WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 03, 1727-1733. Editorial Article ISSN 2277– 7105

OPIORPHIN AS ENDOGENOUS PEPTIDE OF HUMAN SALIVA ACTS AS INHIBITOR OF PROTEASE ENZYMES OF ENKEPHALINS

Kinsuk Sarker and Prof. Dr. Dhrubo Jyoti Sen

Department of Pharmaceutical Chemistry, Shri Sarvajanik Pharmacy College, Gujarat Technological University, Arvind Baug, Mehsana-384001, Gujarat, India.

Article Received on
19 Jan. 2017,

Revised on 09 Feb. 2017,
Accepted on 02 March. 2017

DOI: 10.20959/wjpr20173-8133

***Corresponding Author**
Prof. Dr. Dhrubo Jyoti Sen
Technological University, Arvind Baug, Mehsana-384001, Gujarat, India.

ABSTRACT

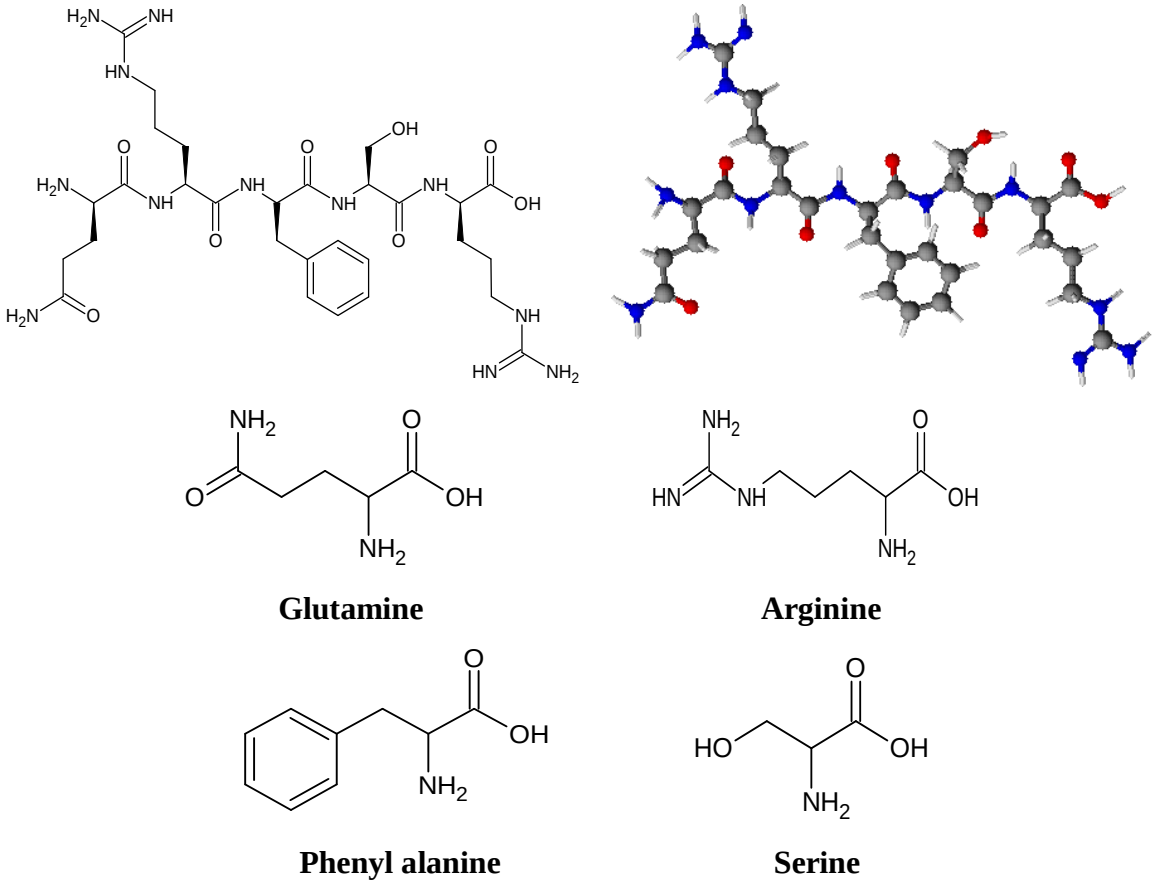
Saliva from humans has yielded a natural painkiller up to six times more powerful than morphine, researchers say. The substance, dubbed opiorphin, may spawn a new generation of natural painkillers that relieve pain as well as morphine but without the addictive and psychological side effects of the traditional drug. When the researchers injected a pain-inducing chemical into rats' paws, 1 milligram of opiorphin per kilogram of body weight achieved the same painkilling effect as 3 milligrams of morphine. The substance was so successful at blocking pain that, in a test involving a platform of upended pins, the rats needed six times as much morphine as opiorphin to render them oblivious to the pain of standing on the needle points. It's pain-suppressive effect is like that of morphine. It may also be an anti-depressive molecule. Opiorphin works in nerve cells of the spine by stopping the usual destruction of natural pain-killing opiates there, called enkephalins. Opiorphin is such a simple molecule that it should be possible to synthesize it and produce large quantities without having to isolate it from saliva. Alternatively, it might be possible to find drugs which trigger patients' bodies to produce more of the molecule themselves.

KEYWORDS: Opiorphin, Peptide, Glutamine, Arginine, Phenyl alanine, Serine, Ecto-endopeptidase, Ecto-aminopeptidase-N, Dipeptidyl peptidase, Enkephalin, Met-enkephalin, Leu-enkephalin.

INTRODUCTION

Opiorphin is [(2S,5S,8S,11S,14S)-14,17-diamino-8-benzyl-2,11-bis(3-guanidinopropyl)-5-(hydroxymethyl)-4,7,10,13,17-pentaoxo-3,6,9,12-tetraazaheptadecan-1-oic acid] which is a

tetra peptide of four amino acids: Glutamine+Arginine+Phenyl alanine+Serine in which the amino acid sequence is like this; Gln-Arg-Phe-Ser-Arg; L-glutaminyl-L-arginyl-L-phenylalanyl-L-seryl-L-arginine.[1,2]



Glutamine **Arginine**

Phenyl alanine **Serine**

Figure-1: Opiorphin and amino acids

The opiorphin molecule has logP= –3.42 because it is highly polar and water soluble so it is easily solubilized in saliva. Molecular Formula=$C_{29}H_{48}N_{12}O_8$, Formula Weight=692.7g, Composition=C(50.28%), H(6.98%), N(24.26%), O(18.48%).

Opiorphin is an endogenous chemical compound first isolated from human saliva. Initial research with mice shows the compound has a pain killing effect greater than that of morphine. It works by stopping the normal breakup of enkephalins, natural pain-killing opioids in the spinal cord. It is a relatively simple molecule consisting of a five-amino acid polypeptide, Gln-Arg-Phe-Ser-Arg in which Arg comes twice to make peptide bond (-CO-NH-).

Opiorphin tetra peptide originates from the N-terminal region of the protein PROL-1 (proline-rich, lacrimal-1). Opiorphin inhibits three proteases: neutral ecto-endopeptidase

(MME), ecto-aminopeptidase-N (ANPEP) and perhaps also a dipeptidyl peptidase DPP3. Such action extends the duration of enkephalin effect where the natural pain killers are released physiologically in response to specific potentially painful stimuli, in contrast with administration of narcotics, which floods the entire body and causes many undesirable adverse reactions, including addiction liability and constipation. In addition, opiorphin may exert anti-depressive and antipanic action.[3,4]

Figure-2: Saliva and Morphine

Therapeutic application of opiorphin in humans would require modifying the molecule to avoid its rapid degradation in the intestine and its poor penetration of the blood brain barrier because it is highly polar in nature (logP= –3.42). This modification is done in the body by transformation of N-terminal glutamine into pyroglutamate. This pyroglutamized form preserves the analgesic properties of opiorphin but with increases pharmaceutical stability. A new painkilling substance has been discovered that is up to six times more potent than morphine when tested in rats — and it's produced naturally by the human body. Natural painkillers are very rare and researchers hope that this recent find might be harnessed as a clinical treatment.[5,6]

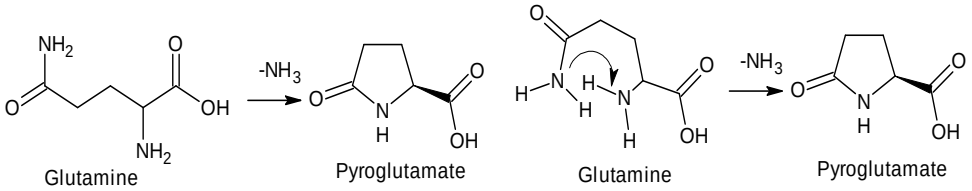


Figure-3: Glutamine converts into pyroglutamate (logP: -1.67 to -2.39)

Naturally produced painkillers might help to avoid some of the side effects experienced by patients treated with synthetic compounds such as morphine, including addiction and tolerance with prolonged use. But the new substance will first have to be tested to confirm whether it will be an effective drug, experts warn.

An **enkephalin** (occasionally spelt encephalin) is a tetrapeptide involved in regulating nociception in the body. The enkephalins are termed endogenous ligands, as they are internally derived and bind to the body's opioid receptors. Discovered in 1975, two forms of enkephalin were discovered, one containing leucine ("leu") and the other containing methionine ("met"). Both are products of the proenkephalin gene. Met-enkephalin is Tyr-Gly-Gly-Phe-**Met** and Leu-enkephalin has Tyr-Gly-Gly-Phe-**Leu**. There are three well-characterized families of opioid peptides produced by the body: enkephalins, endorphins and dynorphin. The met-enkephalin peptide sequence is coded for by the enkephalin gene; the leu-enkephalin peptide sequence is coded for by both the enkephalin gene and the dynorphin gene. The pro opio melano-cortin gene (POMC) also contains the met-enkephalin sequence on the N-terminus of β-endorphin, but the endorphin peptide is not processed into enkephalin. The receptors for enkephalin are the δ-opioid receptors and μ-opioid receptors. Opioid receptors are a group of G-protein-coupled receptors, with other opioids as ligands as well. The other endogenous opioids are dynorphins (that bind to κ-receptors), endorphins (μ-receptors), endomorphins and nociceptin/orphanin FQ. The opioid receptors are ~40% identical to somatostatin receptors (SSTRs).[7,8]

Met-enkephalin, also known as **metenkefalin** (INN), sometimes referred to as **opioid growth factor** (**OGF**), is a naturally occurring, endogenous opioid peptide that has opioid effects of a relatively short duration. It is one of the two forms of enkephalin, the other being Leu-enkephalin. The enkephalins are considered to be the primary endogenous ligands of the δ-opioid receptor, due to their high potency and selectivity for the site over the other endogenous opioids.

Met-enkephalin was discovered and characterized by John Huges, Hans Kosterlitz, et al. in 1975 after a search for endogenous ligands of the opioid receptors. Met-enkephalin is a tetrapeptide with the amino acid sequence Tyr-Gly-Gly-Phe-Met. The tyrosine residue at position 1 is thought to be analogous to the 3-hydroxyl group on morphine. Met-enkephalin is found mainly in the adrenal medulla and throughout the central nervous system (CNS), including in the striatum, cerebral cortex, olfactory tubercle, hippocampus, septum, thalamus and periqueductal gray, as well as the dorsal horn of the spinal cord. It is also present in the periphery, notably in some primary afferent fibres that innervate the pelvic viscera.[9]

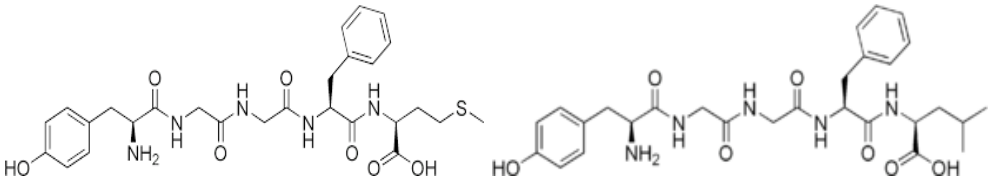


Figure-4: Met-enkephalin and Leu-enkephalin

Met-enkephalin: $C_{27}H_{35}N_5O_7S$:
(2S)-2-[[(2S)-2-[[2-[[2-[[(2S)-2-amino-3-(4-hydroxyphenyl)propanoyl]amino]acetyl]amino]acetyl]amino]-3-phenyl propanoyl]amino]-4-methylsulfanylbutanoic acid.

Biosynthesis

Met-enkephalin is synthesized from proenkephalin via proteolytic cleavage in two metabolic steps. Proenkephalin-A is first reduced by either one of two trypsin-like endopeptidase enzymes, prohormone convertase-1 (P-1) or prohormone convertase-2 (PC-2); then, the resulting intermediates are further reduced by the enzyme carboxypeptidase-E (CPE; previously known as enkephalin convertase (EC)). Proenkephalin-A contains four sequences of met-enkephalin (at the following positions: 100–104; 107–111; 136–140; 210–214) and as a result, its cleavage generates four copies of met-enkephalin peptides at once. In addition, anabolism of proenkephalin-A results in the production of one copy each of two C-terminal-extended met-enkephalin derivatives, the heptapeptide met-enkephalin-arg-phe (261–267) and the octapeptide met-enkephalin-arg-gly-leu (186–193), though whether they affect the opioid receptors in a similar manner as met-enkephalin is not entirely clear.

Clearance

Met- and leu-enkephalin are metabolized by a variety of different enzymes, including aminopeptidase-N (APN), neutral endopeptidase (NEP), dipeptidyl peptidase-3 (DPP3), carboxy peptidase-A6 (CPA-6) and angiotensin converting enzyme (ACE). These enzymes are sometimes referred to as enkephalinases.[10]

Pharmacodynamics

Met-enkephalin is a potent agonist of the δ-opioid receptor and to a lesser extent the μ-opioid receptor, with little to no effect on the κ-opioid receptor. It is through these receptors that met-enkephalin produces its opioid effects, such as analgesia and antidepressant-like effects. It is also the endogenous ligand of the opioid growth factor receptor (OGFR; formerly known as the ζ-opioid receptor), which plays a role in the regulation of tissue growth and regeneration; hence why met-enkephalin is sometimes called OGF instead.

Pharmacokinetics

Met-enkephalin has low bioavailability, is rapidly metabolized and has a very short half life (minutes). These properties are considered undesirable in pharmaceuticals as large doses would need to be administered multiple times an hour to maintain a therapeutically relevant effect, making it unlikely that met-enkephalin will ever be used as a medicine. [D-Ala2]-Met-enkephalinamide (DALA), is a synthetic enkephalin analog which is not susceptible to degradation by brain enzymes and at low doses (5-10µg) caused profound, long-lasting, morphine-like analgesia when microinjected into rat brain.[11]

Leu-enkephalin is an endogenous opioid peptide neurotransmitter with the amino acid sequence Tyr-Gly-Gly-Phe-Leu that is found naturally in the brains of many animals, including humans.

$C_{28}H_{37}N_5O_7$: (2R)-2-[[(2R)-2-[[2-(2R)-2-amino-3-(4-hydroxyphenyl)propanoyl]amino] acetyl]amino]acetyl]amino]-3-phenylpropanoyl]amino]-4-methylpentanoic acid.

It is one of the two forms of enkephalin; the other is met-enkephalin. The tyrosine residue at position-1 is thought to be analogous to the 3-hydroxyl group on morphine. Met-enkephalin [$-CH_2-S-CH_3$] this linkage in side chain is replaced by [$-CH-(CH_3)_2$] in Leu-enkephalin. Leu-enkephalin has agonistic actions at both the μ- and δ-opioid receptors, with significantly greater preference for the latter. It has little to no effect on the κ-opioid receptor.[12]

CONCLUSION

Opiorphin, the word comes from **Opi**um+M**orphin**e which focuses on morphine that comes from opium. It is an endogenous peptide which has four amide (-CO-NH-) linkages produced by amino acids Glutamine, Arginine, Phenyl alanine and Serine having free amino $–NH_2$ and carboxylic acid –COOH that makes the molecule zwitterionic producing amphoteric in nature. So this molecule has logP in negative scale (–3.42), so it can easily solubilize in saliva that can act as inhibitor of protease enzymes of enkephalins both (met-enkephalin & leu-enkephalin) because both enkephalins have same amphoteric nature due to free amino and carboxylic acid groups which possess common functional group effect that can inhibit the protease enzymes by competitive inhibition on opioid receptors. Since the pain pathway follows phospholipase A_2 followed by cyclooxygenase which both are enzymes having same peptide bonds which is present in opiorphin. So it acts by endogenous molecule to act on opioid receptors to inhibit neuronal pain.

REFERENCES

1. Rougeot C, Robert F, Menz L, Bisson JF, Messaoudi M. "Systemically active human opiorphin is a potent yet non-addictive analgesic without drug tolerance effects". J. Physiol. Pharmacol, 2010; 61(4): 483–90.
2. Dickinson DP, Thiesse M. "cDNA cloning of an abundant human lacrimal gland mRNA encoding a novel tear protein". Curr. Eye Res., 1996; 15(4): 377–86.
3. Stanović S, Boranić M, Petrovecki M, et al. "Thiorphan, an inhibitor of neutral endopeptidase/enkephalinase (CD10/CALLA) enhances cell proliferation in bone marrow cultures of patients with acute leukemia in remission".Haematologia (Budap), 2000; 30(1): 1–10.
4. Thanawala V, Kadam VJ, Ghosh R. "Enkephalinase inhibitors: potential agents for the management of pain". Curr Drug Targets, 2008; 9(10): 887–94.
5. Davies KP. "The role of opiorphins (endogenous neutral endopeptidase inhibitors) in urogenital smooth muscle biology". J Sex Med., 2009; 6(3): 286–91.
6. Tian XZ, Chen J, Xiong W, He T, Chen Q. "Effects and underlying mechanisms of human opiorphin on colonic motility and nociception in mice". Peptides, 2009; 30(7): 1348–54.
7. Rougeot C, Robert F, Menz L, Bisson JF, Messaoudi M. "Systemically active human opiorphin is a potent yet non-addictive analgesic without drug tolerance effects". J. Physiol. Pharmacol, 2010; 61(4): 483–90.
8. Popik P, Kamysz E, Kreczko J, Wróbel M. "Human opiorphin: the lack of physiological dependence, tolerance to antinociceptive effects and abuse liability in laboratory mice". Behav. Brain Res., 2010; 213(1): 88–93.
9. Javelot H, Messaoudi M, Garnier S, Rougeot C."Human opiorphin is a naturally occurring antidepressant acting selectively on enkephalin-dependent delta-opioid pathways". J. Physiol. Pharmacol, 2010; 61(3): 355–62.
10. Yang QZ, Lu SS, Tian XZ, Yang AM, Ge WW, Chen Q. "The antidepressant-like effect of human opiorphin via opioid-dependent pathways in mice". Neurosci. Lett, 2011; 489(2): 131–5.
11. Maraschin, Jhonatan Christian; Rangel, Marcel Pereira; Bonfim, Antonio Joaquim; Kitayama, Mariana; Graeff, Frederico Guilherme; Zangrossi Jr., Hélio; Audi, Elisabeth Aparecida. "Opiorphin causes a panicolytic-like effect in rat panic models mediated by μ-opioid receptors in the dorsal periaqueductal gray". Neuropharmacology, 2016; 101: 264–270.
12. Sitbon, Philippe; Van Elstraete, Alain; Hamdi, Leila; Juarez-Perez, Victor; Mazoit, Jean-Xavier; Benhamou, Dan; Rougeot, Catherine. "STR-324, a Stable Analog of Opiorphin, Causes Analgesia in Postoperative Pain by Activating Endogenous Opioid Receptor-dependent Pathways". Anesthesiology, 2016; 125(5): 1017–1029.