Neuropeptide mimetics for pain management*

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Abstract: The discovery of numerous endogenous neuropeptides that participate in the formation, transmission, modulation, and perception of pain signals offers numerous strategies for the development of new analgesics. Nevertheless, the same research has not yet replaced opioids as the gold standard of pain treatment. Therefore, one possible avenue of drug development may shift interest from searching for receptor-selective opioids to creating an arsenal of drugs that target multiple opioid and non-opioid sites simultaneously. The presented short review focuses on the development of potential analgesic peptidomimetic compounds based upon opioid neuropeptides and substance P.

INTRODUCTION

The extension of life expectancy of the elderly and handicapped is a cardinal success of modern health care. Nevertheless, very often the quality of life of such groups in our society is unsatisfactory because of their increased prevalence of chronic pain associated with cancer, osteoporosis, arthritis, postoperative or postinjury pain, including those associated with phantom limbs or spinal cord injury. Chronic severe pain is most destructive for the human psyche. Presently available analgesics are effective in the treatment of acute pain, although even in this setting “analgesic gaps” remain. But for chronic pain there is a need to develop better therapeutic strategies and analgesics than are currently available. Epidemiologic data indicate that even in prosperous, developed nations, 40% of patients with chronic pain are only partially satisfied and 15% are not satisfied at all with the treatments available to them. At the same time, proper treatment of pain is increasingly demanded as a human right by patients, their families, and governments. Therefore, modern medicine urgently needs more effective treatments for pain. In the future, as health-related quality of life assumes increasing importance, progress in the treatment of persistent pain will be a marker of progress in modern health care sciences.

Advances in genetic and pharmacologic analytical techniques have led to the identification of numerous neuropeptide systems that play key roles in pain transmission and modulation [1]. These neuropeptides are created by nature to interact with target cell membrane receptor(s) in well-defined sites of action and for a very limited time. Therefore, most of these endogenous compounds are characterized by low biological barrier permeability and very high susceptibility to enzymatic degradation. These properties strongly limit the possibility of direct application of native neuropeptides as drugs. However,

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progress in structure–activity relationship analytical methods allowed the design and synthesis of a new generation of drugs based upon the primary structures of endogenous compounds. It is now recognized that the neuropeptides play major roles in most physiological processes, including pain and analgesia, mood and affective behavior, appetite, and inflammation. Therefore, today, the design of drugs that interact with neuropeptide systems is one of the most explored avenues in postgenomic medicinal chemistry.

Substance P has been identified as a major neuropeptide responsible for transmission of nociceptive signals. Endogenous opioids are native neuropeptides that are responsible for modulation (generally, suppression) of nociceptive signals. The development of new compounds as potential drugs for pain control based upon these functional antagonists is the goal of this review.

GENERAL APPROACH TO PEPTIDOMIMETICS DEVELOPMENT

The unique sequence of amino acid residues that forms the primary structure of a peptide chain results in compounds that express highly specific biological activities. The amino acid side chains are now recognized to be responsible for specific interactions of peptides with their receptors (Fig. 1A). Indeed, the peptide backbone is only a skeleton that positions amino acid functional side chain groups in specific topographical relations. This perspective has led to many strategies for peptide molecule modifications. Adding bridges in the side chains, changing the chirality of amino acids, and modification of peptide bonds are some examples of possible modifications (Fig. 1B) that result in more enzyme-resistant and/or selective (peptide) peptidomimetics. The identification of functional groups that are necessary for biological activity and further structural analyses of “active” conformation(s) of the neuropeptide could result in nonpeptidic peptidomimetics, in which functional groups are attached to nonpeptidic, organic skeletons (Fig. 1C).

A.

\[
\begin{align*}
R_1 & \quad R_2 \quad R_n \quad R_m \\
\text{AA}_1 &= \text{AA}_2 \cdots \text{AA}_3 \cdots \cdots \text{AA}_n \cdots \text{AA}_{n+1} \cdots \cdots \text{AA}_m \cdots \text{AA}_{m+1}
\end{align*}
\]

Parent peptide

B.

\[
\begin{align*}
R_1 & \quad R_2 \quad R_n \quad R_{\text{pp}} \\
\text{AA}_1 &= \text{AA}_2 \cdots \text{AA}_3 \cdots \cdots \text{AA}_n \cdots \text{DA}_n \cdots \cdots \text{AA}_{n+1} \cdots \cdots \text{AA}_m \cdots \text{AA}_{m+1}
\end{align*}
\]

peptide peptidomimetic (= peptide analog)

Fig. 1 General scheme of transformation (A) natural peptide into (B) peptide peptidomimetic and (C) non-peptide peptidomimetic.

SUBSTANCE P ANALOGS

The undecapeptide substance P (Fig. 2) is a member of the group of endogenous neuropeptides called tachykinins.

Various tachykinins express different receptor selectivity profiles, but all cross-interact with three types of receptors: NK1, NK2, and NK3. Substance P (SP), that expresses highest selectivity for NK1 receptors, is involved in the generation of inflammatory signals, in neurogenic plasma extravasation
such as in migraine, and in the transmission of nociceptive signals. Therefore, the development of NK1 receptor antagonists has been a challenge for many years. The first peptidic SP antagonist was developed over 20 years ago by replacing Phe7 and Gly9 in SP or its C-terminal (4-11) fragment with D-aromatic residues, and changing the chirality of Pro2 or Pro4 [2]. Such antagonists possessed low receptor affinity and poor selectivity, but were widely applied in early tachykinin studies and served as a basis for subsequent development of selective NK1 receptor antagonists. Screening of natural macrocyclic compounds in the fermentation broth of *Streptomyces violaceoniger*, resulted in the discovery of the macrocyclic peptide FK224, that expressed mixed NK1/NK2 antagonist properties [3]. The search for peptidic minimal fragments with antagonist properties to MK1 receptor resulted in the development of very short ligands. The first of this new series was FR113680 (Fig. 3A) [4]. Further screening of tryptophan derivatives led to the discovery of simple aromatic esters of acetylated tryptophan that possess antagonist activities on NK1 receptors (Fig. 3B) [5].

![Amino acid sequences of endogenous tachykinins.](image1)

**Fig. 2** Amino acid sequences of endogenous tachykinins.

An independent search for nonpeptidic peptidomimetics led to the discovery of the selective ligands for the NK1 receptors, RP67580 [6], CP96345 [7], and CP99994 [8] (Fig. 4).

![Examples of peptidomimetics with antagonistic activity to substance P.](image2)

**Fig. 3** Examples of peptide peptidomimetic with antagonistic activity to substance P.

![Examples of nonpeptidic peptidomimetics with antagonistic activity to substance P.](image3)

**Fig. 4** Examples of nonpeptidic peptidomimetics with antagonistic activity to substance P.
On the basis of the above early successes, several additional analogs have been synthesized and tested at preclinical and clinical levels. The study of substance P receptor antagonists has emerged as a field of great promise due to accumulating evidence that NK1 antagonists offer possible new treatment options in therapeutic areas ranging from emesis, and pulmonary disorders to pain, depression, and anxiety. It is hoped that the unique mechanism of action of these agents, which involves modulation of effects mediated by the interaction of the neuropeptide substance P with its G-protein coupled receptor, will offer improvements over existing therapies. However, until now, substance P antagonists have yielded somewhat disappointing results in clinical analgesic trials [9]. These frustrating results may reflect the presence of substance P endogenous feedback mechanisms and/or activation of alternative nociceptive pathways.

ENDOGENOUS OPIOIDS AND THEIR PEPTIDOMIMETICS

Although several neurohormones are involved in nociceptive modulation, the opioid system plays a dominant role and has therefore been a major target for analgesic development. Compared to our knowledge of other neuropeptides, the opioid system is uniquely comprehensive. The structures of peptidomimetics, natural morphine alkaloids, and synthetic fentanyl or methadone (Fig. 5) were known for over a decade before the isolation of endogenous opioid peptides and their families of receptors in the 1970s.

Therefore, unlike the case for other neuropeptide systems, the discovery of endogenous opioid peptide ligands [10] and characterization of three major types (μ, δ, κ) of opioid receptors did not initiate structure–activity studies, but was itself the result of long-standing research.

Opiate alkaloid molecules are very rigid, whereas opioid peptides are very flexible. This fundamental distinction leads to differences in the mechanism of these molecules’ interaction with opioid receptors. Rigid alkaloids probably interact by means of an “induced-fit” mechanism, in which the receptor adopts a local pocket structure to the alkaloid’s conformation, whereas peptides interact via a “zipper” mechanism [11] in which both the peptide molecule and the binding pocket change their structure to optimize their interaction. Because the final biological effect of interactions of both types of molecules is identical, one might speculate that the general structures of the final ligand-receptor complexes are identical or very similar. Therefore, identification of equivalent groups in series of nonpeptidic and peptidic opioid ligands is an important goal of structure–activity relationship studies, that may lead to the development of new opioid analgesics.
Recognition of the common structural motif of a tyramine moiety in both alkaloid and peptide opioids allowed construction of a uniform model of the opioid pharmacophore complex with an opioid receptor [12]. Structure–activity relationship analysis of peptide- and benzomorphan-based opioid molecules concluded that the conformational requirement for the main opioid ligand element, tyramine is shared by all opioid receptors. In all endogenous opioid peptides that possess affinities for opioid receptors, the N-terminal fragment is the same (Tyr-). Differences in amino acid sequences at the C-terminus are responsible for emphasizing selectivity toward one or another opioid receptor type. Thus, C-terminus plays the role of a specific “address” [13] directing the pharmacophore (“message”) toward particular types of receptor. In the case of opioid peptides, two components of the opioid peptide “address” have been defined.

The first opioid address, common to all endogenous opioid peptides, is an aromatic amino acid residue at positions 3 and/or 4. The role of that amino acid residue(s) is to enhance formation of the initial ligand-receptor complex that allows conformational adaptation of tyrosine within its receptor pocket. The topographical relations between the N-terminal tyramine moiety and the aromatic ring of the amino acid(s) in positions 3 and/or 4 are specific for particular opioid receptor types. Though not clearly evident in the case of flexible peptides, the creation of analogs of opioid alkaloids with additional aromatic rings increases their selectivity for particular receptor types. Interestingly, small differences in the topographic location of aromatic rings attached to alkaloids are capable of switching their biological properties from agonist to antagonist. The application of the “message-address” concept to the development of new compounds resulted in the creation of chimeric compounds in which selectivity of nonpeptidic opioid alkaloids were modulated through attachment of various “addresses” [14–17].
MULTITARGET APPROACH TO PAIN TREATMENT

From a clinical point of view, the reduction of side effects such as respiratory depression, gastric dysfunction, tolerance, dependence, and immunosuppression is a strong rationale for the development of new analgesics. The traditional approach to searching for new drugs is to evaluate compounds that will be easy to administer orally, intravenously or intramuscularly; will readily penetrate biological barriers such as the gut–blood and the blood–brain barriers; and that reach target receptors in the central nervous system (CNS). Through the centuries, these requirements of CNS-active drug have not changed significantly. All common methods of peripheral administration of centrally active drugs (oral, transdermal, intravenous) result in widespread systemic distribution. Only a fraction of the total amount of the drug penetrates nonselectively from the periphery to interact with CNS opioid systems. Clearly, high receptor selectivity at the target organ and high blood–brain barrier permeability permit compensation for the nonselective distribution of the drugs. However, central and nervous system sensitization by nociceptive activity (“plasticity”), the multiplicity of nociceptive neurotransmitters and pathways, and individual genetic variability of opioid receptor subtypes and distribution are potential explanations for the lack of success of selective opioid ligand applied to date in clinical pain management. Progress in interventional techniques during the past 20 years allowed the application of drugs directly into the site of desired effect. In 1978, morphine was first injected intrathecally in humans. Since that time, several techniques of direct application of the analgesics to the spinal cord and brain have been developed. These techniques are characterized by fewer side effects at equianalgesic medication doses than occur with traditional systemic drug administration. The introduction of patient-controlled epidural and intrathecal analgesia (PCA) and implantable, programmable pumps for central drug delivery has provided an additional impetus for the popularization of site-specific drug delivery. Site-specific techniques for central drug administration allow the use of low doses of substances whose spectrum of receptor affinities is very broad. Therefore, in clinical practice, the combination of multireceptor-targeted drugs delivered using modern techniques of site-specific application is a most promising evolution of pain management. Although this approach also permits a combination of drugs to be used, the distinct pharmacokinetic and pharmacodynamic profiles of different agents limits the use of many potential mixtures. An attractive solution to this problem is to develop compounds designed with a broad spectrum of receptor affinities. Because a single such molecule comprises covalently fixed pharmacophores, the balance of activities of each pharmacophore is the most critical factor determining the analgesic properties of the entire molecule. Because of structural cross-interaction, the receptor affinities of a new molecule that hybridizes various pharmacophores is not the simple combination of each component. The analysis of necessary elements of each pharmacophore and simulation of interference with the structural elements of other hybridized pharmacophores is an important step in design of new multtarget drugs.
Biphaliin: The peptide peptidomimetic with broad spectrum of opioid affinities

Twenty years ago, a dimeric opioid peptide peptidomimetic with two tetrapeptide pharmacophores connected through a hydrazide bridge was synthesized [18] (Fig. 9).

This compound, termed biphalin, expresses a broad spectrum of opioid receptor affinities (i.e., high, equal affinity for µ- and δ-opioid receptors and lower, but significant affinity for κ-receptors) [19]. Structural analysis showed that both pharmacophores are flexible and can easily adopt conformations to bind to all opioid receptor types [20]. Its broad spectrum of affinities was for many years off-putting to pharmacologists focused upon developing receptor-selective opioid ligands. As described above, recent evolution in understanding the pain system has revealed that nociceptive modulation is a complex process in which all opioid receptors participate. This insight has reactivated pharmacological studies of biphalin, the results of which indicate a unique pharmacological profile. Although it is a uniquely potent analgesic when applied centrally [21], it has very low dependence liability. This constellation of properties, together with its low toxicity in preclinical models, argues for its clinical development as an analgesic drug.

Opioid-substance P hybrids

Extensive, prolonged study of peptidomimetic SP antagonists led to the disappointing conclusion that such compounds themselves are ineffective analgesics. Nevertheless, it has been shown that small quantities of an SP antagonist significantly potentiate the antinociceptive effect of opioid peptides [23]. This observation initiated the search for chimeric compounds that may simultaneously interact with both SP receptors as an antagonist and opioid receptors as an agonist. The first compound of this series hybridized a N-terminal fragment of a casomorphin-related molecule with a C-terminal fragment of an SP antagonist [24]. This chimeric compound indeed had high potency as an analgesic, but its low solubility limited further studies. A recently synthesized new compound, AA501 (Ryc. 10), with such dual properties hybridizes “head-to-head” an opioid tetrapeptide with carbobenzyloxy-tryptophan through a hydrazide bridge. In this molecule, opioid agonist and substance P antagonist pharmacophores partially overlap.
AA501 expresses affinities for µ-opioid and NK1 receptors, and acts in vivo as an analgesic in models of inflammatory and neuropathic pain [25]. These promising results prompted further studies of SP-opioid hybrids. Although SP in general transmits nociceptive signals from the periphery to the brain, and the endogenous opioids modulate that signal, prior studies indicate that autofeedback occurs within SP-containing pathways [26]. Fragments of substance P [27] may induce antinociception in certain models [28] and potentiate morphine analgesia [29]. Therefore, the potential interaction of an SP agonist pharmacophore hybridized with opioid agonist pharmacophore seemed worthy of study. As expected, the final pharmacological properties of such SP agonist/opioid agonist hybrids depend upon the relative activities of these two physiologically antagonistic pharmacophores.

A compound in which the tachykinin pharmacophore dominates induces hyperalgesia [30,31], whereas another compound within which the opioid pharmacophore dominates is antinociceptive [32]. In addition to these expected effects, one compound of this series, termed ESP7 (Fig. 11) showed the intriguing property of achieving analgesia in animals previously made tolerant to the analgesic effects of morphine. The latter unique property suggests that such hybrid compounds offer a previously unexplored avenue to develop drugs for opioid-tolerant patients, or that may find a role in the treatment of opioid dependence.

**CONCLUSIONS**

During the last 30 years, the endogenous systems responsible for pain signal formation, transmission, and modulation have been well characterized. This knowledge has shifted the paradigm for the development of new analgesics. The recognition of various pain mechanisms and nervous system adaptation during pain transmission altered the search for new analgesics from very selective to multitargeted com-

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Neuropeptides and their corresponding peptidomimetics form a very attractive basis to design a new generation of analgesics.

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REFERENCES


