Biochemistry of opioid receptors: binding, regulation and molecular modeling

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Addiction and drug abuse

What is the meaning of drug?

Various meanings of ‘drug’

1) In pharmacy: Given formulations of medicinal plants
2) In medicine: Medicaments in general
3) In society: Natural or synthetic compounds for abuse
Drug addiction and drug abuse

- Chronic or habitual use of any chemical substance to alter states of body or mind for other than medically warranted purposes.
- Psychological dependence is the subjective feeling that the user needs the drug to maintain a feeling of well-being; physical dependence is characterized by tolerance (the need for increasingly larger doses in order to achieve the initial effect) and withdrawal symptoms when the user is abstinent.
**Abused compounds**

- Nicotine
- Alcohol
- Analgesics (opiates)
- Psychostimulants (ecstasy, cocaine)
- Hallucinogens (LSD)
- Inhalants
- Sedato-hypnotics (e.g., barbiturates)
Stimulants:
- Amphetamine
- Ecstasy

Depressants:
- Cocaine
- Morphine
- Heroin

Hallucinogens:
- Mescaline
- LSD
- Psylocibine
- Salvinorin
- PCP

Barbiturates:
- Ergotamine
History of drug abuse

-Wine at ancient Egypt
-Narcotics from 4000 B.C
-Medicinal use of marijuana 2737 B.C. in China
-III. c. B.C.: Theophrastus
-Persian doctors (against dysentery)
-Medieval ages: gift of God (OP)
-Morphine, laudanum, cocaine were completely unregulated and prescribed freely by physicians for a wide variety of ailments.
-But not until the 19th cent. A.D. were the active substances in drugs extracted
-1806: Sertürner isolates morphine (Morpheus)
-During the American Civil War, morphine was used freely
-Use of narcotics and cocaine diminished by the 1920s. The spirit of temperance led to the prohibition of alcohol by the Eighteenth Amendment to the Constitution in 1919, but Prohibition was repealed in 1933.
-1976. discovery of opiate receptors
-1988 Howlett’s group identifies specific THC binding sites in the brain
Opioids

Endogenous: Endorphins, Enkephalins, Dynorphins, Endomorphins
Natural: Opium, Food, Fungi
Synthetic and semi-synthetic: Tramadol, Hydromorphon, Oxycodon

Naloxone, pure opioid antagonist developed by Sankyo in the 1960s. Use for overdose therapy.
Opium alkaloids
(more than 25 natural compounds)

**Morphine** (analgesic)

**Codeine** (antitussive)

**Thebaine** (convulsive, stimulant, chemical precursor for semi-synthetic drugs)

**Papaverine** (smooth muscle relaxant)

**Noscapine** (cough-suppressing)
big O, black stuff, block, gum
Biosynthesis of Morphine:

\[
\text{(R)-reticuline} \quad \text{equiv} \quad \text{salutaridinol}
\]

\[
\text{oripavine} \quad \text{equiv} \quad \text{morphinone}
\]

\[
\text{thebaine} \quad \text{equiv} \quad \text{morphine}
\]
Opiate receptors:

MOP (µ) subtypes: µ₁, µ₂
KOP (κ)
DOP (δ) subtypes: δ₁, δ₂
NOP (N/OFQ)

GPCR:
block the AC, increase the receptor operated K⁺-channel, block of voltage dependent Ca⁺²-channel (hyperpol.)

Adaptation of 2nd messenger mechanism:
tolerance (desensitisation, internalisation, mainly for µ, δ)
Binding pocket and receptor surfaces of MOP receptor
Effect of Opiates

The activation of μ receptors can cause analgesia, physical dependency, respiratory depression, miosis, euphoria, reduce GI motility, etc.

The k receptor is responsible for analgesia, anticonvulsant, dissociative effect, duresis, disphoria, neuroprotection and sedation, etc.

And the third one is the δ receptor, responsible for analgesia, antidepressant, convulsant and physical dependency, etc.
**Analgesic effect**

- maintained consciousness, sedation
- the perception of pain is modified, or may cause total analgesia
- nociceptive pain is more sensitive to opiates than neuropathic pain
- block of ascendant nociceptive transmission + activation of midbrain pain control center (lots of receptor)
- effect also on the peripheral part (receptor upregulation in inflammation)
- NOP receptors control the pain sensation (hypo- or hyperalgesia)
**Mood modifying effect**

- depression, euphoria,
- tranquilant effect
- role of dopamine system (basal ganglia, N. acc, control of emotion and motivation, endogenous opiates+dopamine – reward centre- VTA)

**Other neurological effects**

**Hypothalamus:**

- block of heating center (thermoregulation)

**Neuroendocrine:**

- block of GnRH, CRH (decr. LH, FSH, β-endorphine, ACTH)
- increase of ADH
Pupil:
myosis (pinpoint)
no tolerance

Respiratory:
depression (direct brainstem eff.) a therapeutic dose has no effect in healthy subject. danger in respiratory illness or in combination. decreased resp. rate (even 3-4/min), arrhythmic, Cheyne-Stokes breathing. analgesic and resp. depressive effect are inseparable.
Coughing reflex: inhibited (antitussive)

Vomiting reflex: increased (CTZ), rare in therapeutic dose, mainly ambulant (vestibular stimulus), prevention with phenothiazines.

Cardiovascular effects

vasodilatation, decreased resistance and baroreflex (orthostatic hypotension)
reason: His. liberation, danger of shock in hypovolemia!
decreased cardiac oxygen need
**Gastrointestinal effects**

- **Stomach:**
  - blocked secretion (indirect somatostatin release, blocks ACh)
  - decr. motility and emptying time (12 hours delay), risk of reflux

- **Small int.:**
  - decr. secretion and digestion
  - incr. resting tone, cramps
  - duodenum more sensitive

- **Large int.:**
  - like small int. + dehydration of faeces, constipation
  - incr. anal sphincter tone

*intestinal effects through \( \mu \) and \( \delta \)-receptors, there is no tolerance*
Other smooth muscle effects

urinary bladder, urethra: blocked reflex, incr. tone (catheter)

Skin effects

dilation of skin vessels (flush, sweating)
pruritus (transmitted by the dorsal spinal cord)

Immune system

immunosuppression (through $\mu$-receptors)
risk of infection (naloxone improves the sepsis survival)
Morphine and Heroine Metabolism:

- Morphine
  - Norcodeine glucuronide
    - Norcodeine glucuronide
  - Codeine glucuronide
    - Codeine glucuronide (renal excretion)
  - Morphine 3-glucuronide (extremely potent μ-opioid agonist)
  - Morphine-6-glucuronide
  - Morphine-3-glucuronide

- Codeine
  - Norcodeine
  - Codeine
  - Hydromorphone

- Hydrocodone
  - Morphine
  - Heroin (100% excretion in 2-6 min)

- 6-Monoacetylmorphine
  - Normorphine
  - Normorphine glucuronide
  - Morphine-6-sulfate
  - Morphine-3-sulfate

- Extremely potent μ-opioid agonist

- Renal excretion:
  - Hydrocodone
  - Codeine
  - Morphine

- Potent morphantagonists:
  - Morphine 3-glucuronide
    - GluO
  - Mor mix glucuronide
    - HO

- BRC
- European Union
- European Social Fund
- INVESTING IN YOUR FUTURE
- Hungarian Government
Heroin  white horse, China white; cheese

-Patented medicine

-Bayer, Germany 1897

diacetyl-morphine (DAM)

-Fast hydrolysis (6-MAM)

-Strong lipid solubility

-Excretion with urine

-Strong dependence
**Triads of overdose:**

1. Coma
2. Respiratory depression
3. Pin-like pupil

**Withdrawal and Symptoms**

- 8-12 hours after the last heroin dose:
  - Anxiety, insomnia, midriasis, piloerection, anorexia, tremor, tachycardia, yawning, nasal and tear secretion increased, depression, bone pain, muscle spasm, diarrhoea.
  - later:
  - 7-10 days: Leucocytosis, dehydration, ketosis, pH disturbances, cardiovascular collapse
  - months: Hypotension, bradycardia, hypotermia, midriasis.
Evidence suggest that bioactive compounds can mimic or modulate the effects of the body’s own substances.

Natural opioid peptides or ENDORPHINS (endogenous morphine = endorphin)

Enkephalins (1975)
β-endorphin
Dynorphin …
Neuropeptides: biosynthetic scheme

Propeptid (PP)

Prepropeptid (PPP)

mRNA translation

(gene transcription)

Partially processed peptide

Active peptide

Inactive fragmentum

Active peptide fragment
Enkephalins and opiates
Structural similarities

Leu-enkephalin  Morphine  Met-enkephalin

Leu-enkephalin: 
- Tyr\(^1\)
- Leu\(^5\) to Phe\(^4\)
- Gly\(^3\) to Gly\(^2\)

Morphine: 
- Tyr\(^1\)
- ‘A’-ring

Met-enkephalin: 
- Tyr\(^1\)
- Met\(^5\) to Gly\(^2\)
- Phe\(^4\) to Gly\(^3\)

Chemical structures of Leu-enkephalin, Morphine, and Met-enkephalin are shown with specific amino acid positions labeled.
- Neurotoxic peptides isolated from the venom of the marine cone snail, genus Conus.
- Loop 2 in the structure of conotoxin indicating N-type Voltage Gated Calcium Channel blocking activity.
Prof. Adriano Mollica and his team at Faculty of pharmacy of Chieti-Università -Italy, Have designed and synthetized enkephalin-conotoxin hybrid peptides

**Effect of Enkephalin-Conotoxin Hybrid Peptides on Opioid Receptors**

**Compound1:** H-Tyr-D-Ala-Gly-Phe-Ser-Arg-Leu-Met-Tyr-NH₂

**Compound2:** H-Tyr-D-Ala-Gly-Phe-Arg-Leu-Tyr-NH₂

**Compound3:** H-Ser-Arg-Leu-Met-Tyr-NH₂

**Compound4:** H-Lys-Ser-Arg-Leu-Met-Tyr-NH₂
Radioligand competition binding assays

- fix concentrations of a specific radioligand
- increasing concentrations of unlabeled competitor ligands
Functional $[^{35}\text{S}]\text{GTP} \gamma \text{S}$ binding assays

**Compound 1**

G-protein efficacy

<table>
<thead>
<tr>
<th>Comp. 1 + NTI</th>
<th>Comp. 1 + Cyp</th>
<th>Comp. 1 + nor-BNI</th>
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<tbody>
<tr>
<td>100%</td>
<td>150%</td>
<td>200%</td>
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**Compound 2**

G-protein efficacy

<table>
<thead>
<tr>
<th>Comp. 2 + NTI</th>
<th>Comp. 2 + Cyp</th>
<th>Comp. 2 + nor-BNI</th>
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<tbody>
<tr>
<td>100%</td>
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**Compounds 3 and 4** (conotoxins)

G-protein efficacy

<table>
<thead>
<tr>
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Prof. Mollica and his team found that all compounds exhibited the functional activity at N-type voltage gated calcium channel.

Analgesic effect was proved by pharmacological tests.

Peptide 4 showed the weakest blocking activity.

Compound 1: H-Tyr-D-Ala-Gly-Phe-Ser-Arg-Leu-Met-Tyr-NH₂

Compound 2: H-Tyr-D-Ala-Gly-Phe-Arg-Leu-Tyr-NH₂

Compound 3: H-Ser-Arg-Leu-Met-Tyr-NH₂

Compound 4: H-Lys-Ser-Arg-Leu-Met-Tyr-NH₂
Future perspective

- Reducing side effects of opioids in chronic therapy.
- Decreasing toxicity of conotoxin in combination.
- Administration of these peptides in low dose could block the pain pathway simultaneously by stimulation of opioid receptors and blocking of Voltage Gated Calcium Channels.
Acknowledgment

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Dr. Ferenc Zádor
Stimulant drugs

- Cocaine
- Amphetamine, methamphetamine
- Ephedrine
- MDMA (ecstasy)
Cocaine

Coke, snow, nose candy, flake, blow, big C, lady, white, snowbirds
What are the cocaine effects?

- Block of catecholam. and 5-HT reuptake (PD)
- Increased arousal and performance, decreased fatigue
- Inc. BP, heart rate and self-confidence
- Aggressive behavior, paranoia
- Sustained orgasm (later decreased sexual ability)
- Local anes.
- Decr. Brain activ.
**Amphetamine:** black beauties, hearts, speed, truck drivers, uppers

- Increase D release
- MAOI
- D, NA, 5-HT reuptake inhibition
1920- first synthesis, 1930- medicinal use, 1940- abuse, WW2: pilots use
Abuse: - **typical disco-drug**
- when sleeping is not possible/wanted: truck drivers, artists
Effects: - hyperactivity
- physical and mental productivity increases
- demand for sleeping decreases
- iv „rush”
Overdose: - sy stimulation
- disorientation
- long term: destruction of dopaminergic pathways (6-OH-dopamine metab)
- paranoia, hallucination
- Tolerance: very early
- Dependence: physical not detected psychical very likely
Methamphetamine

meth, ice, crank, chalk, crystal, fire, glass, go fast

-Like amph. potent full agonist of trace amine-associated receptor 1 (TAAR1), a GPCR that regulates brain catecholamine systems.

-Depen. after a single use.

-Anorexia, hyperactivity, mydriasis, excessive sweating, xerostomia, and bruxism (leading to "meth mouth"), arrhythmia, hypertension, hyperthermia, diarrhea, dizziness, numbness, tremor, acne and pallor.

-Placenta, breast milk. Infants smaller head and birth weigh.

-Euphoria, dysphoria, changes in libido, alertness, apprehension, concentration, decr. sense of fatigue, insomnia or wakefulness, self-confidence, sociability
Ephedrine


-1855, 1st chem. synth. of ephedrine by Nagai.

-Bronchodilator, oral-nasal decong. promotes modest short-term weight loss specifically fat loss, not recom., ineffec. in the long term.

-Decre. gastric emptying. Synergi. effect with MTX like caffeine and theophylline.

-C.V, skin, nausea, decr. urination, CNS, dyspnea, pulmonary edema, tremor, hyperglycemic reactions


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MDMA: Ecstasy, E, XTC, Adam (disco and party drug)

-Euphoria, vividness, hyperact., Hallucin. in high dose
-S.e. : like amph.
-Hyperpyrexia (40-42 ºC), panic attack
- hepatotox., neurotox.
- long term: risk of Parkinson-sy

**overdose:**
- collapse, fit on the dance floor
- hypertension, tachyarrythmia
- hyperpyrexia
- muscle spasm, midriasis
- in severe cases DIC, acute renal failure
- first 60 h critical
- no withdrawal syndrome
causes degeneration of 5-HT nerve terminals
Cannabis *sativa*, *C. indica* and *C. ruderalis*

ganja, grass, herb, joint, weed
Boom, gangster, hash
marijuana (THC)

- CB1/2, brain, GI, adipocytes, Leydig c., sperms, ovary, / Leuco., b.stem, spinal cord

- Endogen. ligand: anandamide
  2-Arachidonylglycerol

- Effe.: homeostasis, analg., appetite cont., short term memo. and cognition, emotional processing, neuroprotec., prolif. differ. Surviv. of N and non-N cell

- Most frequent drug
- inhalation, iv equipotent, in 15-30 min effect

- ↓ cognitive function, perception, reaction time, learning, memory
- ↑ Appetite,, amotivational syndrome, antiemetic, muscle relaxant

- Euphoria, „high”, hallucin, acute psychosis (by oral use)

- Self-consciousness, feeling of creativity (not represented in productivity)

- Chronic abuse: ↓ IOP (medicinal use!), ↓ testosterone, hyperphagia, laryngitis, rhinitis

- Withdrawal syndrome:
  - Restlessness, insomnia, anxiety, agitation, nausea, seizures
  - Treatment: psychotherapy, antidepressants
- **Overdose:**
  Not life threatening, anxiety, panic attack

- **Tolerance:** little

- **Dependence:** no physical
Hallucinogens

- Magic Mushroom
- Ergotamine
- Mescaline
- Bufotenin
- LSD

Hallucination

False belief or impression
Notion
Illusion
Delusion
Ergotamine


Post-partum uterine bleeding
Thrombosis and gangrene
Anti-migraine
Ergotism

Ergot-derived drug to stop postnatal bleeding
Lysergic acid diethylamide (LSD)

Possible physical effects of LSD:

**Systemic:**
- Hypothermia
- Fever

**Mouth:**
- Saliva production

**Jaw:**
- Clenching

**Blood:**
- Elevated sugar levels

**Muscles:**
- Hyperreflexia
- Tremors
- Weakness

**Skin:**
- Goose bumps
- Perspiration
- Paresthesia

**Cardiac:**
- Decreased heart rate

**Gastrointestinal:**
- Nausea

- D, 5-HT2-resep. activ.

**Tol.-depen.:** no self administration (animals)

tolerance early
no dependence

Albert Hofmann

acid, blotter, cubes, microdot yellow sunshine, blue heaven
**Magic Mushroom**
purple passion, shrooms, little smoke

Psilocybin mushrooms

No physic. or psycho. Depen.

- Audio, visual, tactile
- Trip

Psilocybin

Psilocin

Gymnopilus luteoviridis

Psilocybe semilanceata

Panaeolus cinctulus
Thank you for your attention!

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