Biochemistry of neurotransmitters

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Neuroscience
2015
References

- This lecture
- Mark’s Basic Medical Biochemistry, 4th ed, pp. 908-918
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, epinephrine, dopamine, histamine, 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.

<table>
<thead>
<tr>
<th>Neuropeptide Families</th>
<th>Opiate Family</th>
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</thead>
<tbody>
<tr>
<td>Tachykinins: substance P, bombesin, substance</td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-Leu-OH</td>
</tr>
<tr>
<td>Insulins: insulin, insulin-like growth factors</td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-Met-OH</td>
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<tr>
<td>Somatostatins: somatostatin, pancreatic polypeptide</td>
<td><strong>Tyr-Gly-Gly-Phe</strong>-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</td>
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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 longer protein by:
  - Proteolytic processing.
  - Vesicular packaging of different proteases with cleavage sequences.
  - Hiding a proteolytic site by post-translational modifications (example: addition of a carbohydrate side chain.)
  - Tissue-specific

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

Vesicles are located further away from the presynaptic membrane and away from place of Ca influx
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 influx of calcium
- Action (short)
- Termination by diffusion, re-uptake, or inactivation
\[ [\text{Ca}^+] = 2 \text{ mM} \]

\[ [\text{Ca}^+] = 50-100 \text{ uM} \]

\[ [\text{Ca}^+] = 0.1 \text{ uM} \]
Presynaptic membrane (thin section)

Synaptic cleft

Vesicle fusions

LDV

SV

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
Leaking
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-hydroxylase)**
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 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) 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:
- 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 spinal cord
- 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

Glutamatergic neuron

LDH → Pyr → Glc
PDH → AcetylCoA
TCA cycle
α-KG
AT → PAG → GLU
GLU → GLU

GABAergic neuron

LDH → Pyr → Glc
PDH → AcetylCoA
TCA cycle
α-KG
AT → PAG → GLU
GLU → GLU

Astrocyte

GluT/SSADH
GABA

Postsynaptic Neuron

GLN → GLU → GLN

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.

![Diagram of histamine synthesis]

**Astrocytes** (MAO) → **Neuron**
Inactivation of histamine
Nitric oxide (NO)

- Glutamate is released (1) and acts on NMDA receptors located on the post-synaptic neuron (2).
- Ca$^{2+}$ 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