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Review

Endogenous opiates, opioids, and immune function: Evolutionary brokerage of defensive behaviors

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Abstract

Recent empirical data have elucidated a compelling physiological basis for endogenously expressed, chemically authentic, morphine and its cognate μ_3 and μ_4 opiate receptors. Cellular "morphinergic" signaling is predominantly targeted to autocrine/paracrine regulatory processes and is reciprocally linked to stimulated production and release of the free radical gas nitric oxide (NO). Additionally, we have recently described a functionally coupled μ_4 opiate receptor/NO regulatory pathway in human multi-lineage progenitor cells in the absence of traditional opioid, neuropeptide, or catecholamine G-protein coupled receptors (GPCRs). These accumulated data not only suggest an evolutionary primacy for morphinergic signaling as a fundamental regulatory mechanism, but identify μ_3 and μ_4 opiate receptors as candidate primordial GPCRs that may have served as prototypic models for diverse families of GPCRs. The present review focuses on the parallel roles of morphinergic signaling and endogenous opioid peptide-mediated regulatory processes in immune function and the development of defensive integrated behaviors including nociception from rudimentary cellular responses to chemical/environmental challenges. Finally, structural similarities between the intracellular domains of μ_3 opiate receptors and chemokine receptors CCR2 and CCR5 provide an additional chemical basis for these contentions.

Keywords: Enkelytin; Morphine; Cancer; Nitric oxide; Chemokine; Cancer

Contents

1. Opioid peptides and immunoactivation

Opioid pentapeptides stimulate cytokine release and immunocyte chemotaxis and induce conformational changes in immunocytes indicative of activation [1]. In particular, [Met]enkephalin and DAMA (d-ala$_2$, met$_5$ enkephalinamide) are potent mediators of immunocyte conformational changes, both in invertebrates and mammals [1]. In addition, immunocytes also contain a novel δ_2-opioid receptor that expresses a preference for naturally occurring δ-opioid substances [1,2]. It is important to note that these activities are phylogenetically ancient and occur both in invertebrates and in mammals—animals that are 500 million years divergent in

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evolution [1]. The similarities even extend to the regulation of immunocyte activation by neutral endopeptidase [3]. Furthermore numerous studies have now found and sequenced mammalian-like opioid precursor fragments, including [Met]- and [Leu]-enkephalin and [Met]-enkephalin-Arg-Phe, in various invertebrates [4–9]. In addition, opioid receptors have also been demonstrated in various invertebrate tissues [1].

1.1. Invertebrate opioids

After more than 23 years of study on invertebrate opioid mechanisms, groups have isolated and sequenced the opioid precursors in invertebrates [10–13]. These reports show that the mammalian-prodynorphin-like molecule of 119 amino acids contains α-neoendorphin-, dynorphin-A- and dynorphin-B-like peptides, which exhibit strong sequence homology (100%, 50% and 76.8%, respectively) with their mammalian counterparts, and has an identical number of [Leu]-enkephalin sequences. The mammalian–like pro-opiomelanocortin (POMC) molecule, and six of its peptides, including adrenocorticotropin (ACTH) and α-melanocyte-stimulating hormone (α-MSH) were also present. Of the six peptides, three showed high sequence similarity to their vertebrate counterparts [Met]enkephalin, α-MSH and ACTH (100%, 84.6% and 70% respectively). Further, immunocytes from *Theromyzon tessulatum* and the marine mussel *Mytilus edulis* contain a mammalian-like proenkephalin molecule [12]. The structure of the leech proenkephalin material demonstrates considerable amino acid sequence similarity to amphibian proenkephalin (e.g., 25.4% with *Xenopus laevis*) but is smaller, at 15 kDa versus 30 kDa. By contrast, *M. edulis* proenkephalin is not only larger (26 kDa) but exhibits a higher sequence identity with guinea-pig proenkephalin (50%). Both of the invertebrate materials possess [Met]enkephalin and [Leu]enkephalin: in a ratio of 3:1 for *M. edulis* and 1:2 in the leech. They also contain sequences that are flanked by dibasic amino acid residues, demonstrating cleavage sites. Finally, the proenkephalin from both the above invertebrates [12] contain the antibacterial peptide enkelytin, which was first isolated by Metz-Boutique and colleagues [14]. The sequence of enkelytin exhibits a 98% sequence identity with mammalian enkelytin.

1.2. Bactericidal activity

The fact that invertebrate proenkephalin also contains enkelytin supports the hypothesis that these molecules first evolved in simpler animals. Indeed, the presence of enkelytin, with its strong antibacterial activity [14], strengthens the association of opioid peptides with immune-related activities. It is possible that immune or neural signaling may lead to enhanced proenkephalin proteolytic processing, freeing both opioid peptides and enkelytin simultaneously (Fig. 1) [15].

In the above scenario, the opioid peptides would stimulate immunocyte chemotaxis and phagocytosis as well as the secretion of cytokines [1], and the simultaneously liberated enkelytin would attack bacteria immediately [16]. This process would allow time for the immunocyte-stimulating capabilities (i.e., recruitment), of the opioid peptides to manifest themselves. This hypothesis is further supported by the presence of specific and similar [Met]enkephalin receptors on immunocytes of invertebrates and vertebrates. Interestingly, this same scenario

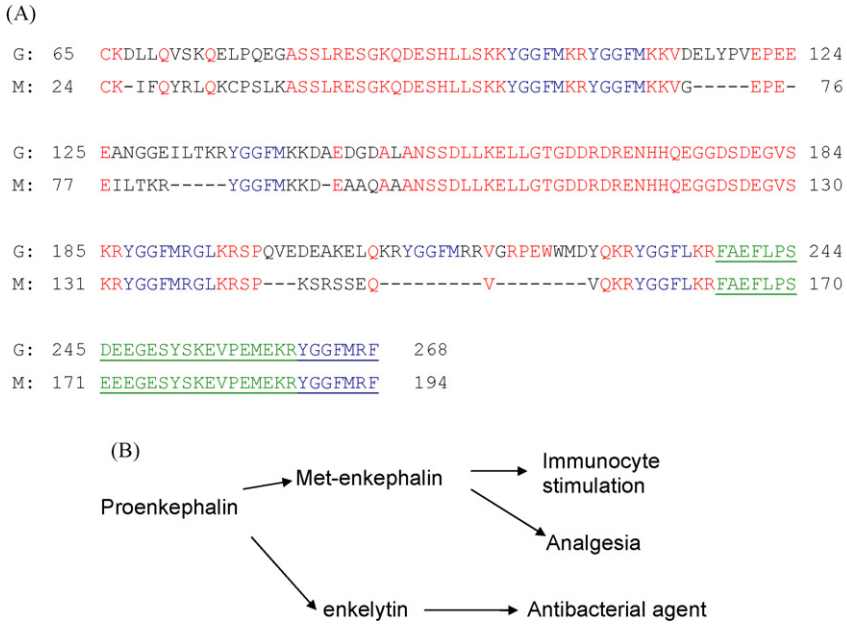

Fig. 1. (A) Comparison of invertebrate and mammalian proenkephalin, demonstrating evolutionarily conserved opioid sequences. Common amino acids are shown in red for comparison with the mammalian material, opioid peptides in blue and the enkelytin (green) sequence, including [Met]enkephalin-Arg-Phe (blue) is underlined, G: Guinea pig, M: *Mytilus edulis*, dashed lines represent spaces in the proenkephalin molecules as determined by a best fit model (figure modified from [16]. (B) Complementary immune actions of [Met]enkephalin and enkelytin. Processing of proenkephalin releases [Met]enkephalin, which induces immunocyte chemotaxis and the release of other signaling molecules (i.e., cytokines), whereas enkelytin exerts an antibacterial action. Within minutes, enkelytin too, is processed to yield [Met]enkephalin-Arg-Phe that further augments the immunocyte response.

may occur in neural tissues and in microglia. In neural tissues, proenkephalin processing may also lead to enkelytin 'liberation', suggesting that neurons may exhibit bactericidal innate immune functions.

Thus, it appears that many mammalian molecular and cellular survival strategies first appeared in invertebrate organisms that evolved at least 500 million years ago. These mechanisms have subsequently evolved to supplement immune actions by covering the latency period before total or partial immune activation occurs. Furthermore, recent studies now indicate that lipopolysaccaride injection, surgical cuts to the integument, or electrical shocks to the ganglia of the leech and mussel all produce enhanced hemolymph levels of both [Met]enkephalin and enkelytin [17]. The same phenomenon is found in human plasma in patients undergoing coronary artery bypass grafting [15]. Taken together, it can be argued that the co-processing and liberation of enkelytin and [Met]enkephalin represents a unified neuroimmune protective response to an immediate threat to the organism, regardless of what form the stimulation takes. Such a unified response might thus represent an important survival strategy.

2. Pain

The concept of pain has proved a difficult issue to approach [18]. Nevertheless, there is a wealth of literature documenting the ability of opioid peptides in ameliorating the sensation of pain [19,20]. In this regard, why, then, is an antibacterial peptide found within proenkephalin, a naturally occurring analgesic inducing molecule, and why has this association endured for at least 500 million years? The close association of enkelytin and opioid peptides, (i.e., [Met]enkephalin), probably reflects the fact that both types of molecules have evolved to fight the presence of microbes. Bacteria and viruses are persistent factors in the environment and are a threat to any organism, regardless of the evolutionary time period. Thus, in order to survive and reproduce, organisms had to evolve processes to combat this immediate non-cognitive threat. The association of enkephalins, enkelytin and other opioid peptides probably represents such a successful strategy (see [16]). In addition to nociception or pain as a factor in such an analysis, analgesia or even pleasure may have evolved to interpret the environmental situation [21,22].

2.1. Opioid immune actions and pain taken together

In part, the meaningfulness of these observations is upheld by neuropeptide processing. Opioid precursors present in evolutionarily diverse immunocytes [11–13] are also found with their processing enzymes, e.g., neutral endopeptidase 24.11 (NEP) and angiotensin converting enzyme [13,23,3]. Thus, activated immune tissues can rapidly produce high levels of these peptides without waiting for their genetic expression, indicating their prominence in proinflammatory events. The complexity of the processing cascade can be observed with NEP, in that it may not only be responsible for cleaving the precursor but the active peptide fragments that result from its action, e.g., [Met]enkephalin and MSH [24,25]. In some cases, the actual inactive products may act as competitive inhibitors to further limit the activity of the prime enzyme, adding the dimension of micro-environmental control [26]. The concentration of the particular enzymes, or immunocytes representing portable enzyme carriers, e.g., granulocytes carrying NEP, is equally important for consideration. The number of cells present at a site of antigenic challenge may influence neuropeptide processing to the point where small peptides, i.e., [Met]enkephalin, can no longer be found. In such an event, presumably after the peak of a proinflammatory response, the same enzyme may down regulate this process, since the smaller immunocyte-activating peptides cannot be found. This process may also limit the spread of chemotactic agents, thus limiting immunocyte recruitment. Given these considerations, it becomes even more important to study early immune events for neuropeptide processing [27] as indicated by the presence of enkelytin.

In conclusion, the association of opioid peptides and enkelytin in proenkephalin suggest that, evolutionarily, the opioid pentapeptides may have originated as immune signaling molecules. The association of the pentapeptides with pain may serve to indicate that nociception may have evolved later or was involved with a creature sense of discomfort. The significance of these processes is noted by their intact presence in organisms 500 million years divergent in evolution. Finally, as pain emerged as an evolvement of the noxious stimulus in mammals its motivational properties, leading to priority setting, were evident, placing opioid peptides and morphine into the role of dampening this sensation so that, once addressed, healing could take place. Thus, by dampening pain opioid peptides could still exert their immune stimulatory actions. Regardless of stimulation, dampening this sensation became critical to survival, allowing an organisms energy to be focused into alleviating this sensation. This can be noted by distracting organisms, which may momentarily allow them to "escape" the sensation, until it can emerge and fulfill its motivational role. Taken together, this counter intuitive hypothesis, explains the variation in opioid and opiate signaling in regard to immune and vascular regulation, while both induce nociception.

3. Endogenous morphine

Studies on the pharmacological properties of morphine and morphine-like substances have long been exclusively concerned with the effects of exogenous opiates, a family of important analgesic and antinociceptive drugs. The capacity of synthesizing morphine was always thought of as being restricted to plants. The first report on the occurrence of this molecule in a vertebrate animal, a toad, was made by Spector [28]. In another review the history of this field is discussed in detail and we refer the readers to this report [29]. Briefly, morphine biosynthesis occurs in animals as shown in human neuroblastoma cells, white blood cells and in invertebrate ganglia [30–32]. In an earlier report, both morphine-like and codeine-like molecules were reported in the nervous tissue and hemolymph of the marine mollusc *M. edulis* as well [33].

The first demonstration by Kosterlitz and coworkers [34] that exogenous morphine can bind to receptors in the mammalian

brain indicated that it shares these sites with those used by endogenous opioid substances (e.g., enkephalins). Since then, growing information on the multiplicity of available receptor types has led to the understanding that, depending on their site of action, opioid peptides as well as opiate alkaloids may bind to more than one opiate receptor subtype [35]. Different degrees of selectivity have been recognized in various signal molecules by comparing affinity constants as well as relative strength in competitive binding assays. The detection of a new subtype of opiate receptor, μ_3, in the human immune system as well as that of an invertebrate, was not entirely unexpected. Earlier studies on the immuno-stimulatory effects of specific neuropeptides (Met-enkephalin, deltorphin I) on human granulocytes, as well as immunocytes of two invertebrate species, *M. edulis* and *Leucophaea maderae* [2,36,37], have demonstrated them to be mediated by a subtype of δ-receptor, δ_2.

3.1. Opiate alkaloids and the immune system

The concept of a functional relationship between endogenous opiates and the immune system are based on the demonstration of such material in the circulation and of special opiate receptors (μ_3) on immune cells of vertebrates and invertebrates [33,38]. Effects of exogenous morphine on cells of this system have been discovered almost 100 years ago [39,40]. Additional information on this subject can be summarized as follows: injection of vertebrate animals with morphine resulted in deficient macrophage function [41] and alteration of T-cell activity [42]. This drug was also shown to antagonize IL-1α- or TNF-α-induced chemotaxis in human granulocytes and monocytes [43,44], as well as *M. edulis* and *L. maderae* immunocytes [1,33]. The effects of opiate alkaloids on these cells differed from those of various opioid peptides tested. Moreover, morphine has recently been demonstrated to upregulate neutral endopeptidase 24.11 in human granulocytes [45], an observation made many years earlier in neural tissues [46]. In animals tested thus far, the administration of morphine tended to inhibit or reduce immunocyte activity, i.e., chemotaxis, cellular velocity, phagocytosis [1]. T-cell deficits in heroin addicts were shown to consist of their inability to form rosettes on sheep erythrocytes [47,48]. By contrast, the opioid peptide Met-enkephalin enhanced rosette formation [47,49]. It is of interest that opiate alkaloids tend to act at higher concentrations (10^{-8} M) than, for example, Met-enkephalin which stimulates immunocyte activity at 10^{-11} M [2,50,51].

One important activity of morphine observed in the immune system is down regulation leading to various types of opportunistic infections (see [52,53]). In addition, morphine brings about various specific alterations in immune competence. It has been shown to inhibit the expression of antigenic markers for both T-helper and T-suppresser cells. Morphine has also been shown to inhibit the respiratory burst of these cells [54]. In addition this compound was found to suppress antibody production in mice in response to the T-cell-dependent antigen trinitrophenyl-ovalbumin [42]. Furthermore, the administration of morphine has been shown to increase the plasma levels of CRH, ACTH and glucocorticoid [55–59].

3.2. Functional roles of endogenous opiates

In this discussion of the possible activities of endogenous opiates we are guided by information collected in numerous studies on the pharmacological responses to the administration of exogenous morphine and related drugs, discussed in the preceding section. One feature that appears to be characteristic of exogenous opiate compounds, exemplified by their known antinociceptic effects, is that they lower thresholds under a variety of physiological and pathological conditions. It is, therefore, reasonable to speculate that endogenous opiates may act in a similar capacity, wherever a situation calls for it.

The presence of opiate alkaloids in the circulation and of special opiate receptors on immunocytes, demonstrated in vertebrates as well as invertebrates, enables these compounds to participate directly in autoimmunoregulatory activities. These direct activities may be judged to be largely of an inhibitory nature [1,51]. In addition, circulating opiates may contribute to the sum total of directives mediated by signal molecules reaching the central nervous system from various sources, including the immune system and adrenal tissue [60].

The direct and indirect down regulating activities attributed to endogenous morphine should be considered in the context with those of other chemical mediators known to act in the same capacity. One of these inhibitory molecules is the cytokine interleukin-10 (IL-10), which is released by macrophages to counteract excessive immunostimulation caused by other cytokines which, under certain conditions of activation, are produced and released by the same cells (see [61,62]). Another inhibitory signal molecule produced by immunocytes is ACTH [25] which, like IL-10, can be considered to participate in autoimmunoregulatory activities.

The question arises in which manner and under which circumstances the immunosuppressive activity of endogenous morphine is called into action. It is reasonable to speculate that the need for an additional control system may arise under conditions making unusual demands. There seems to be general agreement on the fact that serious or life threatening challenges create a state of alertness, brought about by the instant release of stimulatory messenger molecules (opioid peptides and others see earlier discussion), during which all available energies are directed toward meeting the emergency. What should be considered to be equally important is that these stimulatory signals need to be stopped as soon as they are no longer required, so as to prepare the organism for a subsequent challenge. An endogenous morphine material would seem to be an appropriate candidate to meet this demand [51].

For example, during major surgical interventions, the immunosuppressive effect of ACTH and IL-10 produced by immunocytes may not suffice to lower the hyperstimulation of granulocytes and macrophages attributable to their release of IL-1 and TNF due to this trauma. It seems reasonable to suggest that, under these circumstances, morphine may be called upon to down regulate the process so as to restore the normal level of activity. The validity of this proposal is supported by tests carried out with blood samples taken from patients during cardiopulmonary bypass operations. In preparations exposed to

morphine, signs of cellular activity were less pronounced than in untreated samples [1,44,63–65]. Furthermore, studies in vertebrates showed a marked increase in morphine concentration in the spinal cord of rats suffering from chronic pain elicited by experimental arthritis [66]. The same was true for animals subjected to prolonged food deprivation in which the morphine content of brains was higher than in controls [67] (see also [68]).

The insights gained from the study of the various traumatic situations cited suggest that in the hierarchy of available down regulating mechanisms, morphine operates as a strong backup system. The observation that this secondary system goes into effect after a latency period during which endogenous opiate levels rise, is in line with the fact that the μ_3 opiate receptor has an affinity constant in the range of 10^{-8} M. Furthermore, in numerous reports we demonstrate that morphine stimulation via μ_3 is, in part, coupled to NO release, accounting for its immune, vascular and neural down regulating properties [69–76]. The reports demonstrate that within the behavioral response of the tissue to a "proinflammatory-type" stimulus, the tissue cause NO release to counter this physiological state, down regulating the activated immunocytes as an example. Thus, it would appear that a normal function of cNOS-derived NO is to limit immune activation [74]. In this regard, we surmise that the basal, unstimulated levels of NO produced by these tissues may limit micro-environmental noise [74,77,78] by maintaining cells in a mild inhibitory state. Thus, when called upon morphine may enhance this inhibitory state by augmenting NO normal basal actions.

We have hypothesized that certain classes of cells are constitutively activated and can respond to micro-environmental changes [74]. This low level of NO production may provide a major pathway to dampen micro-environmental "noise" that would otherwise non-specifically and inappropriately lead to increased activation. In this regard, NO may modulate the threshold required for activation of these cells [74] and the magnitude of the subsequent response [79]. A diminished level of NO would then represent a disinhibition process that results in an overcoming of the inhibitory influence by changing the level of NO production and the corresponding levels of excitatory signal required for cellular activation (see [74]) such as brought about by opioid peptide activation with an enkelytin presence. Notable examples abound in the literature. Indeed exposure of cells to lipopolysaccaride (LPS) triggers an excitatory signal that reduces the constitutive production of NO and activation of these cells occurs [80,81].

Evidently, the availability of a network of effective immunostimulatory, i.e., opioid peptides, agents has great survival value for vertebrates and invertebrates alike. It is, therefore, understandable that the development of its elements, including those operating in immunoregulation, can be traced far back on the evolutionary scale. The need for the operation of more than one immunosuppressive mechanism is as obvious as that for the availability of effective immunostimulatory agents. It is our hypothesis that it is one of morphine's important tasks to meet this vital demand. It would also be advantageous during this "recovery" period that the dampening of pain continues so that the motivational stimulation or hyperactive awareness of the sensation does not hamper the organism's "relaxation/recovery".

In addition to those discussed here, endogenous morphine and related opiates may be presumed to engage in a variety of other activities, for example some operating within the confines of the nervous system. However, most of these questions are still unanswered, and efforts for their solution will undoubtedly gain momentum in years to come.

4. Conclusion

We have hypothesized how evolutionary pressure was instrumental in forming functional links between cellular/tissue activation, inhibitory tone, nociception, and antinociception. This primarily involved multiple survival mechanisms by which primordial/progenitor cell types obtained the capability to regulate their responsiveness to environmental threats with minimal perturbations of metabolic homeostasis. Accordingly, nature has provided a multi-purpose chemical messenger/protein modifier in the form of the free radical gas NO. Cells that emerged with the ability to temporally recruit and regulate NO expression within discrete microdomains possessed a major survival strategy that has been sustained throughout the course of evolutionary adaptations.

Additionally, we propose the existence of several evolutionary linkages between tissue activation, cellular inhibition, antinociception and cancer progression. Primordial, multipotential cell types, before the emergence of specialized plant and animal cells/organ systems, required selective mechanisms to limit their responsiveness to environmental noise. Notably, evoked release of NO in an exquisitely graded fashion is capable of this regulatory role and cells that emerged with the potential for recruitment of NO as a multi-faceted autocrine/paracrine signaling molecule were provided with extremely positive evolutionary adaptations. In parallel fashion, endogenously expressed, chemically authentic, morphine was fashioned as a key cellular signaling molecule, responsible for regulating intermediary metabolic functions, including mitochondrial respiratory rate [82]. The widespread expression of morphine by plants, invertebrate and vertebrate cells/organ systems strongly indicates a high level of evolutionary conservation of morphine and related morphinan alkaloids as essential chemical factors required for normal growth and development [83].

Recent complementary studies have provided biochemical, molecular, and pharmacological characterization of a two unique six-transmembrane helical (TMH) domain opiate receptors expressed from the μ opioid receptor (MOR) gene [84]. Designated μ_3 and μ_4 receptors, both protein species are Class A rhodopsin-like members of the superfamily of G-protein coupled receptors (GPCRs) but are selectively tailored to mediate the cellular regulatory effects of endogenous morphine and related morphinan alkaloids via stimulation of nitric oxide (NO) production and release [84]. Both μ_3 and μ_4 receptors lack an amino acid sequence of approximately 90 amino acids that constitute the extracellular N-terminal and TMH1 domains and part of the first intracellular loop (IL) of the μ_1 receptor, but retain the empirically defined ligand-binding

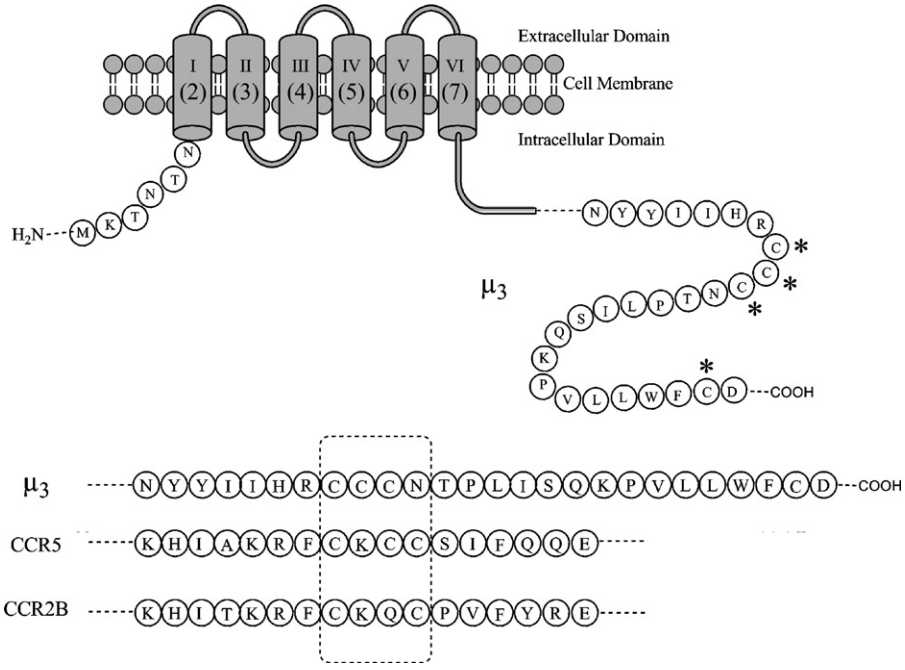

Fig. 2. Sequence alignment of cysteine clusters in the C-terminal intracellular domains of the μ_3 opiate receptor in relation to the human chemokine CCR5 and CCR2b receptors. Upper graphic includes a schematic representation of the μ_3 opiate receptor. Trans-membrane helical (TMH) domains of the μ_3 opiate receptor are numbered I–VI and correspond to conserved TMH domains 2–7of the μ_1 receptor. Conserved extracellular loops (ELs), intracellular loops (ILs), and C-terminal intracellular sequences common to traditional μ_1 and μ_3 receptors are represented by thick grey lines. The unique 26 amino acid C-terminal intracellular domain of the μ_3 receptor is represented by the single letter amino acid code with starred cysteine residues. Similarly, the conserved intracellular N-terminus of the μ_3 receptor expressed from Exon 1 of the μ receptor gene is represented by the single letter amino acid code. Lower graphic provides a sequence alignment of homologous cysteine clusters contained within the C-terminal intracellular domains of the μ_3 opiate receptor and internal amino sequences 314–330 of the human CCR5 and CCR2b receptors, as adapted from [87,91].

pocket distributed across conserved TMH2, TMH3, and TMH7 domains of the μ1 sequence. Additionally, the receptor proteins are terminated by unique intracellular C-terminal amino acid sequences that serve as putative coupling or docking domains required for constitutive NO synthase (cNOS) activation (Fig. 2). Because the recognition profile of μ_3 and μ_4 receptors is restricted to rigid benzylisoquinoline (BIQ) alkaloids typified by morphine and its extended family of chemical congeners, it is hypothesized that conformational stabilization provided by interaction of extended extracellular N-terminal protein domains and the extracellular loops is required for binding of endogenous opioid peptides as well as synthetic flexible opiate alkaloids.

Cellular expression of "truncated" six-TMH domain opiate receptors had been previously alluded to by molecular studies of μ receptor-encoding mRNA slice variants [85,86]. These accumulated data suggest that μ_3 and μ_4 receptors may be representative members of a "budding" family of primordial GPCR responsible for autocrine/paracrine regulation of cellular metabolic activity via local circuit Ca^{2+} gating and NO feedback inhibition. In combination with complementary analyses demonstrating *de novo* synthesis of chemically authentic morphine by diverse classes of animal cells [29], the establishment a short loop "morphinergic" regulatory pathway mediated by morphine and naturally expressed active morphine congeners such as its 6-glucuronide conjugate and their cognate μ_3 and μ_4 receptors receives significant validation.

We currently propose that the expression of endogenous morphine by animal and human cells is designed to mediate homeopathic regulation of metabolic activity via activation of cognate μ_3 and μ_4 receptors that serve as transductive conduits for short-circuit Ca^{2+} fluxes. The profound implications of the our recent demonstration of a μ_4 receptor/NO-coupled regulatory pathway in human MLPC indicate that comparative phylogenetic analysis of the μ receptor gene may provide answers as to whether six-TMH domain μ_3 and μ_4 receptors are prototypic evolutionary models that have given rise to seven-TMH domain μ, δ, and κ receptors. We have also hypothesized that the primordial "morphinergic" signaling pathway served as a prototypic model by which diverse catecholamine signaling pathways were formulated [83]. Furthermore, it appears that the unique cysteine cluster found at the C-terminal tail or intracellular domain of the μ_3 opiate receptor bears a striking sequence homology to similar cysteine clusters within the C-terminal domains of the CCR2B and CCR5 chemokine receptors [87]. In the case of CCR5, mutational analysis demonstrates that receptor function is critically linked to maintain the integrity of the intracellular cysteine residues. Additionally, the cysteine cluster on the C-terminal tail of the μ_3 receptor represents a potential nitrosylation domain as well as a docking site for covalent attachment to cNOS, further supporting the case for functional coupling of these signal molecule systems [88,89]. Similar criteria are presumably applicable to structure/function relationships of the μ_3 opiate receptor and establish evolutionary linkages

between opiate and chemokine signaling processes early during evolution. Ongoing studies to support the evolutionary primacy and retention of endogenous morphine and its cognate μ_3 and μ_4 receptors in relation to chemokine systems are in progress.

In conclusion, prior studies have monitored the inhibitory effects of morphine exposure on tumor growth in human patient populations and in selected animal models (see [90]). These key observations require re-examination and re-interpretation in light of recent empirical evidence supporting the de novo expression of chemically authentic morphine by animal cells [30,31] as well as the opiate receptor subtypes discussed in the present report. Structural homologies of μ_3 and μ_4 receptors to major species chemokine receptors provide us with a critical linkage of the morphine cancer data to chemokine signaling processes (Fig. 2). In sum, these novel findings suggest that the emergence carcinogenic processes, in part, may appear via alteration of homeostatic cellular processes involving morphinergic/NO signaling.

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