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Research Article

From morphine to endogenous opioid peptides, e.g., endorphins: the endless quest for the perfect painkiller

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Abstract. Opium was known since the Neolithic era and in 5th century wild Papaver use was reported to induce sleep and relieving pain. First active component isolated from Opium was morphine, the paradigm of a natural product discovered 150 years before isolation of endogenous opioid ligands, brain pentapeptide enkephalins. Since then many endorphin peptides and their mode of action were discovered. Native endorphins were characterized thanks to the synthetic antagonist naloxone.

Keywords. Opium, morphine, peptides, peptidomimetics, analgesics.

MORPHINE: A PARADIGMATIC EXAMPLE OF A NATURAL PRODUCT MIMICKING ENDOGENOUS MOLECULES

The story of morphine (Figure 1) and its analogues is a paradigmatic example of the classical pathway to drug development undertaken by those researchers starting from a natural compound. In particular the phases of development and optimisation of morphine-like drugs are exemplified as follows:

1. Recognition of the pharmacological activity of a plant
2. Extraction and identification of the active ingredient
3. Studies on synthesis (partial and total)
4. Structure-activity relationship studies (through synthetic analogues)
5. Development of analogues as drugs to optimize activity and decrease side effects
6. Receptor theories; 'rational' synthesis of analogues/structure-based design

Morphine was isolated for the first time from the opium poppy by Friedrich Wilhelm Adam Sertürner (Figure 2).

In a letter to the editor of the Journal Der Pharmacie Für Ärzte Und Apotheker (Vol. 13, 1805), he reported on the isolation of a substance from opium with alkaline character. In 1806, Sertürner moved to Einbeck, Ger-

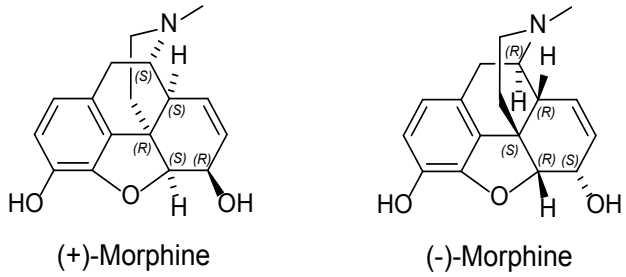

Figure 1. Structures of the bioactive (-)-Morphine and of the inactive (+)-Morphine.

Figure 2. Friedrich Wilhelm Adam Sertürner (1783-1841) who isolated the substance from opium with alkaline character that he called "Morphine", a name with a clear mythological reference: the Greek God of dreams Morpheus.

many where he was assistant to the tenant of the magistrate's pharmacy. In 1809, he became pharmacist and, since the tenant was already 75 years old, he intended to take charge of the pharmacy. However, he was not successful. During the invasion of Napoleon Bonaparte's troops into Germany, French legislation became the law of the land in those parts, which fell under French government and Sertürner was allowed to open a second pharmacy. Therefore Sertürner continued his research work on morphine in Einbeck and published the results in two papers. In one of these (1817), he reported his observations on the use of a drug in humans that he called for the first time "morphine", name with a clear mythological reference. The French chemist Gay-Lussac was interested in Sertürner's publication and ordered a French translation, which earned Sertürner the scientific breakthrough of morphine. He is recognized the pioneer in alkaloid research, and for that he received a doctor degree from the university of Jena in 1817 when he published the isolation of pure morphine from opium after at least thirteen years of research and a nearly disastrous trial on himself and three teenagers, leading to pain in the region of the stomach, exhaustion, and severe narcosis that came close to fainting as described in details by Sertürner.[1-4]

HISTORICAL BACKGROUND

The poppy and possibly its bioactivity was known since the Neolithic era, since seeds were found in tombs dating to 4200 BC. It was certainly cultivated in Mesopotamia, Persia, India and China and widely used as sleeping or sedative remedies, but also used in religious and spiritual rituals.

The five-volume De Materia Medica written by Pedanius Dioscorides, remained in use from the 1st to the 16th centuries, described opium and the wide range of its uses. In the 5th century Pseudo-Apuleius refers to the use of wild poppy *Papaver agreste* for inducing sleep and relieving pain.

The Persian Abu Bakr Muhammad ibn Zakariyya al-Razi (865-925 AD; 251-313 AH) also known as Rhazes because of the place where he was born and died, i.e., Rayy, near Teheran. He studied medicine in Baghdad and became one of the greatest physicians of the medieval period, writing over 200 works; half of them on medicine, but others on topics that included philosophy, theology, mathematics, astronomy and alchemy.[5] He made use of opium in anesthesia and in "In the Absence of a Physician" (a home medical manual directed toward ordinary citizens for self-treatment), he recommended the use of opium for treatment of melancholy. All the leading physicians of Baghdad used opium that was considered particularly effective for diseases of the intestines and of the eyes, but it also featured in a number of remedies to treat gout and painful joints. Al-Razi gave recipes for gout and the joints based on ointments that were applied to the painful areas with a damp piece of paper or cloth to keep the medication moist. A good paste that al-Razi described contained equal parts of opium and liquid storax (Liquidambar orientalis).[6] The renowned Andulasian opthamologic surgeon Abu al-Qasim al-Zahrawi ("Abulcasis", 936–1013 AD) relied on opium as a surgical anaesthetics and wrote a treatise, al-Tasrif, that influenced medical thought well into the 16th century.

In 1527 the Swiss physician, alchemist and astrologer Philippus Aureolus Theophrastus Bombastus von Hohenheim (1493-1541) who called himself Paracelsus, introduced to Western medicine *Laudanum* or opium tincture returning from Arabia with a famous sword,

within the ball of which he kept "Stones of Immortality" composed of *opium thebaicum*, citrus juice, and "quintessence of gold". The name *Laudanum* was invented by Paracelsus from the latin "laudare" or was a corrupted form of ladanum (from the Persian ladan), a resinous juice or gum obtained from various kinds of the Cistus shrub (by M. Ray, Editor of Encyclopaedia Britannica, 2017). The term is used now to describe the alcoholic tincture of opium, a 10% solution of opium powder dissolved in high-proof distilled spirits. It was used as an analgesic substance and Paracelsus understood that opium was more soluble in alcohol and reported the first evidence of dependence. Laudanum was a major part of the pharmacopeia into the 20th century. It was a common drug of abuse during the Victorian era. Paracelsus considered himself an alchemist and his ideas were not always well accepted by the medical community. However he was the first to introduce chemistry into medicine in the 16th century. Most of his work was published only after his death (Figure 3) and Peder Sorensen in 1571 in "Idea medicinæ philosophicae" started emphasizing Paracelsus's pioneering work in Chemical Medicine.[7]

Laudanum became the basis of many popular patented medicines of the 19th century (Figure 4).

The English physician Thomas Dover (1660-1742) was the first to market in England a powder, the Dover's powder, a preparation of opium and ipecacuanha, the later was added for its emetic properties to limit its use.[8] Its recreational use was therefore widespread, Godfrey's Cordial was sold freely,[9] and opium was freely imported from India, like tea or tobacco.

In the 17th century in China the use of smoking opium was widely spread, also because of the prohibition of tobacco (1644); consumption was very high and opium was imported from India via Canton by English and American merchants. The blockade of the importation by Chinese authorities caused the Opium War (1839-1842). Interestingly in 1841, the US president William Henry Harrison was treated with laudanum.[10] Moreover, in the American Civil War, the Union Army used 2.8 million ounces of opium tincture and powder and about 500,000 opium pills.[11]

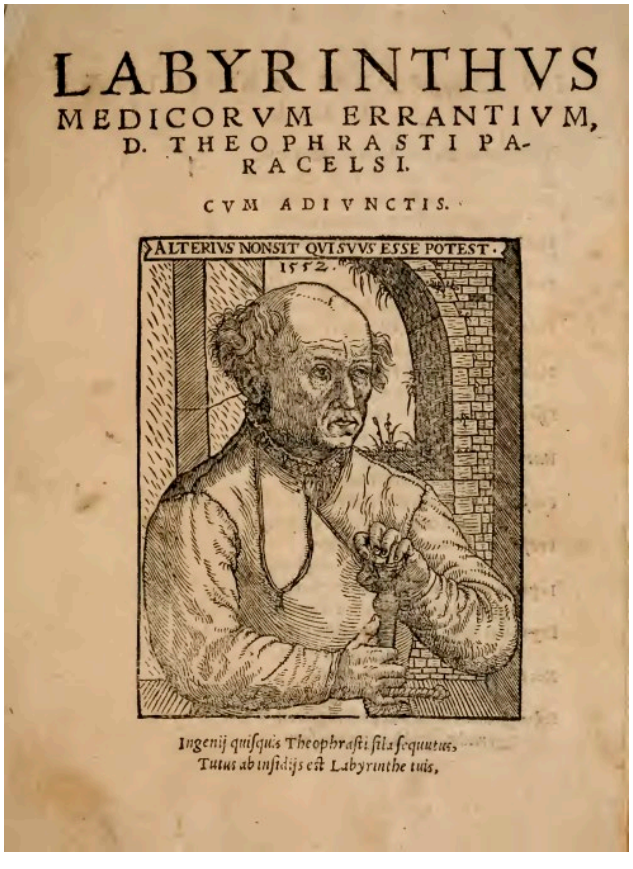

Figure 3. Cover of the *Labyrinthus Medicorum Errantium* by D. Theophrasti Paracelsi.

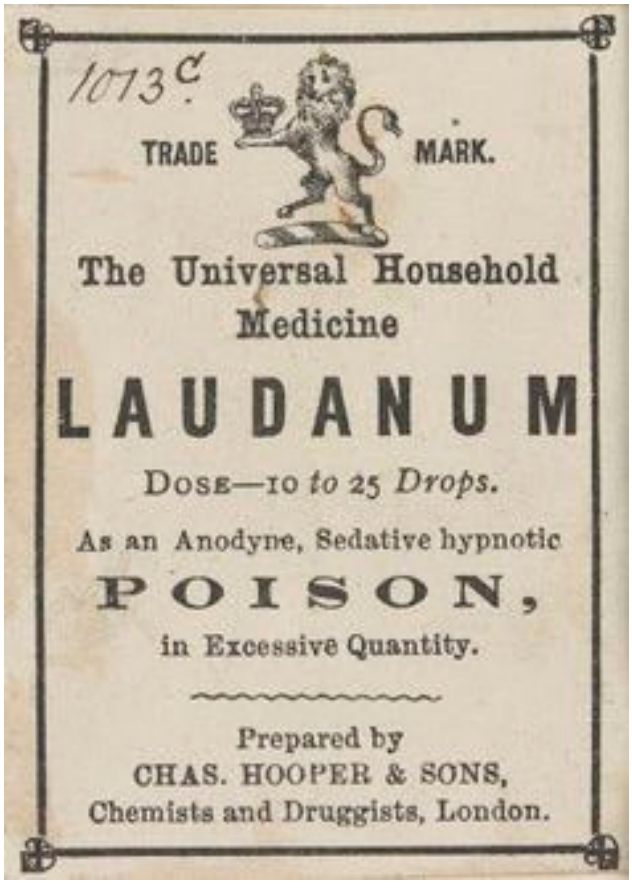

Figure 4. Label of Laudanum bottle prepared by Chas. Hooper & Sons, Chemists and Druggists, London.

OPIOIDS AND OPIATES

Opium is the latex or rubber obtained by etching the immature capsules of *Papaver somniferum* (Figure 5). It contains various alkaloids with analgesic action, of which the most relevant one is morphine.

The term opiate (widely used until the 1980s) describes any natural or synthetic agent derived from morphine.

In 1833 MacFarlane prepared morphine on a commercial scale,[12] and in 1853 injectable morphine comes into use during the American Civil War.[13] Noteworthy is that in 1898, Bayer registered diamorphine (diacetylmorphine) the name of heroin in Germany as an antitussive (cough suppressing) drug.[14] The Harrison Narcotics Act, which was passed in 1914 and took effect in 1915 marked the beginning of federal narcotics control in USA. This act aimed to control each phase of production and distribution of opium, morphine, heroin and any new derivatives that could have similar biological activity. The first drug prohibition federal law in USA was the Smoking Opium Exclusion Act. It passed in 1909 and prohibited the importation of opium prepared for smoking in the United States.[15]

FROM MORPHINE TO CODEINE AND TO SEMI-SYNTHETIC ANALOGUES VIA THEBAINE

Morphine structures

Morphine is an opium phenantrenic alkaloid with 5 stereogenic centers (*), and therefore with the theoretical possibility of presenting $2^5 = 32$ stereoisomers. Practically, geometric restrictions limit the possibilities to 16 stereoisomers. The natural bioactive enantiomer is (-)-Morphine (5R, 6S, 9R, 13S, 14R) (Fig. 1). Isomorphine is the epimer in which the absolute configuration at C-6 is R (hydroxyl in position b). The synthetic enantiomer of (-)-Morphine, the (+)-Morphine (Fig. 1) has about 10,000 times less affinity than the natural (-)-Morphine and possesses no functional efficacy when tested at concentrations up to 100 fold the effective dose of natural morphine.

In 1925 Robinson and Gulland determined for the first time its structure[16] and only more than 25 years later, i.e. in 1952, Gates performed its first chemical synthesis.[17] It took additional 20 years to univocally determine morphine X-ray structure.[18]

In 1832 Codeine (Figure 6) was isolated from opium and characterized by Pierre-Jean Robiquet, a French pharmacist who discovered other important natural substances such as asparagine, amygdaline in bitter almonds, caffeine, etc.[19] He reported that he was commissioned by the "Société de Pharmacie" to examine the procedure to extract morphine that was proposed by William Gregory (1803-1838) in Edinburgh and that during his routine work he discovered codeine as a powder crystallized after evaporation of the mother liquor left after treatment with KOH and washing with water. Therefore, for the first time he discovered that morphine was not the only active ingredient in opium. He named the new ingredient codeine and M. Kunckel demonstrated its strong action on the spinal cord and that it did not paralyze the back parts as morphine did.[20]

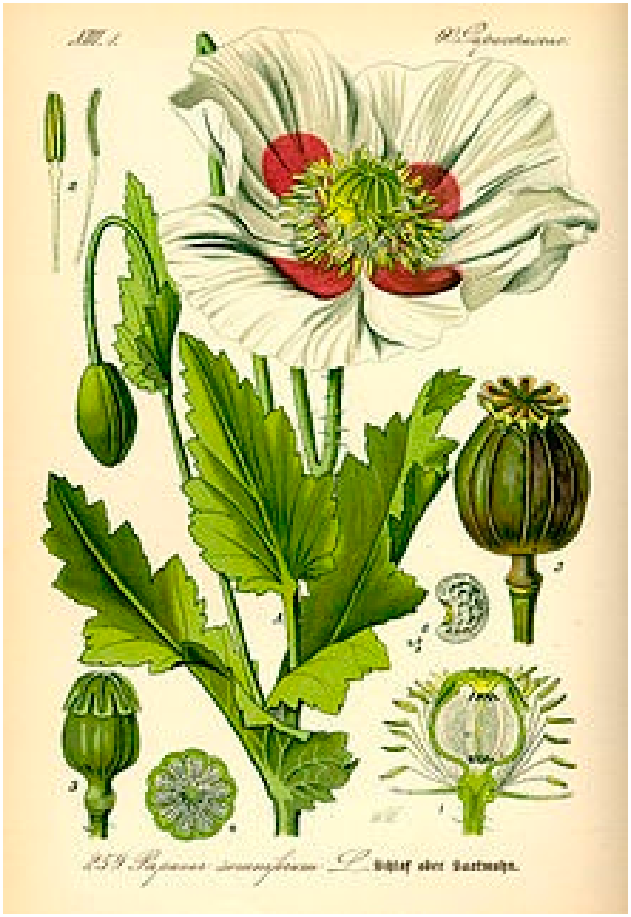

Figure 5. *Papaver somniferum.*

Thebaine (Figure 7) is a minor constituent of opium similar to morphine and codeine but with a weak analgesic action. Its significance comes essentially from its industrial use as the starting material to produce semi-synthetic drugs such as codeine but also the opiate antagonist naloxone (see below).

Codeine is none other than methyl-morphine. Codeine itself is oxidized into codeinone, and the methyl ether of the enol form of codeinone is thebaine.

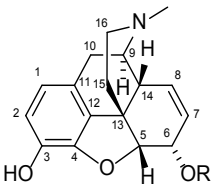

Figure 6. R = H: Morphine; R = CH_3: Codeine. Adapted from Ref. 21.

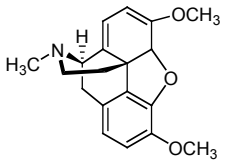

Figure 7. Thebaine or paramorphine (0.3%) weak analgesic action. Adapted from Ref. 21.

It was not until 1927 that a compound identified as the dimethyl ether of morphine was finally isolated from the mixture of the products of hydrogenation of opium.[22] To conclude this enumeration of the most striking chemical interrelations between morphine, codeine and thebaine, it should be recalled that, as Knorr demonstrated in 1909,[23] treatment with acids transforms thebaine into codeinone.

In 1874 heroin was prepared as first example of a semi-synthetic opioid by the English chemist and physicist, C.R.A. Wright, at St. Mary's Hospital Medical School in London.[24] Wright synthesized heroin (diacetylmorphine) after mixing and simmering morphine with acetic anhydride (Figure 8). Heroin displayed 5-fold the analgesic activity of morphine.

First tests of heroin were conducted on dogs and rabbits showed severe side-effects and C. R. A. Wright stopped the experiments. However, in 1897 heroin was rediscovered by Felix Hoffmann at the Bayer pharmaceutical company in Elberfeld (Germany) acetylating morphine with the objective of producing codeine. Therefore heroin, the same compound discovered by Wright was not patentable. Before the extreme addictiveness of heroin was recognized, from 1898 to 1910 heroin was marketed by Bayer as a non-addictive morphine substitute and cough medicine for children,[25] to prepare patients for anesthesia, and to control certain mental disorders (Figure 9).

A range of synthetic opioids such as methadone (1937), pethidine (1939), fentanyl (late 1950s), and derivatives thereof have been introduced, and were targeted for certain specialized applications.

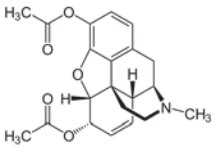

Figure 8. Heroin. Adapted from Ref. 21.

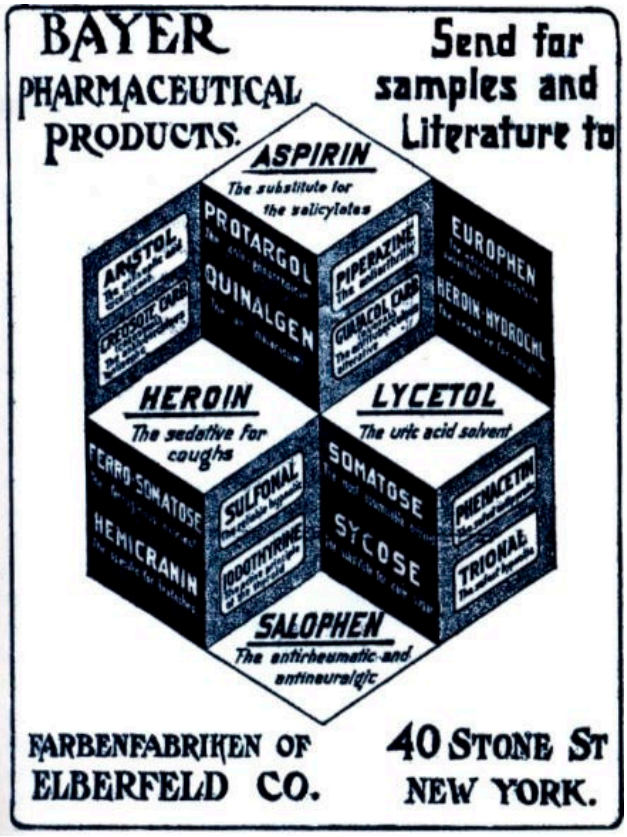

Figure 9. Advertisement for Bayer Heroin: the sedative for coughs.

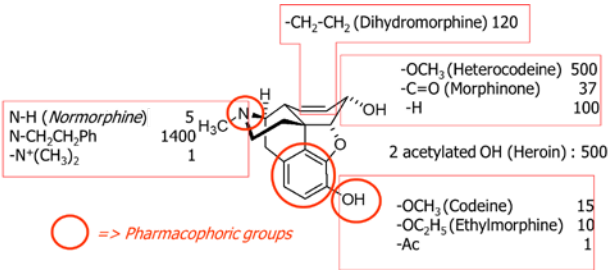

Figure 10. Structure-Activity Relationship: modifications in the morphine structure and analgesic effect. Morphine = 100 as a reference.

Morphine pharmacological profile

Morphine is the most studied molecule of natural origin in the last two hundred years, with the aim of discovering a central analgesic orally active molecule, free of side effects and not addictive.

The main effects of morphine are: analgesia, euphoria and dysphoria (psychological distress), sedation, respiratory depression (the first cause of death due to morphine overdose), depression of cough reflexes, nausea and vomit, physical and psychological dependence, miosis (constriction of the pupil), constipation (reduction of intestinal motility), spasms of the biliary tract, stimuli and difficulties in urination, stimulation of histamine release with consequent vasodilation, bronchial constriction, redness and itching, effects on the endocrine system (decreased libido, impotence, amenorrhea) and immunosuppression.

Morphine can be administered orally (the analgesic potency is reduced to about 5-30% of that obtained by parenteral administration); subcutaneously and intramuscolarly absorption is effective with the inconvenience of tissue irritation; intravenous administration via slow infusion is preferred for better analgesic coverage and reduction in the risk of overdose.

The antalgic therapy uses drugs belonging to different classes: non-steroidal anti-inflammatory drugs (NSAIDs), opioids (morphine-like) and local anesthetics. Local anesthetics (e.g. lidocaine) clearly have a mechanism of action external to the Central Nervous System (CNS), as they affect transient pharmacological blockade of nerve conduction from peripheral receptor sites. The assumptions underlying the historical distinction between NSAIDs, endowed with peripheral activity, and opioids, active at the CNS, no longer seem to be justified. In fact, the central effects of some NSAIDs have been demonstrated, as well as the existence of peripheral opioid receptors.

FROM AGONIST TO ANTAGONIST ACTIVITY: ON THE WAY TO THE DISCOVERY OF ENDORPHINS AND OPIOID RECEPTORS

Observing the relationship between structure and analgesic activity not only in different opium components but also in the semi-synthetic analogues derived from morphine (Fig. 9), it is reasonable to think that a natural plant originated product such as morphine could be further optimized for interaction with a mammalian opiate receptor (for a long time unknown) for which it is not the natural ligand.

Interestingly on June 4, 1970 the use of narcotic antagonists in the treatment of heroin addiction was debated in a symposium sponsored by the National Institute of Mental Health and the Department of Psychiatry, New York Medical College. Many molecules were tested and Cyclazocine apperared to be the most promising one (Figure 11).

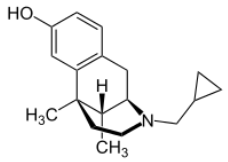

Figure 11. Cyclazocine.

Cyclazocine was found to be a clinically effective and protracted opiate antagonist whose effect lasted for at least 24h. In addition it exhibited unpleasant initial side-effects including dizziness, headaches and hallucinations that were disproportionately intensified as the dose was increased and reappeared when it was discontinued. It was concluded by the review of the clinical data that an ideal antagonist would be one exhibiting antagonistic efficacy for weeks or months, without agonistic activity.[26]

Efforts to avoid these side-effects led clinicians to the use of the first "pure" antagonist: naloxone (Figure 12). Originally synthesized in the private laboratory of Mozes Judah Lewenstein and subsequently developed by Endo Laboratories, Garden City, Naloxone has no pharmacological properties of its own but it abolishes or prevents the hallucinations, euphoria, respiratory depression, nausea, convulsions and other effects produced by narcotics. It can also abolish these effects when they are produced by other antagonists. Naloxone is synthesized from thebaine, which explains its high cost.[27]

Naloxone is a specific opiate antagonist that has no residual agonist activity. It is a competitive antagonist at the different receptors: μ, δ, and k opioid receptors above described. It was introduced in 1973 and is used to inhibit the effect of narcotics on the CNS (see below).

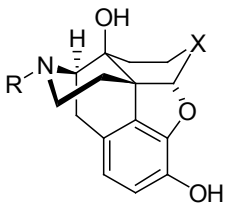

Figure 12. Morphine agonist Oximorphone and antagonist Naloxone. **Oximorphone** (X = CO, R = CH_3) agonist. **Naloxone** (X = CO, R = CH_2-CH=CH_2) antagonist used in opiates overdose.

Narcan, an injectable form of naloxone is currently used to fight the opioid epidemic and substance abuse to reverse drug overdose and addiction.[28]

FROM MORPHINE ANTAGONIST NALOXONE TO ENDOGENOUS OPIOID PEPTIDES: THE DISCOVERY OF ENDORPHINS

After the advent of the pioneering work of Robert B. Merrifield,[29] who introduced the solid-phase synthesis of peptides, the easiest pathway to develop drugs acting at peptidergic receptors is based on characterization of endogenous bioactive peptides that can be synthesized and subsequently structurally stabilized to increase in vivo half-life, limiting rapid metabolism and excretion and fine tuning their biological properties.

However, this general trend was not observed in the case of morphine, since evidences of the existence of endogenous substances with morphine-like activity (endorphins) were obtained only in the 70's, after decades of use of morphine and its derivatives for both recreation and therapeutic applications, extensively described above. Of particular significance was the observation that naloxone was able to antagonize the analgesia induced by electrical stimulation of specific areas of the brain (gray periaqueductal area). The emerging hypothesis suggests that under stress conditions (electrical stimuli), the release of endogenous substances with an activity profile similar to that of morphine is activated. This is the paradigmatic example that exogenous natural products are able to mimic the activity of endogenous molecules.

In 1975, Hans W. Kosterlitz and his former student John Hughes discovered Enkephalins, natural ligands for opiate receptors that were characterized as the pentapeptides H–Tyr–Gly–Gly–Phe–Met–OH and H–Tyr–Gly–Gly–Phe–Leu–OH (Figure 13). The structure was elucidated by the determination of the amino acid sequence of natural enkephalins by the dansyl–Edman procedure and mass spectrometry and followed by synthesis and demonstrating the complete chemical and biological equivalence of the natural and synthetic peptides. These morphine-like peptides that can be antagonized by opiate antagonists, such as naloxone, are naturally occurring substances in the brain, which affect how we feel pleasure and help fight pain.[30] Independently Solomon H. Snyder identified the same two peptides in bovine brain.[31-33] Kosterlitz, Hughes and Snyder shared the prestigious Albert Lasker Prize in 1978 for this research that paved the way for the development of new kinds of nonaddictive analgesic.

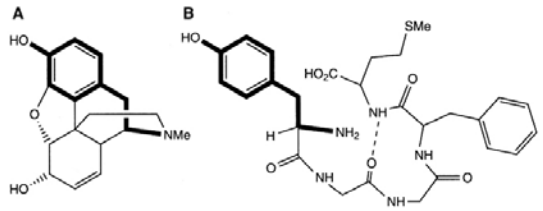

Figure 13. Similarities of the pharmacophoric feature in bold of morphine (A) and [Met^5]enkephalin (B).

Interestingly, only one single C-terminal amino acid residue is the difference between [Met^5]enkephalin and [Leu^5]enkephalin. Both peptides induce in vivo a deep analgesia in rat (completely antagonized by naloxone), but the activity is short-lived, because of a fast degradation by blood and cerebral peptidases. [Met^5]enkephalin has about 30% of the morphine potency and is about 3-fold more potent than [Leu^5]enkephalin.

The N-terminal tyrosine residue was found to be the main pharmacophoric determinant of opioid peptides, a structural feature shared also by morphine and analogues (Figure 13). In fact, this portion is strictly maintained by all the brain opioid peptides discovered subsequently (Figure 14).

The brain opioid peptides discovered in the '70s led to the implementation of a new nomenclature. In fact, the endogenous peptides were initially considered not related to morphine from a structural point of view, but their pharmacological actions are similar to those of morphine, as they are ligands of the same receptors.

The term opioid has over the years been used to indicate a substance that is pharmacologically similar to opium or to morphine, both of an endogenous nature and of a synthetic or semi-synthetic nature. Enkephalins

[Leu^5]enkephalin	Tyr-Gly-Gly-Phe-Leu
[Met^5]enkephalin	Tyr-Gly-Gly-Phe-Met
Dinorphin A(1-17)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln
Dinorphin B(1-8)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile
Dinorphin (1-13)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr
α-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys
β-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro
β_h-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu- Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Gln

Figure 14. Endogenous Opioid Peptides: Endorphins.[34]

and other brain peptides discovered later are collectively known as endorphins (Figure 13).

Endorphins are among the brain chemicals known as neurotransmitters, which are molecules inducing electrical signals from one neuron to the subsequent within the nervous system. At least 20 types of endorphins have been discovered in humans. Endorphins can be found in the pituitary gland, in other parts of the brain, or distributed throughout the nervous system. Stress and pain are the two most common factors leading to the release of endorphins. Endorphins bind and interact with the opiate receptors in the brain leading to nociception, a perception of analegesia. As such they act similarly to drugs such as morphine and codeine. In contrast to the opiate drugs, however, activation of the opiate receptors by the body's endorphins does not lead to addiction or tolerance.

THE CHARACTERIZATION OF OPIOID RECEPTORS

Identification in the 70's of endorphins and in particular enkephalins, as the endogenous substances with morphine-like activity enabled the search and characterization of the opioid receptors. Therefore it was possible to classify the opioid receptors into three types, called μ, δ, κ.[35] The genes of these receptors were cloned and the relative transcripts showed similarities of more than 60% of the nucleotide sequence.

All opioid receptors belong to the superfamily of G protein coupled receptors (GPCR), whose α-subunits are of the Gi/0 type. Using these transductional couplings, opioid receptors control the activity of effectors such as adenylate cyclase (inhibition) and some ion channels (Ca^{2+} and K^{+}).

μ Opioid receptors

The μ receptors are the most widespread and abundant and mediate most of the opioid analgesic effects. Morphine and naloxone have a weak μ selectivity. However, μ opioid receptors are located also outside the CNS, in numerous intramural nerve plexes (gastrointestinal tract, biliary tract, urogenital pathways, circulatory and respiratory systems). Accordingly, it was not possible to confirm the elegant hypothesis, that a single opioid receptor subtype could be the unique mediator of "pure" analgesic activity. Based on this hypothesis, a selective agonist of this receptor subtype would be the "perfect" analgesic, devoid of side-effects, induced by activation of different receptor subtypes by non-selective compounds. In fact, several attempts to develop such a selective agonist by modification of morphine failed, simply because each receptor subtype mediates many different biological effects.

κ Opioid receptors

The hypothesis that suggested their existence was advanced by Martin,[36] and was based on the different effects produced by Morphine and some structurally-related analogues (for example, ketociclazocine). They have been so named to highlight the ketonic nature of the compounds that activate them selectively (in particular EKC, ethyl ketociclazocine).

They play an important role in receiving and processing the primary afferent pain information. In the brain they integrate the ascending pain information and inhibit the painful sensations that descend to the spinal cord. Also located in the limbic system and in the cerebral cortex, they are involved in affective and emotional states and in the awareness of analgesia.

Function of receptor κ. They exert a modulation role of the processes of: analgesia, diuresis, hypothermia, neuroendocrine secretions, feeding (activation of k receptors causes an increase in appetite, inhibited by nor-BNI, a selective k antagonist.

κ-Agonists. In various animal models, the κ-agonists cause sedation at lower concentrations than the μ-agonists. The increase in diuresis, induced in a characteristic way by the κ-agonists, is consequent to the inhibition of the release of VP from the neurohypophysis.

The withdrawal symptoms from κ-agonists are different and less severe than those that occur with agonists μ. A further advantage of their use is linked to the absence of respiratory depression and constipation.

A disadvantage, found when κ-agonists are administered, is the occurrence of dysphoric and psychotomimetic effects in humans. This is due to a lack of selectivity, as many of these substances also interact with δ-receptors.

The use of κ-agonists with arylacetamide structure induces neuro-protection from cerebral ischemic damages.

The endogenous ligands of the **κ**-receptor belong to the dynorphin and neoendorphin families. The first peptide to be isolated from the pig's pituitary gland was Dinorphin – (1-13) in 1979;[37] in 1982 the Dynorphin-(1-17) was isolated,[38] and the neoendorphin was first isolated in 1979 from the porcine hypothalamus but an incorrect sequence was assigned.[39] The sequence was then assigned corrected in 1981 by the same research group.[40-41]

δ Opioid receptors

The distribution of δ opioid receptors (studied by autoradiographic techniques with tritiated and iodinated radioligands) at the CNS level is pre-eminent in the more evolutionary younger cerebral structures (olfactory bulb, caudate nucleus, neopallio, putamen), while it is relatively poor in midbrain and in the medulla oblongata. Following the observation of their almost total absence, for example in reptiles and birds, this receptor subtype would have developed fully later than the other opioid receptors.

Function of δ Receptor. δ Opioid receptors play a role in regulating the processes of analgesia, motor coordination, intestinal motility, smell, respiration, cognitive function, emotional state.

OPIOID-RECEPTOR-LIKE

In addition to the three classic opioid receptors (μ, δ, κ), a new opioid receptor named Opioid-Receptor-Like (ORL) was discovered in 1994.[42] It is also coupled to G proteins and has a high sequence homology (> 60%) compared to μ, δ, κ. However, the typical opioid ligands (peptides and non-peptides) do not bind to ORL. It is present in all brain regions and in the spinal cord. It is located in the intestine, in the vas deferens, in the liver and in the spleen. It was not found in skeletal muscles, in the esophagus, in the kidneys and in the adrenal glands.

An endogenous agonist characterized to be an eptadecapeptide structure (see comparison with Dinorphin A) called Orphanin-FQ (OFQ) or Nociceptin (NC) has been identified.

OFQ (or NC) is generated by pro-Orphanin-FQ (or pro-Nociceptin). The pharmacological profile of NC has not yet been fully defined, but NC has analgesic activity.

OFQ: Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln

DinA: Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln

SYNAPSIS AND ENDORPHINS RELEASE

As depitected in the general scheme reported in Figure 15, pro-opioid proteins are synthesized in the cell nucleus (1) and are transported by microtubular system (2) to the nerve terminal. (3) The active endogenous opioids (E) are released from the pro-opioid proteins by the "process" proteins that are specific proteinases, (4) they are transported and stored in the presynaptic vesicles, and (5) are released when the presynaptic neurons

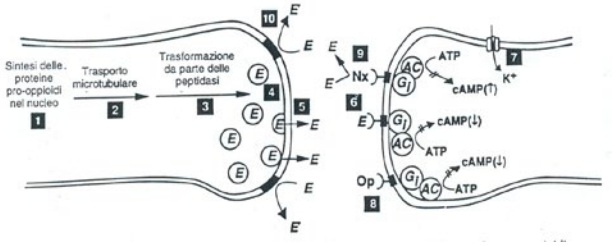

Figure 15. General scheme of peptidergic synapses.[43]

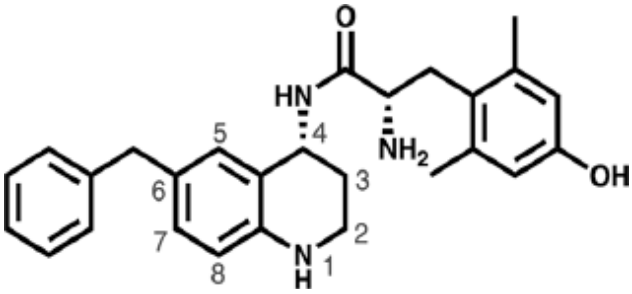

Figure 16. Peptidomimetic lead compound concomitantly acting as μ-opioid receptor agonist and δ-opioid receptor antagonist.[44]

are excited. (6) Endogenous opioid peptides bind to the postsynaptic receptor, activating the inhibitory G protein (Gi) that induces inactivation of adenylyl cyclase, inhibiting release of cAMP and (7) influence the influx of potassium ions through the cell membrane. The overall effect is hyperpolarization of postsynaptic neuron and inhibition of cell excitation. (8) Exogenous opioids (Op) such as morphine bind to the opioid receptors and simulate the action of (E). (9) Opioid antagonists such as naloxone (Nx) bind to receptors and competitively inhibit the action of (E) and (Op). (10) The action of (E) is interrupted by a membrane-bound peptidase, which hydrolyzes the peptide bond Gly3-Tyr4 in enkephalin and leads to its inactivation.

TOWARD THE PERFECT PAINKILLER: DUAL ACTIVITY PEPTIDOMIMETICS

Pharmacological studies suggest that the δ-opioid receptor plays a key role in modulating some side effects associated with opioids including analgesic tolerance and physical dependence.

In fact coadministration of δ-opioid receptor antagonist with morphine attenuate analgesic tolerance, physical dependence and drug-seeking behavior. Accordingly recent studies aimed to develop molecules concomitantly acting as μ-opioid receptor agonist and δ-opioid receptor

antagonist. In particular recently Mosberg *et al.* reported a family of peptidomimetics (Figure 16) producing long-lasting and dose dependent antinociception in mice after peripheral administration.[44]

The bioavailable molecules recently described are promising leads in the search for future drug candidates endowed with dual activity as μ-receptor agonist/δ-receptor antagonist. These novel peptidomimetics can represent the long sought painkillers.

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