</td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Gly-Gln-His-OH</td>
</tr>
<tr>
<td>Dynorphin</td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH</td>
</tr>
</tbody>
</table>
Stages of action

- **Synthesis (ER (1) as pre-propeptides then propeptides and then in Golgi apparatus (2))**
- **Packaging into large-dense core vesicles (with modifying enzymes)**
- **Transport (3) (fast-axonal transport)**
  - During the transport, proteases cleave the precursor neuropeptide into the final mature form (4).
- **Release (5)**
  - Release is gradual over time in response to general increases in the level of intracellular calcium.
- **Action (prolonged)**
  - Mainly via GPCR
- **Termination by diffusion and degradation (6)**
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.
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 or internal sources and at higher concentrations than required for small-molecule neurotransmitters.
The levels of regulation of neuropeptide expression

- Diffusion
- Proteolysis
- Proteases may or may not be released
- Minutes-hours
- Hours-days
- msec-sec-minutes
- Nucleus
  - Transcription factor binding
  - Changes in mRNA synthesis or splicing
  - Additional precursors, enzymes expressed
- Ribosomes/ER
  - Translation rate changes with pre-existing or new mRNA
- Golgi/TGN
  - Oligosaccharide maturation
  - Aggregation of prepropeptide with enzymes, other granule proteins
- Degradation in cell
- LDCV
  - Processing Storage Degradation?
SMALL-MOLECULE NEUROTRANSMITTERS
Stages of action

- Synthesis of enzymes in ER (1) and Golgi apparatus (2) where they are modified (3).
- Transport of **soluble** enzymes via slow axonal transport (4)
- Neurotransmitters precursors are taken up via transporter proteins located in the plasma membrane of the nerve terminal (5), and the neurotransmitter is synthesized in the presynaptic nerve terminal then packaged in small synaptic vesicles (6).
- 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**
Ca^+ = 2 mM

Ca^+ = 0.1 uM

Ca^+ = 50

[Ca+] = 50-100 uM

[Ca+] = 0.1 uM

Cell Soma

Nucleus

Trans-Golgi network

Axonal transport

Initial processing

Final processing

Storage

Initial synthesis

Ribosome

Endoplasmic reticulum

Small synaptic vesicle

Large dense core vesicle

Secretion
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)
Tyrosine (hydroxy-phenylalanine) is present in all food products and synthesized from phenylalanine. It enters the neuron by active transport.
50% Most

10% Some

Na+-dependent dopamine co-transporter (DAT)

Cocaine Amphetamine
LDCV

Amphetamine

Na+-dependent norepinephrine co-transporter (DAT)
Leaking
The catecholamines (dopamine and epinephrine) are transported into vesicles by an ATP-dependent process linked to a proton pump.

Protons are pumped into the vesicles by a vesicular ATPase (V-ATPase).

The protons then exchange for the positively-charged catecholamine via the transporter VMAT (vesicular monoamine transporter).
COMT and MAO

Inactivation is dependent on SAM, vitamin B12 and folate.

Parkinson's disease
Regulation

• **Tyrosine hydroxylase**
  
  – **Short term:**
    
    • Inhibition by free cytosolic catecholamines, which compete with BH4 binding to enzyme
    
    • Activation by depolarization, which activates several protein kinases including PKC, PKA, Ca-calmodulin-dependent kinases that phosphorylate tyrosine hydroxylase. This makes the enzyme bind more tightly BH4 and, consequently, less sensitive to endproduct inhibition.

  – **Long-term (plus dopamine β-hydroxylase)**
    
    Prolonged sympathetic neuronal activity increases the amounts of tyrosine hydroxylase and dopamine – hydroxylase mRNAs.
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.
Melatonin

Serotonin is synthesized in the pineal gland and serves as a precursor for the synthesis of melatonin, which is a neurohormone involved in regulating:

- sleep patterns
- Seasonal and circadian (daily) rhythms
- Dark-light cycle
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 $\rightarrow$ Krebs cycle $\rightarrow$ dehydrogenation of $\alpha$-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

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

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

- Choline + 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**

Astrocytes (MAO) → Neuron

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).
- Ca\textsuperscript{2+} enters the postsynaptic neuron activating NOS (3), which forms NO from arginine (4).
- NO stimulates guanylate cyclase forming cGMP (5), which results in a physiological response (6).
- NO can diffuse out: a) to the presynaptic terminal (\textit{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 $[\text{Ca}^{+2}]$ for release
- Site of synthesis, modification, action
- Fate
# Neuropeptides vs. Neurotransmitters

<table>
<thead>
<tr>
<th></th>
<th>Neuropeptides</th>
<th>Neurotransmitters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size</strong></td>
<td>Larger molecules (3 to 36 amino acids)</td>
<td>Smaller molecules of different precursors</td>
</tr>
<tr>
<td><strong>Re-uptake</strong></td>
<td>Not –re-taken up again</td>
<td>Possible re-uptake</td>
</tr>
<tr>
<td><strong>Post-release modification</strong></td>
<td>Yes by extracellular peptidases</td>
<td>No modifications</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Large dense core vesicles</td>
<td>Small synapse vesicles</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td>Anywhere in the neuron</td>
<td>In the presynaptic axon terminal</td>
</tr>
<tr>
<td><strong>Secretions</strong></td>
<td></td>
<td>Co-released</td>
</tr>
<tr>
<td><strong>Action</strong></td>
<td>Slow-acting</td>
<td>Fast-acting</td>
</tr>
<tr>
<td><strong>Synthesis</strong></td>
<td>Ribosomes, ER, Golgi bodies, etc.</td>
<td>Cytoplasm of presynaptic end</td>
</tr>
<tr>
<td><strong>Efficiency of signaling</strong></td>
<td>More efficient</td>
<td>Less efficient</td>
</tr>
<tr>
<td><strong>Concentrations</strong></td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td><strong>Diffusion at Release Site</strong></td>
<td>Diffuse away</td>
<td>Do not diffuse far</td>
</tr>
</tbody>
</table>