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REVIEW ARTICLE

REVIEW ON HUMAN OPIORPHIN: A POTENT NON-ADDICTIVE ENDOGENOUS PEPTIDE ANALGESIC WITHOUT DRUG TOLERANCE EFFECTS

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ABSTRACT:

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Opiods analgesics such as morphine, have the biggest disadvantage of addiction and tolerance with prolonged use. It has been reported that Human Opiorphin is a potent non-addictive endogenous peptide analgesic without drug tolerance effects. This paper summarizes the recent scientific findings about Opiorphins.
Keywords: Pain, Opiorphin, endogenous peptide.

INTRODUCTION

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage[1]. According to etiology and pathophysiology pain is of two types acute and chronic pain[2],[3]. The drugs which are used to reduce or alleviate the acute and chronic pain are called Analgesics[4],[5],[6]. Prostaglandins are knowns as pro-inflammatory chemicals which are generally produced by body as a response to tissue damage. They have important role in process of pain and inflammation. Analgesic effect can be obtained by diminishing the production of Prostaglandins[7].

Opioid analgesics are commonly used analgesic agents to alleviate pain. Opioid drugs like morphine and its derivatives act on opioid receptors and reduces the pain. Opioid receptors are of three different classes: mu(μ), delta(δ) and kappa(κ) receptors. Drugs bind to these receptors and are responsible for producing analgesic effect[4],[5],[6]. Opioids receptor activation can be done by decreasing the production of cAMP by inhibiting adenylate cyclase. Opioids cause hyperpolarization of target cell by increasing the conduction of potassium and make the cell less responsive to depolarizing pulses by inhibiting the calcium influx. Due to this the release of neurotransmitters from neurons is

declined and reduces the generation of impulse[8].

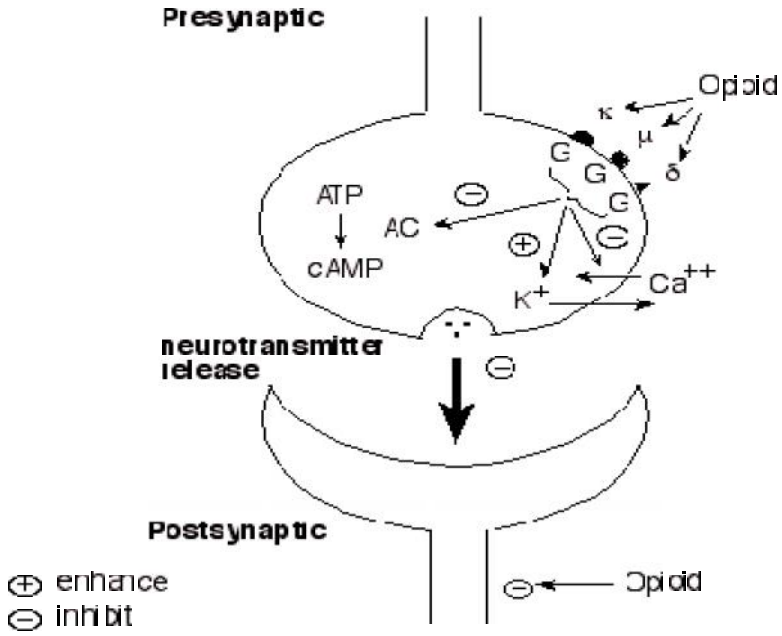

Fig No: 1 Mechanism of action of opioid receptors adapted from[9].

Opioid receptor agonists have limited use as they develop tolerance and dependence. They show some adverse effects like respiratory depression and constipations[2],[3],[10]. Analgesics are also used for treating various pains like spinal cord injury, Osteoarthritis, Rheumatoid arthritis, Back pain etc[6].

Endogenous opioid peptides: Endogenous opioid peptides bind to repectors preferentially.

Endomorphines binds extensively to mu(μ) receptors[11] while enkephalins binds to Delta(δ) receptors. Enkephalinergic derivatives have pain killer effect. Dynorphins binds specifically to Kappa(κ)[12].

Receptor subtype	Functions	Opioid Peptide Affinity
μ(mu)	Supraspinal and spinal cord analgesia; sedation; inhibition of respiration; slowed gastrointestinal transit.	Endorphins>enkephalins>dynorphins
δ(delta)	Supraspinal and spinal analgesia; modulation of hormones and neurotransmitter release.	Enkephalins>endorphins>dynorphins
K(kappa)	Supraspinal and spinal analgesia;psychotomimetic effects; slowed gastrointestinal transit.	Dynorphins>>endorphins and enkephalins.

Table 1: adapted form[13].

Enkephalins are the endogenous opioid peptides interacts with mu(μ) and delta(δ) opioid receptors, and plays a key role in control of pain perception[14].

Sialorphin is the first identified natural enkephalin degrading inhibitor found in rats. Sialorphin a QHNPR peptide (Gln-His-Asn-Pro-Arg) produce potent antinociception by inhibiting enkephalin degradation[15]. It is an androgen peptide extracted from saliva and urine excreted by submandibular and prostate gland of adult rat. Sialorphin also found to obtain in milk and placenta[16]. Along with potent analgesic activity, it has key role in male sexual behavior and pain perception[15],[17],[18]. Due to its limitations a functional homologue is discovered in human saliva[19]. Human Saliva secreted from oral cavity is a combination of water electrolytes, proteins and enzymes[20]. This is the important biological fluid containing most potent painkiller "Opiorphin"[21].

Biochemical Approach for isolation of Opiorphin[22]:

The centre for Biomedical research at Pasteur Institute, Laboratory of pharmacology of Pain, Department of Structural Biology and Chemistry, Paris cedex, France, started isolation of Opiorphin by following clinical research protocol. Human saliva was collected from healthy individuals followed by successive extraction and HPLC chromatographic procedure for the separation of major salivary component which can inhibit the human Neutral endopeptidase (NEP) activity. Finally the product was purified by micro-sequencing, it was proved the existence of pentapeptide having QRFSR sequence. QRFSR pentapeptide was named as Opiorphin. Activity of opiorphin was similar to that of QHNPR pentapeptide, Sialorphin obtained from rat. Opiorphin was obtained from selective maturation of human Proline rich, lacrimal 1 (PROL 1) it was revealed by in-silico genomic analysis. Using pharmacological methods it was revealed that Opiorphin pentapeptide was a dual inhibitor of both Neutral endopeptidase(NEP) and aminopeptidase N(AP-N). Thus it protects the circulating enkephalins from degradation[22].

Peptide Synthesis:

Sample preparation plays a major role in quantifying simple molecules from complex biological fluids as their quality mainly depends on the sensitivity and specificity of analysis. As the biological fluids contain more number of proteins it is difficult to isolate small molecules like Opiorphin[16].

Initially biological sample was de-frosted at $4^{o}C$, and then it was treated according to following conditions:

Acid-methanol extraction:

To one volume sample four volumes of 0.1% trifluoroacetic acid(TFA) in methanol was added at $4^{o}C$ and then mixture was processed by rapid stirring, it resulted in the precipitation and elimination of high molecular weight proteins that were denatured in acid-methanol solution. The soluble low molecular weight molecules in methanol phase were separated from the precipitate by centrifugation at 4700rpm for 30 mins at $4^{o}C$ and were lyophilized at $-110^{o}C$ for 48hrs[16].

C18 Solid-phase Extraction[23]:

Acidified and clarified biological samples

Applied to C18-seppak catridges

Pre-conditioned with three successive cycles of methanol and pure water, maintained in 0.1% TFA- Water.

Analytes were el → 100% methanol containing 0.1% TFA

Fraction were collected at 4°C

Frozen at -80°C

&

Lyophilised at -110°C for 48hrs.

Dried extracts were resuspended in 250µl pyrolyzed water at 4°C

Centrifuge for 30mins at 4000 rpm and +4°C to quantify Opiorphin related components[23]

Reverse phase C18 HPLC Chromatography[23]:

RP-HPLC coupled with PDA and radiometric detection was used to separate and identify the opiorphin related molecules.

Stationary Phase: C18 bonded analytical column

Mobile phase : acetonitrile in presence of 0.1% trifluoroacetic acid(TFA)

- C18 analytical columns were used under TFA 0.1% water solvent-equilibrium conditions and the resuspended extracts obtained were applied.
- Elution was carried out using 25mins Linear gradient techniques from 0-50% acetonitrile, containing 0.1% TFA at 1ml/min flowrate. The various compounds were eluted and isolated according to their hydrophobic nature.
- The HPLC system was Thermo-regulated at 12°C.
- Each fraction measuring 1ml was collected and lyophilized at -110°C for 48hrs.
- The eluted fractions were collected at 1min time interval, lyophilized at -110°C for 48hrs and the chromatographic profile was analysed by the peak heights.

Opiorphin:

Chemical formula	$C_{29}H_{48}N_{12}O_8$
Molecular Weight	692.36 $g.mol^{-1}$
Amino acid sequence	Gln-Arg-Phe-Ser-Arg
Solubility	Soluble in Water
Appearance	White powder

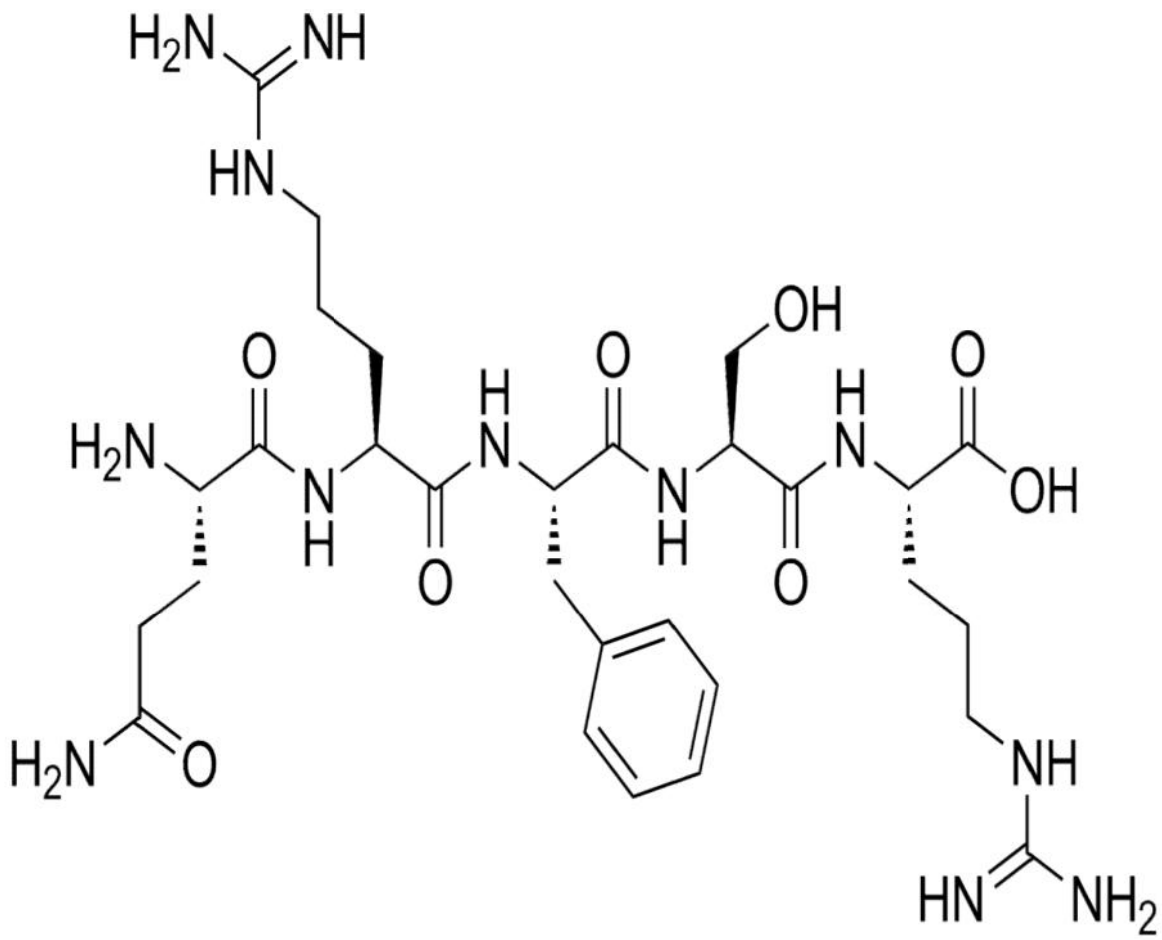

Fig No:2 OPIORPHIN adapted from[24]

Opiorphin, a QRFSR pentapeptide (Gln-Arg-Phe-Ser-Arg) obtained from N-terminal of protein Proline rich, lacrimal 1 was a natural anti-nociceptive modulator of opioid dependent pathway[21].

Mechanism of action of Opiorphin:

Opiorphin is a natural inhibitor of enkephalin inactivating enzymes like neutral ecto-endopeptidase and ecto-aminopeptidase[25]. It increases the half-life of enkephalins[26],[27] and thereby increases the binding capacity of enkephalin related opioid peptides to opioid receptors and shows the analgesic effect by activating endogenous opioid pathway[28]. In addition to analgesic activity, it possess anti-panic and anti-depressant activity[26].

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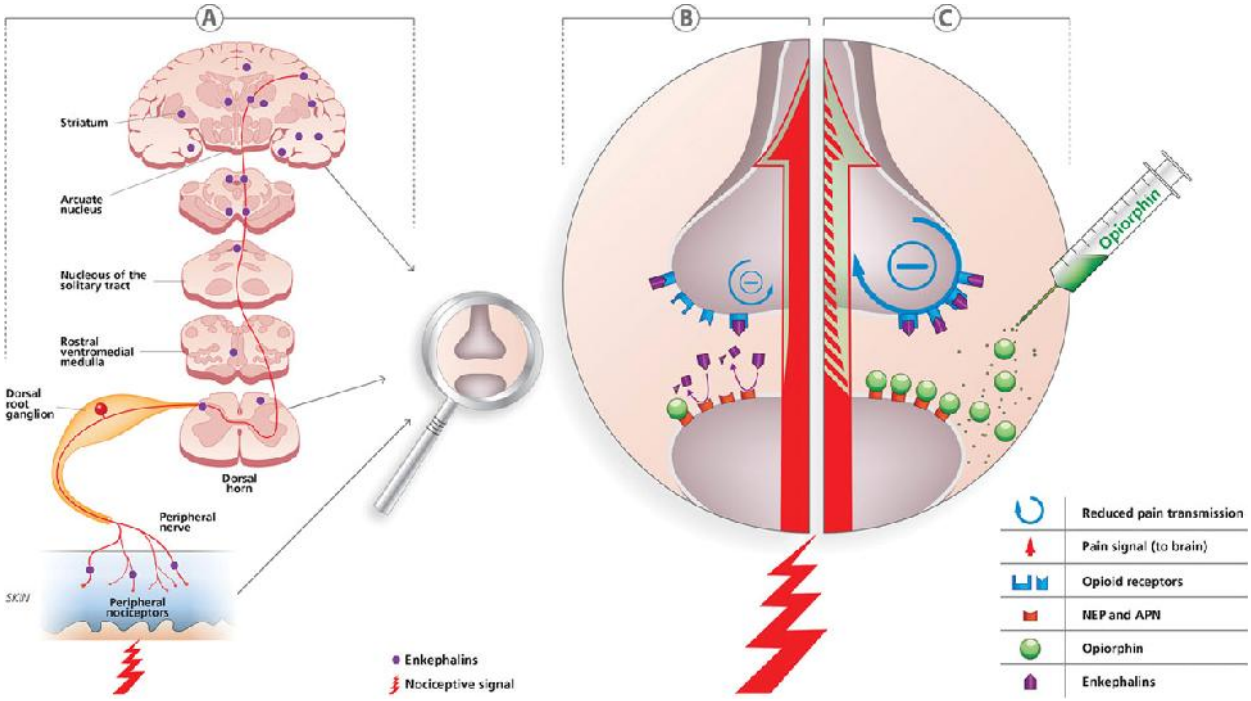
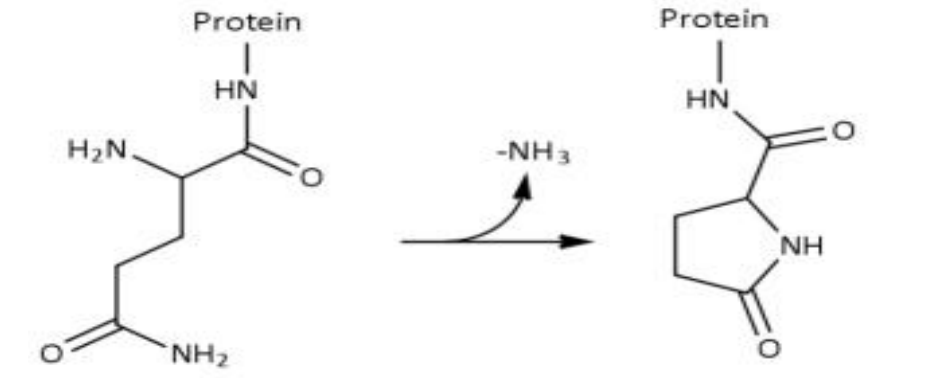


Fig No:3 Mechanism of action of Opiorphin adapted from[29]

Compared with other opioid drugs opiorphin does not cause addiction, drug tolerance and constipation. So opiorphin was considered as more potent drug compared to other opioid drugs like morphine[15]**.** Opiorphin was identified in male reproductive system, mammary glands and lachrymal glands, so in addition to saliva it occurs in semen, milk, tears and urine. Opiorphin secretion and concentration was differentiated based on gender and type of organ[16].

Opiorphin required modification of molecule to show therapeutic use, due to drawbacks of peptides like rapid degradation by peptidases which affect their clinical use and poor penetration of blood brain barrier. N-terminal glutamine is transformed to pyroglutamate form which improves pharmaceutical stability like improves half-life and bioavailability[30].



Glutamine **Pyroglutamate**

Fig No: 4 Conversion of glutamine to Pyroglutamate adapted from[31]

Structure Activity Relationship[32]:

Structure Activity Relationship of Opiorphin was studied to know about the aminoacid residues and functional groups required to show inhibitory effect towards NEP and AP-N human ectopeptidases. The inhibitory activity of modified compounds was evaluated.

The importance of N-terminal amine group of the NH_2-QRFSR peptide in the inhibitory potency of Opiorphin towards rhAP-N:

- The inhibitory potency of Opiorphin peptide reduces towards AP-N ectopeptidases on pyroglutamylation($pGlu^1$-RFSR), octanoylation(($(CH_2)_8$-QRFSR), biotinylation(biotine-[$(CH_2)_6$]-QRFSR), Acetylation(Ac-QRFSR),of amine group.
- On the other hand pyroglutamylation and octanoylation shows the inhibitory activity equal to Opiorphin native peptide towards rhNEP[32].

The Significance of free C-terminal carboxyl group in inhibitory potency towards rhNEP:

- The inhibitory activity of compound towards rhNEP will be diminished on amidation of the C-terminal (QRFSR-$CONH_2$).

The role played by aromatic side chain of Phe^3 residue in inhibitory potency towards rhNEP and rhAP-N:

- Substitution with Tyr residue(QRYSR) forms a compound with 8 fold decrease in rhAP-N inhibition potency and slight decrease in rhNEP inhibition potency.
- If it is substituted with an Ala residue led to compound with completely reduced inhibitory potency towards both rhNEP and rhAP-N[32].

The importance of RFS central residue of QRFSR peptide in inhibitory potency:

- The compounds QRGPR-QHNPR-QRFPR showed inhibitory activity towards rhAP-N. On the other hand activity is low or diminished towards rhNEP.

The role played by the guanidium side chains of the Arg^2(R^2) and Arg^5(R^5) residues in inhibitory potency towards rhAPN:

- Substitution with amino acid side chain of Lys residue led to compounds with 10 folds decrease in rhAPN inhibitory activity and equal inhibitory potency towards rhNEP.

From the above consequences it is clear that, Phe^3plays key role in opiorphin interaction with rhNEP and rhAP-N. FSR tri-aminoacid possess minimum NEP inhibition activity. FSR peptide is 10 times more active than the QRFSR peptide in its inhibitory potency. Aminoacid sequence of opiorphin is required for rhAP-N inhibition, change in sequence decreases the inhibition activity[23].

By the addition of N-terminal Zn-chelating group, a cys-thiol group and replacing the first peptide bond by polyethylene substitutes, a $[CH_2]_6$ linker and substitution by Ser^4 by Ser-O-$[CH_2]_8$,results in a high performing C-[$(CH_2)_6$]-QRF[S-O-$[CH_2]_8$]-Ra opiorphin analog shows 10 folds increased inbitory potency towards rhAP-N and more than 40 folds increase in inhibitory potency towards NEP compared to native QRFSR peptide. This peptide analog has more plasma stability and shows analgesic activity in formalin-induced rat pain model[23].

Few analogs have been developed by modifying Opiorphin: At position 4 serine is replaced by Proline form P_1 residue and two more analogs are formed by insertion of alanine residue in place of Gln(P_2) and His(P_3) in Sialorphin sequence[33].

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Table No:2 Analogs of Opiorphin adapted from[33]

Peptide	Sequence	Formula
Opiorphin	QRFSR	$C_{29}H_{48}N_{12}O_8$
Sialorphin	QHNPR	$C_{26}H_{42}N_{12}O_8$
P_1	QRFPR	$C_{31}H_{50}N_{12}O_7$
P_2	AHNPR	$C_{24}H_{39}N_{11}O_7$
P_3	QANPR	$C_{23}H_{40}N_{10}O_8$

]

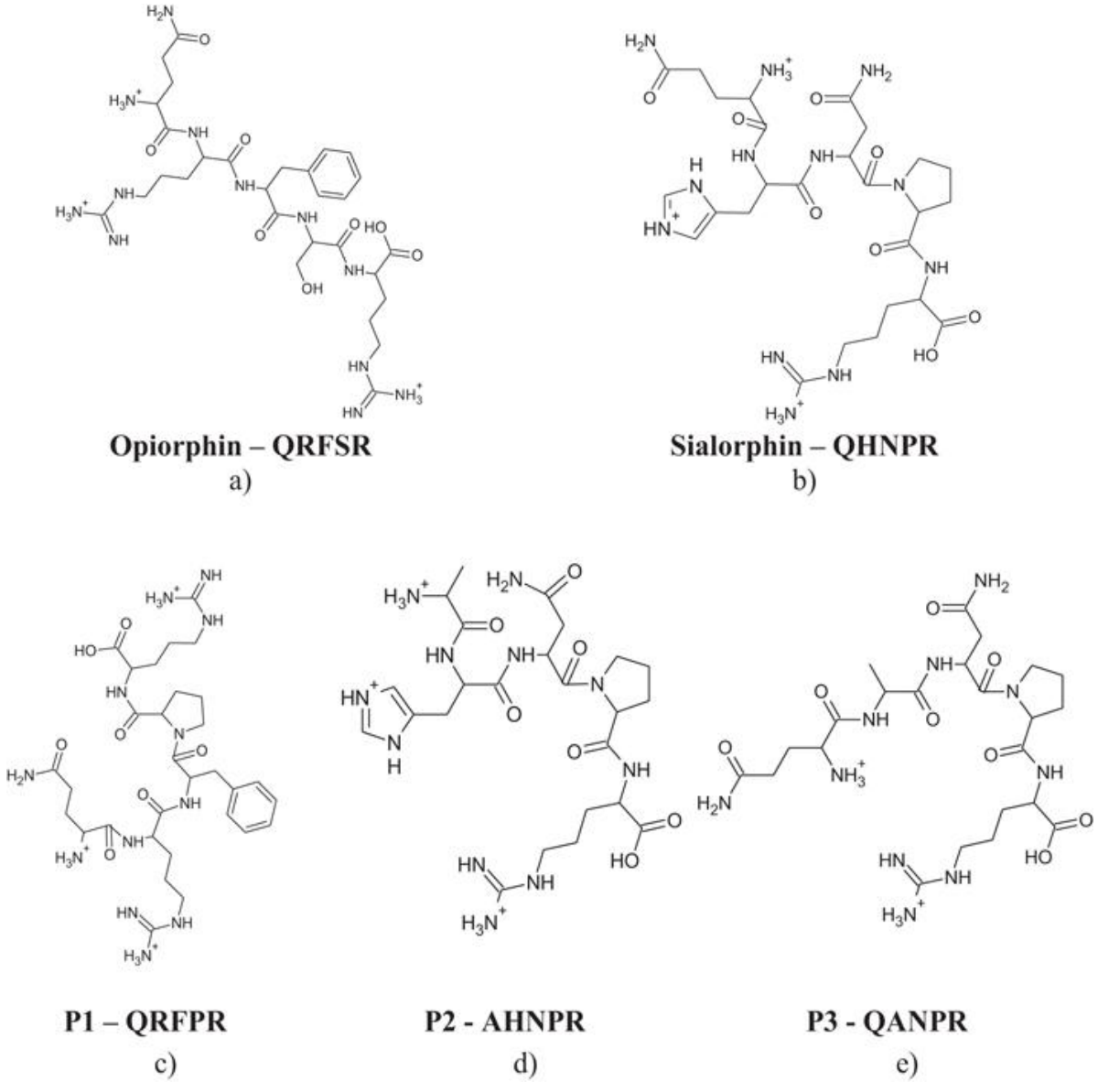

Fig No:5 Opiorphin Analogs adapted from[33]

ANIMAL STUDIES:

Tail Flick Test:

Tail flick test was conducted to evaluate analgesic effect of Opiorphin. Albino mice of either male or female of 20-25grams weight were selected and divided them into 5 groups, each group has 6 mice. Group I was treated with 0.2ml distilled water served as control. Group II, III, IV served as treated were given 0.2ml, 0.4ml and 0.5ml doses of saliva collected from healthy human and finally group V was treated with 300mg/60kg acetyl salicylic acid[34].

The doses were given at a time to all 5 groups and the test was conducted at 0mins and then for every 30mins upto 180mins. Mice was strained with cuff hold and the tail was dipped in water bath at 50^{o} temperature and tail flick was observed for evaluation of analgesic effect[35].This model was used to evaluate central pain were the pain response was from spinal origin. This method is simple and inexpensive[34]. The results were evaluated statistically by ANOVA test followed by Tukey's multiple comparision test[34].

Table No:3 Effect of saliva adapted from [34]

Dose of Saliva	Time(to produce maximum effect)
0.2ml	90mins
0.4ml	120mins
0.6ml	180mins

0.4ml of saliva produces maximum effect at 120mins similar to that of standard drug[34].

Formalin induced pain model:

Formalin-induced pain model was to analyze the analgesic potency of opiorphin analogs, it was conducted by selecting Male Wistar rats of 250-280gms weight, after acclimatization period of 7 days, they were weighed and randomly placed for treatment in room with 12hrs alternative light and dark cycles. Opiorphin analogs was administered systematically by dissolving in vehicle solution(55% 0f PBS 100Mm-45% of Acetic acid 0.01N), at a dose 2mg/kg in 10-15 mins prior to behavioral test and similarly Morphine HCL is given at a dose of 2mg/kg. Group of 8 rats were used for experiment, 50µl of a 2.5% formalin solution was injected under the surface of left hind paw after I.V. injection the duration of formalin-injected paw licking and the number of inflamed paw flinches and body tremors were recorded for a period of 60 min after formalin administration[23].It was observed that systematic administration of opiorphin analog[C]-[$(CH_2)_6$]-QRF-[S-O-$(CH_2)_8$]-R at 2mg/kg reduces the pain throughout 30-60 mins time period. Previously this model was used to test Opiorphin[23].

CONCLUSION:

Literature studies of human opiorphin when compared with acetyl salicylic acid and its analogue [C]-[$(CH_2)_6$]-QRF-[S-O-$(CH_2)_8$]-R compared with Morphine, both have shown to produce an analgesic effect using tail flick and formalin induced pain models respectively . The mechanism of action may be activation of the endogenous opioid pathways by inhibiting the breakdown of the endogenous enkephalin..

This study gives us hope that in future there may be methods to relieve pain especially free of addiction and adverse effects. Opiorphin is a promising molecule that does not have any addiction , tolerance and constipating actions and also offers an additional advantage as it known to possess anti-panic and anti-depressive activity.Further researches and experimental studies can be carried out by isolating opiorphin molecule from saliva as it is a simple molecule and its analgesic effects should be compared with morphine.

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