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Research Article

ENDOMORPHIN-2, ITS RELATED TETRAPEPTIDE DERIVATIVES, TOPICAL ANALGESIC EFFECT FOR INSTANT RELIEF OF VARIOUS PAINS

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ABSTRACT

Background and Purpose: Endomorphin-1 and endomorphin-2 are two endogenous tetrapeptide amides which have been shown to have analgesic effect by systemic injection in rats. Our study could determine if endomorphins or their related derivatives have any analgesic effect on topical application in human subjects.

Experimental Approach: Certain endomorphin-related tetrapeptide derivatives were synthesized, and formulated as suitable topical formulations. The compositions were topically applied to human skin to determine if the compositions could provide any analgesic effect.

Results and Conclusions: We have discovered that endomorphin-2 and certain related derivatives including N-acetyl-endomorphin-2, N-pyroglutamyl- endomorphin-2 and N-benzoyl-endomorphin-2 hydrazide but not endomorphin-1 or related derivatives, on topical application to areas of pain provide complete, instantaneous and durable relief of various kinds of pain. The various kinds of pain include common headache, hangover headache, osteoarthritis, psoriatic arthritis, dental extraction, joint sprains, muscle stress and sunburn.

Implications: The above endomorphin-2 derivatives can be used instead of morphine or other opiates for pain control in numerous conditions.

INTRODUCTION:

Animal studies have shown that two endogenous opioid tetrapeptides, endomorphin-1and endomorphin-2, have a very specific and high affinity to μ-opioid receptors for analgesic effect after systemic injection, and that their affinities to delta or kappa 1-opioid receptors are very minimal (Zadina, Hackler, and Kastin, 1997; Goldberg, Rossi, Letchworth, Mathis, Ryan-Moro, Leventhal , et al ,1998; Zadina, Martin-Schild , Gerall, Kastin, Hackler , Ge, et al, 1999). Both endomorphin-1 and endomorphin-2 have tetrapeptide sequences in amide form as **Tyr-Pro-Trp-Phe-NH_2** and **Tyr-Pro-Phe-Phe-NH_2**, respectively.

The neuroanatomical distribution of endomorphins reflects their potential endogenous role in many major physiological processes; these include (a). perception of pain, (b). responses related to stress, and (c). complex functions such as reward, arousal, and vigilance, as well as autonomic, cognitive, neuroendocrine, and limbic homeostasis (Zadina, 2002; Fichna, Janecka, Costentin, and Do Rego, 2007). The endomorphins have been found in the brain, stored in neurons and axon terminals. The most outstanding effect of the endomorphins is their antinociceptive action, acting in both central and peripheral neurons. Additionally, the endomorphins can cause vasodilatation by stimulating nitric oxide release from the endothelium..

The μ receptor has been discovered to have two subtypes, namely μ_1 and μ_2. Studies have shown that μ_2-opioid receptors would be stimulated by both endomorphin-1 and endomorphin-2, whereas μ_1-opioid receptors would be stimulated only by endomorphin-2. Further studies have revealed that μ_1-opioid receptors mediate supraspinal analgesia and modulate acetylcholine and prolactin release, whereas μ_2-opioid receptors mediate spinal analgesia, respiratory depression, and inhibition of gastrointestinal transit (Sakurada , Zadina , Kastin , Katsuyama , Fujimura , Murayama , et al, 1999; Przewlocki, Labuz, Mika, Przewlocka, Tomboly, and Toth 1999)

Opioids such as morphine, acting at the mu opioid receptor, are the most effective analgesics. Adverse side effects however severely limit their use. Of particular importance, widespread abuse has caused major medical, societal, and economic problems. Respiratory depression from overdoses has caused fatalities. Tolerance to higher doses complicates treatment and increases the risk of serious side effects. Motor and cognitive impairment are especially problematic for older adults. Despite the host of negative side effects, opioids are commonly used for acute and chronic pain conditions (Fichna, Janecka, Costentin, and Do Rego, 2007; Zadina, Nilges, Morgenweck, Zhang, Hackler, and Fasold, 2015).

Separation of analgesia from unwanted side effects has long been an unachieved goal of opioid research. Rats spend more time in a compartment when they have received morphine, but treatment with endomorphin does not induce this behavior (Sakurada, Zadina, Kastin, Katsuyama, Fujimura, Murayama, et al, 1999).

Rats injected with morphine had significantly impaired motor skills and impaired breathing, while rats receiving endomorphins experienced no impaired motor skills or substantial respiratory depression. The pain relief provided by the endomorphins was equal to or greater than that by morphine, and it is time for nonaddictive relief of pain (Zadina, Nilges, Morgenweck, Zhang, Hackler, and Fasold, 2015; Grosser, Woolf, and FitzGerald 2017)

Initiation of the studies reported herein was motivated by several years of personal endurance of pain by both authors, one of whom having debilitating osteoarthritis of knees and the other having had disabling traumatic damage of the lower back. Pain in the one with knee osteoarthritis was severe, such that lower doses of an opioid was required daily to provide partial but sufficient relief to enable him to continue lifelong pursuits in laboratory, clinical and pharmaceutical research. Pain in the other, due to lower back injuries, was alleviated to tolerable levels by physical therapy and by weekly acupuncture treatment, all of which have enabled him also to continue lifelong pursuits in biochemical and pharmaceutical research. Therefore, both authors have had compelling interest and desire personally to test

on themselves peptide compounds with analgesic potential.

METHODS

The endomorphin-2 and related tetrapeptide derivatives were synthesized by automated equipment and were chemically identified by mass spectroscopy and HPLC, etc. Purities were more than 98%. For topical administration, a tetrapeptide derivative was typically dissolved in an aqueous solution consisting of water 40 parts, ethanol 40 parts and propylene glycol 20 parts by volume (WEP442), or an anhydrous solution consisting of ethanol 70 parts and propylene glycol 30 parts by volume (EP73). When propylene glycol is replaced by PEG-200 in the above aqueous solution, the vehicle is WEG442. Thus, N-acetyl-endomorphin-2 (N-Ac-Tyr-Pro-Phe-Phe-NH_2) and N-pyroglutamyl- endomorphin-2 (N-Pg-Tyr-Pro-Phe-Phe-NH_2) at various concentrations such as 0.5%-1% were readily formulated. For example, when N-pyroglutamyl- endomorphin-2, one gram was dissolved in 99 ml WEP 442, the solution thus formulated contained 1% N-Pg-Tyr-Pro-Phe-Phe-NH_2, in WEP442. N-Benzoyl- endomorphin-2 hydrazide (N-Bz-Tyr-Pro-Phe-Phe-$NHNH_2$) 0.5% in WEP442 was formulated in the same way. Other compositions containing different tetrapeptide derivatives at various concentrations ranging from 0.5 to 4% were readily formulated in the same way.

To formulate lotion, a tetrapeptide derivative, 1 g was dissolved in propylene glycol 33 ml, diisopropyl adipate 33 ml, and the solution thus obtained was mixed with an oil-in-water emulsion 33 g. The lotion thus formulated contained N-Pg-Tyr-Pro-Phe-Phe-NH_2, 1% in lotion.

The authors have tested on their own skin first all the topical formulations for safety including non-irritating, non-allergenic and non-toxic attributes before they were tested by anyone else.

Test solutions were topically applied by the subjects to skin area(s) of pain expression such as frontal, temporal or parietal skin areas for headache; fingers; toes; wrists; ankles; elbows; knees; areas of sunburn, etc. Subjects were those who were brought to our attention because of their having a painful condition or those who sought us out after having learned of our progress in finding effective topical analgesic solutions. Instructions to subjects were to apply the test solution with fingers and hands to the area(s) of pain in an amount to moisten the skin surface thoroughly and to determine degree of pain relief (zero to complete), the rapidity of relief, and the duration of relief. Many subjects who applied test solutions for accidentally traumatized digits or other sites were using, or had used, the solution for relief of pain in chronic conditions. Hence the solutions were immediately available to them. One of the authors involved himself with self-inflicted injures to the skin in order to explore whether pain from such skin-localized injury would be relieved by the same test solutions found to relieve pain of other conditions. These injuries included (a) numerous simultaneous insertions of 27G needles to depth of 1 mm, and (b) intradermal injections of 0.1 ml of 2% NaCl aqueous solution.

RESULTS

Numerous endomorphin derivatives have been tested against numerous kinds of painful conditions (Table). Two derivatives, N-Ac-Tyr-Pro-Phe-Phe-NH_2 and N-Pg-Tyr-Pro-Phe-Phe-NH_2, have been tested most extensively to date, in concentrations of 0.5% to 4%, in both aqueous and non-aqueous vehicles. In use against pain from psoriatic arthritis of fingers, wrists and ankles duration of complete analgesia from a single application of 4% solutions has been as long as 19 hours. We have also discovered that N-Bz-Tyr-Pro-Phe-Phe-$NHNH_2$ is so far the most effective analgesic agent.

Table 1. Conditions wherein pain was completely or partially relieved by topical endomorphin-2 and related tetrapeptide derivatives.

Conditions or Disorders	Number of Cases
Arthritis:	
Osteoarthritis; fingers, knees, shoulders, ankles	21
Psoriatic arthritis; joints of fingers, wrists, ankles, knees	7
Meniscus injury and degeneration	4
Headache:	
Migraine	4
Non-migraine, tension, hangover etc.	14
Dental/toothache	4
Lipoma	1
Muscle pain, soreness	3
Pharyngitis	1
Sprains, bruises: hands, feet, fingers, toes, etc.	18
Sunburn, thermal burn,	3
Viral infection: herpes zoster	1
Wounds: post-operative, post-subdermal injection sites	3

After topical application of an effective test solution the pain was diminished or disappeared either instantly or within a few minutes, and the absence of pain typically lasted for hours. In cases of pain from recently induced trauma or muscular stress the pain never returned. For recurrent chronic pain the test solution would be reapplied to the same skin area(s), with lasting analgesic effect again.

Following are some examples:

Example 1: Analgesic effect on pain from herpes zoster.

A male subject, age 93, developed herpes zoster involving the temporal branch of the tri-geminal nerve with severe pain involving the areas of left temporal, left forehead, left scalp, left neck and left ear canal. Touching of left anterior scalp hair provoked sharp pain.

The subject, by use of a dropper, topically applied an anhydrous composition containing N-Ac-Tyr-Pro-Phe-Phe-NH_2 0.5% in EP73 in amount sufficient to wet the skin surfaces. In less than a minute of topical application, the pain disappeared completely. Relief of pain lasted for up to 1 hour. Multiple repeated topical applications of the same formulation gave the same analgesic result with complete relief of pain that lasted for longer periods of up to 6 hours.

Example 2: Analgesic effect on headache

A female subject, age 55, developed an ordinary tension type of headache early one afternoon. Such headaches usually would persist for several hours, being relieved incompletely by treatment with oral drugs such as ibuprofen and/or acetaminophen. In this instance she topically applied N-Ac-Tyr-Pro-Phe-Phe-NH_2 1% in EP73 to the frontal and temporal areas. Within minutes the headache pain disappeared completely. She stayed pain free over the next 18 hours.

Example 3: Analgesic effect on severe migraine headache

A female subject, age 46, gave a history of severe migraine attacks which occurred every 1-3 weeks over a 10-15 year interval. One day, at approximately 8 AM visual aura, nausea, and pain in frontal scalp, forehead and frontal sinuses began and within 5-10 minutes became very severe. At that time the subject topically applied N-Ac-Tyr-Pro-Phe-Phe-NH_2 1% in WEP442 to the involved areas. In about 6-7 minutes, as she prepared to make another

topical application, pain and all other symptoms suddenly disappeared completely and did not return. At follow-up, 3 days later, she remained free of any headache and symptoms.

Example 4: Comparative analgesic efficacy on pain of psoriatic arthritis

A female subject, age 54, with severe generalized psoriasis beginning at about the age of 16, had received various treatments over the years that included methotrexate, other antimetabolites, and most recently drugs known as biologics. While these drugs have provided intervals of partial relief, none had provided lasting benefit, particularly on her painful arthritis which had developed in both wrists and in six interphalangeal finger joints. Seven related tetrapeptide derivatives, 0.5% to 1% in vehicle WEP 442, one at a time were topically applied to the fingers and wrists of both hands. Analgesic efficacy of each was judged to be as follows.

(1) N-Ac-Tyr-Pro-Phe-Phe-NH_2 provided rapid complete relief of pain, i.e. within 2-4 minutes, lasting for 6-7 hours. Analgesic efficacy was rated by subject as 95-100%..

(2) N-Ac-Tyr-Pro-Phe-Phe-$NHNH_2$ provided slow relief of pain, i.e. within about 10 minutes. Almost complete relief of pain occurred and lasted for 6-7 hours. Efficacy was rated by subject as 90%.

(3) N-Pg-Tyr-Pro-Phe-Phe-NH_2 provided rapid relief of pain, i.e. within 2-4 minutes, and lasted for 6-7 hours. Subject rated analgesic efficacy as 95-100%.

(4) N-Pg-Tyr-Pro-Trp-Phe-NH_2, an endomorphin-1 related derivative provided slow, minimal relief of pain. Analgesic efficacy was judged by subject to be 0-10%.

(5) N-Bz-Tyr-Pro-Phe-Phe-$NHNH_2$ provided rapid complete relief of pains within 2-5 minutes, lasting for about 24 hours. Efficacy was rated by subject the best as 100%.

(6) N-Bz-Tyr-Pro-Phe-Phe-NHOH also provided rapid complete relief of pains within 2-5 minutes, lasting for about 10 hours. Efficacy was rated by subject as 90%.

(7) N-Ac-Tyr-Pro-Phe-Phe-$NHCH_2CH_2CH_3$ provided slower relief of pain, within 5 minutes, and lasted for less than 6 hours. Subject rated analgesic efficacy as 75%.

Example 5: Analgesic effect on painful tendonitis of right wrist and right thumb

A female subject, age 46, with painful tendonitis after playing tennis, topically applied N-Pg-Tyr-Pro-Phe-Phe-NH_2 1% in WEP 442 to the right thumb and to the entire wrist. Within 5-10 minutes all pain and discomfort from any hand movements were gone and did not return within a 24 hour follow up period.

Example 6: Complete relief of knee pain in psoriasis.

A 29 year woman with plaque psoriasis of scalp, elbows and knees for about 10 years had painful knees for the past 4-5 years. Knee pain had gradually worsened. Topical applications of N-Ac-Tyr-Pro-Phe-Phe-NH_2 0.5% in WEP442 had relieved her knee pain for about 6 hours, which now permitted her to pursue recreational hiking. After hiking for several hours knee pain would re-occur, but was immediately relieved by topical application of the above same formulation.

Example 7: Analgesic effect on pain from psoriatic arthritis

A 59 year old woman with plaque psoriasis since early teenage had chronic foot pain for 10-15 years, and neck pain for 4-5 years, the latter being aggravated by occupationally keeping her neck to one side as she held a phone against her head. Topical applications of N-Pg-Tyr-Pro-Phe-Phe-NH_2 0.5% in WEG 442 to the feet and neck had provided almost complete relief of pain for 8-10 hours, consistently over one month of daily use, 2-3 times daily.

Example 8: Analgesic effect on arthritic pain of hands.

A male subject, age 72, a veteran guitar player who in earlier years was able to play his instrument for about an hour without interruption in group session performances. Over recent years his physical capability in playing time gradually was reduced to about 20 minutes, due to worsening painful arthritis of his finger/hand joints. He was provided N-Pg-Tyr-Pro-Phe-Phe-NH_2 0.5% in WEG 442 which he topically applied to his hands and fingers before

beginning to play. Such applications enabled him to play his guitar for 1 hour pain free. He also reported that the quality of his performance improved concomitantly.

Example 9: Analgesic effect on sunburn pain

A female subject, age 54, presented with severe sunburn of anterior legs after exposure to sunlight without sunscreen protection. Topical application of N-Ac-tyr-Pro-Phe-Phe-NH_2 1% in WEP442 to the inflamed skin provided complete relief of pain almost instantaneously. Absence of pain lasted for about 3 hours. Repeated applications provided the same relief.

Example 10: Analgesic effect on acute pains from foot bruise and fractured patella.

A 79 year old female subject suffered severe pain in left knee and in arch of left foot following a forward fall to a wooden floor. Diagnoses of multiple fractures of patella and bruised foot were made by x-ray. Surgical repair of patella was done, and several incisions were closed with adhesive. Intense pain and of both knee and arch of foot persisted. Topical applications to both sites of N-Pg-Tyr-Pro-Phe-Phe-NH_2 0.5% in WEP 442 provided immediate, complete and sustained relief of knee pain and foot pain for more than 6 hours. Topical applications 3-4 times daily allowed the subject to pursue rehabilitation exercises pain free.

Example 11: Analgesic effect on osteoarthritic knee by intra-articular injection

A male subject, age 94, with osteoarthritis of the knees that progressively worsened over the years, evaluated the analgesic effectiveness of many topical preparations of tetrapeptide derivatives of the invention on numerous occasions. After such experiences he decided to self-inject his knees with a sterile aqueous preparation of N-Bz-Tyr-Pro-Phe-Phe-$NHNH_2$ at a concentration of 0.08% (0.8 mg/ml) in water.

Each knee was injected with 1 ml (0.8 mg) of the preparation via the sub-patellar route. There was no discomfort from the injections as compared with other injections.The pains in both knees gradually disappeared and the pain relief lasted for 11 hours. The knees felt more comfortable than usual after 24 hours. The improvement from intra-articular injection was judged to be more than 90% by clinical evaluation.

The foregoing result suggests that endomorphin-2 tetrapeptide derivatives may be therapeutically effective for long term treatment of osteoarthritic pain by in situ administration. We are now engaged in explorations of systemic analgesic therapy with derivatives administered by other routes.

We estimate that the depth of the site from pain where complete relief is achieved by topical application is about 1 cm. Degrees of analgesia achieved in various kinds of knee pains are illustrative:

(a). Pain in earlier phases of osteoarthritis have been relieved rapidly and completely by topical applications of active derivative 1% solutions. As disease has progressed to “bone-on-bone” status, relief of pain still has occurred rapidly but degree of relief has become less than complete.

(b). Pain from an injured meniscus, during days and weeks following injury, has been rapidly and completely relieved by applications of active derivative 1% solutions. In one case surgical remedy was delayed and degenerative changes extended deeper and deeper, at which time relief of pain by topical applications became progressively less than complete.

(c). In contrast is the case in which intense pain from the patella with multiple fractures was completely relieved by topical applications of active derivative 1% solution during a 3 week interval, at the end of which time all pain spontaneously ceased.

The following endomorphin-2 and related tetrapeptide derivatives have been tested and found to be therapeutically effective for various pain conditions:

(1). Endomorphin-2: Tyr-Pro-Phe-Phe-NH_2

(2). Endomorphin-2 related tetrapeptide derivatives:

(a). N-acetyl (N-Ac) derivatives; N-Ac-Tyr-Pro-Phe-Phe-NH_2; N-Ac-Tyr-Pro-Phe-Phe-$NHCH_3$; N-Ac-Tyr-Pro-Phe-Phe-$NHCH_2CH_3$; N-Ac-Tyr-Pro-Phe-Phe-NHOH; N-Ac-Tyr-Pro-Phe-Phe-$NHNH_2$; N-Ac-Tyr-Pro-Phe-Phe-NHNHAc

(b). N-pyroglutamyl (N-Pg) derivatives; N-Pg-Tyr-Pro-Phe-Phe-NH_2; N-Pg-Tyr-Pro-Phe-Phe-$NHCH_3$; N-Pg-Tyr-Pro-Phe-Phe-$NHCH_2CH_3$; N-Pg-Tyr-Pro-Phe-Phe-NHOH; N-Pg-Tyr-Pro-Phe-Phe-$NHNH_2$; N-Pg-Tyr-Pro-Phe-Phe-NHNHAc

(c). N-propanoyl (N-Pa) derivatives; N-Pa-Tyr-Pro-Phe-Phe-NH_2; N-Pa-Tyr-Pro-Phe-Phe-$NHCH_3$; N-Pa-Tyr-Pro-Phe-Phe-$NHCH_2CH_3$; N-Pa-Tyr-Pro-Phe-Phe-NHOH; N-Pa-Tyr-Pro-Phe-Phe-$NHNH_2$;

(d). N-benzoyl (N-Bz) derivatives; N-Bz-Tyr-Pro-Phe-Phe-NH_2; N-Bz-Tyr-Pro-Phe-Phe-$NHCH_3$; N-Bz-Tyr-Pro-Phe-Phe-NHOH; N-Bz-Tyr-Pro-Phe-Phe-$NHNH_2$;

Degree of pain relief for ordinary headaches has been complete in all cases to date. Degree of relief of migraine pain however has been variable, from case to case and in individual cases. Our testing reveals that endomorphin-1 (**Tyr-Pro-Trp-Phe-NH_2**) or its derivative is **not** therapeutically effective as an analgesic substance on topical administration in human subjects. Such finding appears to be in conflict with earlier reports about the analgesic effect of endomorphin-1 by systemic injections in animals

Our findings are that endomorphin-2 and its derivatives are therapeutically effective as analgesic substances to eradicate pain on topical application in human subjects, the findings heretofore unknown to us. There has been no description or implication heretofore about analgesic effect or lack thereof, in animal or human studies, by topical application.

Our studies further reveal that the most active endomorphin-2 related derivatives has been when the amino terminal group is substituted by N-acyl group such as N-acetyl, N-pyroglutamyl or N-benzoyl; the carboxyl group is in amide or hydrazide including unsubstituted or substituted form; and that derivative is less or not effective when the carboxyl group is in a free acid or an ester form. We have also found that a larger radical at the carboxyl group, such as phenethyl amide, renders the derivative less or not effective as a topical analgesic substance.

We wish to emphasize that endomorphin-2 and its derivatives, but not endomorphin-1 or its derivative, are therapeutically effective as analgesic substances to **promptly** eradicate pain on topical administration in human subjects. The mechanism of this unique performance has not been ascertained.

DISCUSSION

Topical analgesic effect of an endomorphin-2 or related tetrapeptide derivative appears to be mediated through binding with μ-opioid receptor in the nerve endings at the outermost levels of the epidermis, because the effect is so rapid. The absence or diminished amount of μ-receptor glycoprotein in damaged epidermis of skin may explain the absence of analgesic effect in skin injured by scalpel incision, or traumatized by intradermal injection of hypertonic saline.

It appears that the μ opioid receptor in the epidermis is predominantly μ_1 opioid receptor which binds only to endomorphin-2, but not to endomorphin-1 for supraspinal analgesia effect (and also for modulating the release of acetylcholine) because endomorphin-1 has very minimal or no analgesic effect on topical application to skin.

The instances of immediate and complete relief of pain at deeper sites, as with muscle strains or patellar bone fractures, suggest that endomorphin-2, or its related tetrapeptide, binds to μ-receptor in nerve endings in epidermis, which in turn suppresses or inactivates pain recognition throughout the nerve fiber to tissue levels as deep as about one centimeter. This perception of mechanism of action from this study seems reasonable insofar as it is unlikely that the

tetrapeptide molecule could penetrate deep enough to reach target levels without extreme dilution. The efficacy and variation of efficacy in individual subjects may depend on the concentration of μ-receptor glycoprotein present in the nerve endings of the epidermis. There is great need today for analgesic agents that have no toxic effects and which could be used in place of opioids. Agents we have uncovered in this study should help fulfill such need.

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