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Review

Endogenous morphine/nitric oxide-coupled regulation of cellular physiology and gene expression: Implications for cancer biology

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Abstract

Cancer is a simplistic, yet complicated, process that promotes uncontrolled growth. In this regard, this unconstrained proliferation may represent primitive phenomena whereby cellular regulation is suspended or compromised. Given the new empirical evidence for a morphinergic presence and its profound modulatory actions on several cellular processes it is not an overstatement to hypothesize that morphine may represent a key chemical messenger in the process of modulating proliferation of diverse cells. This has been recently demonstrated by the finding of a novel opiate-alkaloid selective receptor subtype in human multilineage progenitor cells (MLPC). Adding to the significance of morphinergic signaling are the findings of its presence in plant, invertebrate and vertebrate cells, which also have been shown to synthesize this messenger as well. Interestingly, we and others have shown that some cancerous tissues contain morphine. Furthermore, in medullary histolytic reticulosis, which is exemplified by cells having hyperactivity, the mu3 (μ_3) opiate select receptor was not present. Thus, it would appear that morphinergic signaling has inserted itself in many processes taking a long time to evolve, including those regulating the proliferation of cells across diverse phyla.

Keywords: Endogenous morphine; μ_3 Opiate receptor; Nitric oxide; Stem cell; Cancer

Contents

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1. Historical context and clinical implications

Morphine and chemically related opiate alkaloids represent time-tested analgesic principles for management of severe pain associated with metastatic disease [1,2]. A substantial body of accumulated evidence, however, documents a cadre of cellular/physiological effects associated with pharmacological administration of morphine and related opiate alkaloids that lie outside the realm of anti-nociception and appear to be mediated by indirect and/or "non-traditional" mechanisms. Notably,

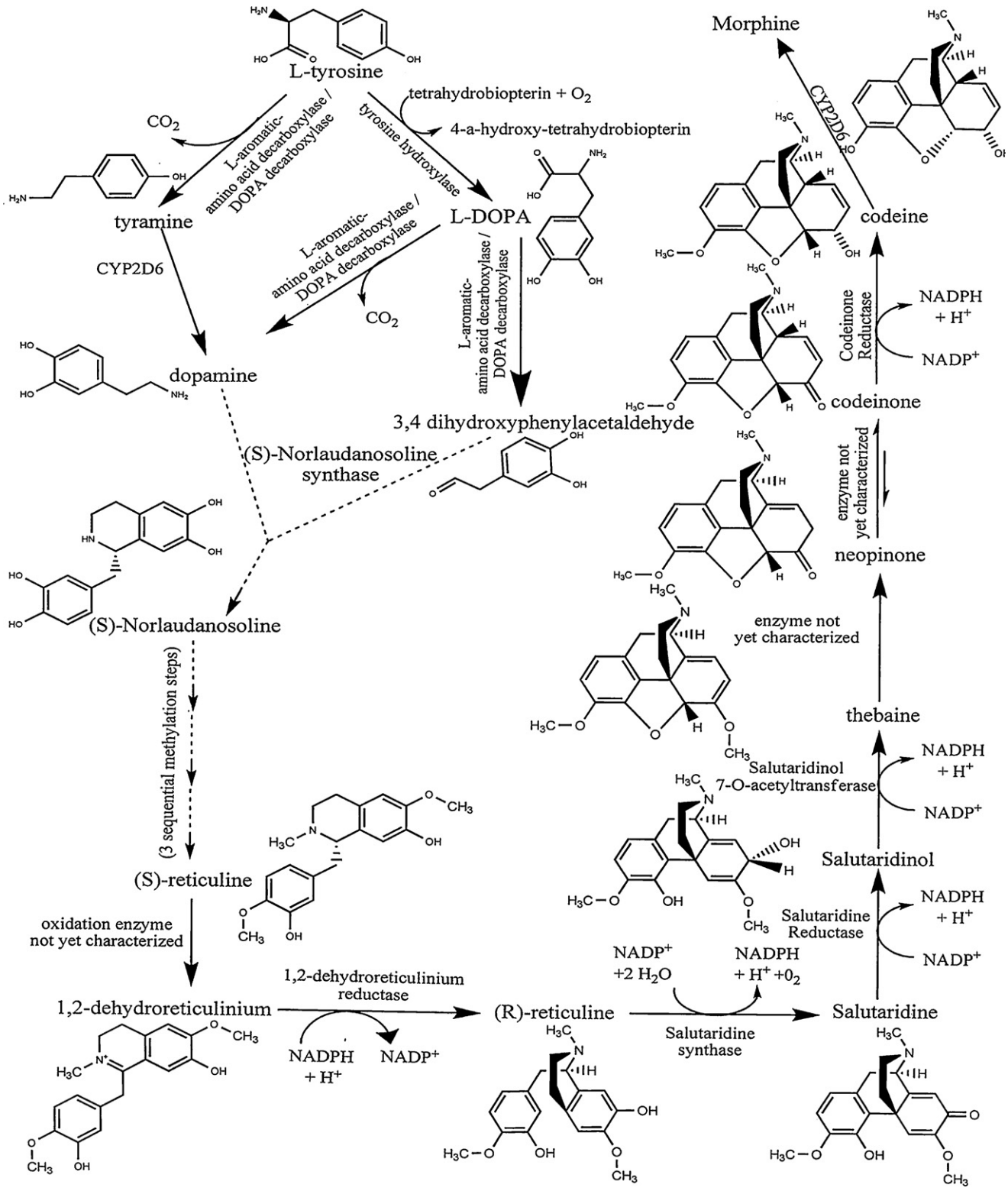

Fig. 1. Detailed schematic indicating relevant enzymes and chemical intermediates in the morphine biosynthetic pathway.

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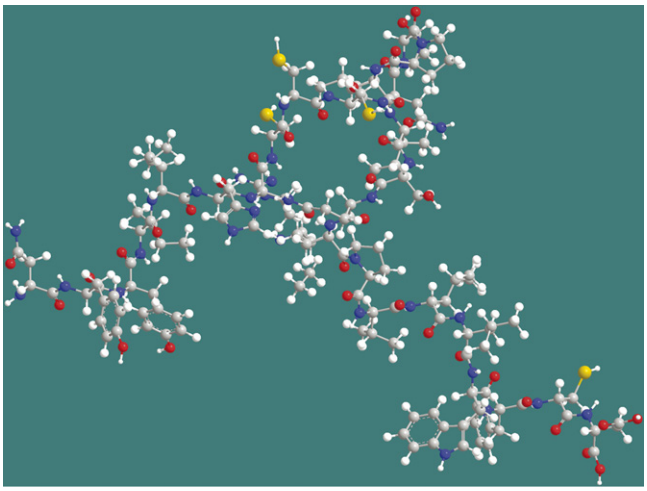

Above: A 3-dimensional 26 amino acid sequence representing the unique intracellular domain of the μ3 receptor

Right: A 3-dimensional 4 amino acid sequence representing the unique intracellular domain of a μ3-like receptor

White=H; Grey=C; Blue=N; Red=O; Yellow=S

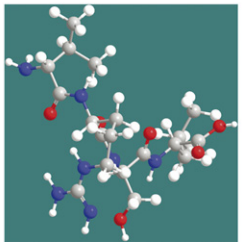

Fig. 2.

complementary studies have monitored the inhibitory effects of morphine treatment on tumor growth in human patient populations and in selected animal models. 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 [3,4].

Because animal cells and complex organ systems have the ability to carry out *de novo* synthesis of morphine via highly regulated enzyme-linked catalytic steps (Fig. 1) [3,4], the clinical relevance of physiological and/or pharmacological perturbations of ongoing morphinergic cellular signaling pathways to cancer biology becomes compellingly evident [5–8]. Accordingly, the present review will develop a brief schematic in support of morphine's essential role as an autocrine/paracrine regulatory factor devoted to hierarchal integration of cellular function [9,10] via intimate association with a novel μ_3 opioid receptor subtype functionally coupled to stimulated production and release of the primordial signaling molecule nitric oxide (NO) (Fig. 2). Several newly formulated hypotheses are proposed to explain the anti-proliferative effects of morphinergic signaling based on recently published empirical evidence combined with re-evaluation of clinical and pre-clinical databases.

2. Morphine, inhibition of tumor progression, and immune competence

Complementary pre-clinical and clinical studies have monitored inhibitory effects of pharmacologically administered morphine on tumor growth. For example, a pre-clinical murine animal model of tumor progression has demonstrated that repeated administration of morphine results in significantly diminished tumor cell-induced tissue destruction [11]. Pre-clinical observations are complemented by a series of clinical studies demonstrating that both pre- and post-operative administration of analgesic dosages of morphine as an adjuvant to cancer surgery operationally reduces systemic cell dissemination of tumor cells [12–14]. An additional clinical study proposes an indirect analgesic or extra-analgesic mechanism for the observed anti-metastatic effects of administered morphine, potentially involving enhanced host resistance to tumor cells entering the systemic circulation during surgical manipulation [15].

Morphine has been demonstrated to decrease cell growth of human breast cancer cells *in vitro*, despite significant cellular expression of traditional μ_1 receptors [16]. The question was raised whether the anti-proliferative effects of morphine could be mediated through interaction with other receptor systems, particularly, since receptor binding studies revealed that only some tumors express μ receptors [17]. For example, Manneckjee and Minna found that the inhibitory effects of morphine on lung cancer growth could be reversed by nicotine, pointing towards an opioid-acetylcholine interplay on systemic or receptor levels [18]. In another study, it has been shown that morphine may exert its anti-proliferative effects on breast cancer through interaction with the somatostatin receptor SSTR2, suggesting a functional interaction of morphine with the inhibitory somatostatinergic system [19]. This hypothesis is supported by findings that show a direct inhibitory effect of somatostatin analogues on human tumor growth [20–22]. The critical question, however, whether morphine acts via μ receptor binding to exert its potentially anti-proliferative, anti-neoplastic functions, is elucidated by recent findings that suggest other physiologically active μ opioid receptor splice variants to play a critical role, variants that may be present and operational in immune and vascular tissues and that had gone previously undetected [23,24]. Thus, when examining the underlying molecular mechanisms of morphine down regulation, particularly in cancer, it seems necessary to also look for and consider these receptor variants. In any case, however, it appears that morphine has the potential to be protective in many tissues in response to stress and injuries, including cancer damage [2,10,11].

The complexity of morphine-tumor interaction can also be observed in angiogenesis: morphine may enhance angiogenesis by μ receptor activation, and this mechanism may be protective, e.g., in wound healing, where tissue injury is overcome [25]. However, morphine also potentially induces angiogenesis in cancer, thereby possibly facilitating tumor growth and metastatic potential [26]. In part, this effect may be due to rebound excitation, following morphine's immediate action, which is down regulating in nature [10,27,28]. After morphine-stimulated down regulation, e.g., immune suppression, this rebound excitation may allow cells, i.e., immune system, to restore enhanced surveillance [29,30]. This capacity clearly would have survival value, and it may be further supported by the fact that this mechanism can already be observed in organisms that evolved 500 million years before man [29].

Pharmacological administration of morphine and related opiate alkaloids has been demonstrated to mediate positive

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effects on host immunity against proliferation of malignant cells [13,31,32]. For example, temporally dependent morphine treatment enhances human cytotoxic T lymphocyte activity and interferon gamma production *in vitro*, resulting in enhanced T cell-mediated immune responses [32]. Furthermore, it has been suggested that morphine's anti-metastatic effects when preemptively administered prior to cancer surgery involve functional recruitment of large granular lymphocytes and natural killer (NK) cells [13]. The contribution of NK cell activation to the anti-proliferative effects of pharmacologically administered morphine, however, remains a controversial issue and requires further investigation [13,31,32]. The same caveat applies to potential role morphine-dependent enhancements of lymphokine activated killer cell activity [31]. Interestingly, a relatively recent study indicates that the synthetic phenylpiperidine μ_1 receptor opiate agonist fentanyl suppresses NK cell activity, thereby increasing the risk of tumor metastasis [33]. The opposite pharmacological effects of the naturally occurring opiate-alkaloid morphine and the synthetic opioid analgesic on NK cell activity linked to *in vivo* tumor progression may be functionally linked to their differential effects on μ_3 receptor activation. As discussed below, morphine and related morphinan opiate alkaloids are highly efficacious activators of the μ_3 receptor, whereas fentanyl and related synthetic opioids are observed to be without effect.

Finally, opioid peptides, in general, appear to have proinflammatory capabilities throughout the body, whereas opiate alkaloids like morphine may represent a class of immune and vascular systems' anti-inflammatory, i.e., inhibitory or down regulating, key players [10,34,35]. Hence, both – opioids and opiates – theoretically present themselves as potential candidates for tumor growth modification.

3. Apoptotic and transcriptional effects of morphine administration on tumor growth

Researchers recently have proposed a protective role for morphine against tumor growth and metastasis through induction of apoptosis in tumor cells [36,37]. This conclusion also derived from findings related to chronic morphine administration in latent pain situations, where morphine's cytotoxic effects may be partially associated with apoptosis induction [36]. Again, we see the double-edged sword in morphine physiology and regulation, however, the specific mechanisms by which morphine influences tumor growth are far from being fully understood. This fact may also be due to a lack of realization of an endogenous autoregulatory morphine signaling system [51,63,98].

Morphine inhibits tumor necrosis factor (TNF)-alpha mRNA expression and release, which attenuates the *in vitro* growth of various cancer cell lines [38]. Furthermore, TNF-alpha is regulated by the (pro)inflammatory transcription factor nuclear factor kappa B (NFkB) [39,40], which augments cell proliferation and is regulated, among others, by morphine or its derivatives: Inhibition of NFkB in human cancer cell lines – related to morphine exposure – is associated with higher apoptosis induction [41]. Similarly, it has been shown that an inhibition of NFkB attenuates apoptosis resistance in lymphoid cells [42]. Finally, we demonstrated that morphine can directly inhibit NFkB actions via NO release [43,44] (also see below).

4. Endogenous morphine expression by animal cells

Based on the historical belief that morphine was not present endogenously in animals, studies on the pharmacologic properties of morphine and morphine-like substances had focused on the effects of exogenous opiates, a family of important analgesic and anti-nociceptive drugs. This focus changed following the discovery [45] that morphine binds to the same receptors used by endogenous opioid peptides. An important step forward was the demonstration of endogenous opiates in various vertebrate tissues, including the nervous and immune systems [46–48]. At this turning point, a quest began to identify the possible roles played by this new group of endogenous messenger molecules under both physiological and pathological conditions.

Our recent work provides compelling *prima facie* evidence that *de novo* synthesis of chemically authentic morphine is realized by diverse animal cellular systems from L-tyrosine-derived small molecules within a strikingly similar biochemical pathway to that described in opium poppy (Fig. 1) [4,81,82,90]. Furthermore, we have monitored a functional linkage of *de novo* morphine synthesis to evoked release of the alkaloid upon physiological demand, suggesting that low steady-state levels of morphine in many, if not all, mammalian organ systems, indicate dynamic utilization and turnover of releasable cellular pools of morphine.

Recent studies from our group employing well established *ex vivo* invertebrate nervous tissue preparations and primary cultures of human white blood cells [3,4,49] and those by Zenk and coworkers using human tumor-derived cell lines [50,51] have markedly facilitated the formulation of an evidence-based model of *de novo* formation of endogenous morphine in animal cells with remarkable similarities to the well-characterized enzymatic pathway described in *Papaver somniferum* [8]. Key observations from these studies indicate that L-tyrosine, its monoamine homolog tyramine, and their respective catechol derivatives, 3,4-dihydroxy-L-phenylalanine (L-DOPA) and dopamine (DA) serve as substrates for *de novo* morphine production and that pharmacological characterization of tyramine utilization as a morphine precursor indicates one or more catalytic steps mediated by microsomal cytochrome P450 2D6 (CYP2D6) [3,4].

The significance of tyramine as a biosynthetic intermediate is validated by *in vitro* enzyme kinetic studies demonstrating dopamine formation via CYP2D6-catalyzed ring hydroxylation of tyramine [52–55] which in turn lends support to the existence of a potentially important tyrosine hydroxylase-independent pool of cytosolic DA that is available for endogenous morphine expression [3,4,49]. These data are complemented by metabolic labeling/isotope enrichment studies employing SH-SY5Y neuroblastoma cells [50,51], indicating asymmetric isotopic labeling of the benzyl and isoquinoline chemical domains of newly formed morphine that is operationally determined by the type of L-tyrosine-derived precursor molecule that

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is employed: L-tyrosine and L-DOPA are incorporated in both the benzyl and isoquinoline chemical domains of morphine, whereas dopamine and tyramine are only incorporated into the isoquinoline domain.

Taken together, we have formulated a hypothesis, which states that endogenous morphine, used as either a hormone or neurotransmitter down regulates immune, vascular, neural and gut tissues under normal and following trauma situations [10,27,34,56,57]. In this regard, as noted in these reports, tolerance is viewed as a mechanism to ensure that morphine's continued presence does not permanently limit a needed tissue excitatory state, since excitation may be required to overcome a traumatic event, which morphine would continue to down regulate.

5. Biochemical characterization of a novel opiate-alkaloid selective μ_3 receptor subtype in invertebrate and mammalian tissues

The demonstration of endogenous opiates, i.e., morphine, codeine, in various vertebrate tissues, including the nervous system [14,51,91–95], is quite important for establishing the significance of the μ_3 opiate receptor subtype. There is a body of evidence that shows opiate alkaloids such as morphine, morphine-3- and 6-glucuronide, as well as the morphine putative precursor molecules (thebaine, salutaridine, norcocolarine, reticuline, tetrahydropapoverine (THP) and codeine) exist in vertebrates [58–61]. In invertebrates, specifically *Mytilus edulis*, the presence of morphine, morphine 6-glucuronide, morphine 3-glucuronide, codeine, THP and reticuline also have been reported [10,48,62–64]. Endogenous opiate levels can be induced to change following stimulation [10]. Morphine has also been found in human plasma [65,66], suggesting a hormonal action with immune, vascular and gut tissues as targets [57,67].

Classical opioid receptors are linked to trimeric G proteins that in turn modulate Ca^{++} and K^{+} channels, adenylyl cyclase, and probably other signal transduction systems [68]. In this regard, the μ_3 receptor is also linked to G protein, based on guanine nucleotide effects on agonist binding to the receptor [69]. The μ_3 receptor differed from previously described neuronal opioid receptor subtypes in that it exhibited essentially no or exceedingly low affinity for naturally occurring endogenous opioid peptides or their analogues [70]. However, the opiate-alkaloid binding that was present was naloxone sensitive, demonstrating its opiate receptor properties [34]. Also, and of crucial importance, these properties corresponded to effects of opiates, i.e., morphine, on immunocytes that were not mimicked by opioid peptides. In contrast, each of the other opioid receptor subtypes binds at least one of the endogenous opioid peptides with high affinity. Furthermore, certain opiate alkaloids, benzomorphans and other drugs bind to classical opioid receptors but not to the G protein coupled μ_3 receptor [48,69,71–73].

Binding sites for this novel morphine (opiate-alkaloid selective, opioid peptide-insensitive) receptor, designated the μ_3 receptor were first reported to be present in human peripheral blood monocytes and in invertebrate immunocytes and later on other cell types [48,69,71,73–75]. The newly discovered opioid peptides endomorphin-1, -2 and orphanin FQ also do not bind to this opiate receptor subtype [76]. 6-Glucuronide, not the 3-glucuronide metabolite of morphine, binds to the μ_3 receptor. The synthetic phenylpiperidine μ_1 opiate agonist fentanyl does not bind to this receptor as well [69,73,74].

6. Structural and functional elucidation of a novel opiate-alkaloid selective μ_3 receptor subtype in invertebrate and mammalian tissues

Traditional opioid receptors have been classified as three major types, i.e., μ, delta, and kappa, and heterogeneous μ, delta, and kappa subtypes have also been described [68,77–82]. Historically, amino acid sequences of μ, delta, and kappa opioid receptors from several species have been derived from their respective cloned cDNA sequences [83–86].

The μ_3 receptor cDNA, compared with μ_1, is truncated at the 5′-end, missing several hundred nucleotides, but the middle and conserved region sequences are identical with μ_1 [24]. The 3′ end of μ_3 exhibits 100% identity to a portion of the 3′ end of the μ_1 variant, followed by a new fragment of 263 bases, and then a 201-bp fragment of the 3′ end of the μ_1 gene untranslated region [24]. As observed presently, the μ_3 opiate receptor represents a transmembrane G protein-coupled protein with a novel linkage to constitutive nitric oxide synthase (cNOS) [34,48,69,73,75,87–89]. The novelty and selectivity of this G protein-coupled, naloxone-sensitive receptor was made apparent when a variety of opioid peptides were found to be ineffective in displacing specifically bound 3H-dihydromorphine (DHM) as well as in stimulating NO release, whereas opiate alkaloids were quite potent [24,48]. In this report, we found that only one set of μ-specific primers used in the PCR reactions (from map position 896–1336) yielded a specific PCR product [23]. This segment of the cDNA encodes the third extracellular loop of the receptor that is important for μ agonist selectivity [90,91].

To determine if the cDNA clone we isolated was functional and had the biochemical properties expected of the μ_3 receptor, the cDNA was expressed in a heterologous system (Cos-1 cells). Morphine administration to Cos-1 cells transfected with the μ_3 receptor cDNA was observed to effect "real-time" release of NO into the cellular bath medium, as monitored by a NO-selective amperometric nanoprobe [24]. In contrast, morphine administration was observed to have no effect on NO release from Cos-1 cells transfected with a control cDNA construct in a naloxone antagonizable manner [24]. The addition of naturally occurring Met- and Leu-enkephalins, as well as the delta opioid receptor peptide agonist DPDPE, did not stimulate NO release in the controls or transfected cells [24]. These results show that transfection of the novel cDNA clone isolated from human testes conferred the expected opiate-alkaloid selective and opioid peptide-insensitive characteristics of the μ_3 opiate receptor [10,23,70,92]. In addition, we analyzed human heart endothelial tissue, human leukocytes, and Cos-1 transfected cells for expression of the μ_3 receptor-encoding mRNA by Northern blot

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analysis [24]. A band of the expected size of approximately 1.45 kb was observed in the various tissues [24]. The selectivity of the μ_3 opiate receptor subtype, therefore, provides further evidence for the status of morphine as an endogenous signaling molecule [10].

7. Demonstration of functionally coupled μ_3 receptor/nitric oxide (NO) cellular signaling in invertebrate and mammalian tissues

There has been in the literature an association of NO with morphine actions. Peripheral morphine analgesia involves NO-stimulated increases in intracellular cGMP [93]. Nitric oxide has been associated with anti-nociception [94] as well as tolerance and dependence [95]. In addition, the morphine-induced suppression of splenic lymphocyte proliferation has been shown to involve enhanced NO expression [96]. Morphine and NO have been functionally linked in the regulation of physiological processes in the gastrointestinal system [57,97]. Furthermore, morphine, not opioid peptides, stimulate constitutive NO release in macrophages, granulocytes, various types of human and rat endothelial cells, invertebrate neurons and immunocytes and in rat median eminence fragments, all in a naloxone antagonizable manner [28,34,76,98–104]. These data suggest that the μ_3 receptor is functionally coupled to stimulated NO production and release in these cells via Ca^{++} stimulated activation of cNOS. Furthermore, morphine's actions in these diverse tissues complements what is known about NO mediating immune and vascular functions, namely that it can down regulate them from an excitatory state or prevent the excitatory state from occurring [27,28,34,104]. These reports NO is also involved in cell growth and maturation, thus implicating morphine as well [5,6,105]. Additional information on opiate-alkaloid signaling substances can be summarized as follows: Injection of vertebrate animals with morphine results in deficient macrophage function [106] and an alteration of T-cell activity [107]. Morphine also antagonizes interleukin-1alpha or tumor necrosis factor-alpha-induced chemotaxis in human granulocytes and monocytes [108,109].

8. Demonstration of biologically relevant morphine/NO-coupled cellular signaling in cancer cells

NO, when released through cNOS pathways, has anti-inflammatory, anti-proliferative capacities [110,111]. However, the specific mechanisms by which increases in NOS activity and the subsequent production of NO may actually cause cytostasis are not clear. This effect, in parts, may be due to the NO-associated inhibition of NFkB [40,111]. NO stabilizes the NFkB inhibitor, i.e., inhibitory kappa B alpha (IkBa), and this increased NFkB inhibition, as well as the correlated decrease of NFkB binding to the DNA, may attenuate apoptosis resistance in cancer cells (as described), possibly leading to down regulation of tumor growth [10,43,44,112,113]. In addition, the anti-proliferative action of NO is associated with the inhibition of polyamine formation (e.g., via ornithine decarboxylase inhibition) and the deterioration of other critical pathways, including down regulation of arginase activity, thereby decreasing tumor growth [110]. These pathways and their endogenous control are complex: Some breast cancer cells lines, for example, were shown to have high arginase activity and very low NOS activity [114]. NO derived from macrophages is known to have tumoricidal activity, and polyamines may promote the growth of tumor cells [115]. Therefore, arginase may be playing a role in promoting tumor growth by inhibiting the production of NO. This may represent a significant fact, given morphine's ability to stimulate cNOS-derived NO release, since this action may not be observed in the presence of arginase [116,117].

Morphine-coupled NO production and release has been shown to be neuroprotective via inhibitory effects on the cellular expression of beta secretase-1 (BACE-1), a key protease involved in maturation of beta amyloid protein in the CNS of Alzheimer patients [118]. Furthermore, inhibitors of beta and gamma secretase decrease endothelial cell proliferation, oppose sprouting of microvessel outgrowths, and inhibit formation of capillary structure [119]. In addition, these inhibitors significantly attenuated growth and vascularization of xenotransplanted human glioblastoma and lung adenocarcinoma into nude mice [119]. Thus, it is hypothesized that the two proteases (secretases) play a role in angiogenesis, since they are also highly expressed in the endothelium of neoforming vessels [119]. It appears, hence, that one of the pathways involved in the anti-tumoral potential of morphine may be the NO-dependent modulation of secretase activity, i.e., decreased angiogenesis. Furthermore, an effect of NO on the induction of anti-angiogenic endostatin has been demonstrated – an effect that has been linked to the inhibition of proliferation and migration of endothelial cells, again illustrating the anti-proliferative potential of NO [120,121]. Given this potential and the physiological morphine–NO coupling, it is comprehensible that biomedical strategies are now looked for that will strengthen a role for opiates and/or NO as potential tumoricidal agents.

A crucial feature of cancer progression and metastasis is the disruption of extracellular matrix and the spreading of proliferating cancer cells. Extracellular matrix, however, is remodeled by matrix metalloproteinases (MMP), and therefore, MMP modulation has become a main target of cancer research [122]. Hence, MMP-2 is under the control of NO, and cNOS activity here seems to be controlled by opioids in a non-opioid receptor manner [122]. Yet, morphine attenuates metalloproteinase activity via the NO/cNOS system as it has been shown, for example, in sarcoma cells [122], again pointing towards possible clinical implications, i.e., for the treatment of sarcomas. In fact, morphine, not opioid-peptides, is coupled to constitutive NO release via the μ_3 receptor in endothelial and immunocompetent cells [24,73,87,88]. Expression of this opioid receptor subtype has been found in human specimens of cancer tissue, e.g., non-small-cell lung carcinoma [112], and thus it has been suggested that the anti-cancer effects or morphine are related, in parts, to cNOS-derived NO release coupled to μ_3 activity [112]. However, increased NO production in lung carcinoma may also indicate that cancer cells use endogenous opiates to down regulate the immune response to tumor growth, i.e., deterioration of host tumor defense capacities. Again, one may realize

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the complexity of morphine-nitric oxide interactions in cancer, given the fact that excitatory rebound capacities have also been shown for morphine (as described), and additionally, NO can be produced/released via different pathways that partially act antagonistic, i.e., constitutive (cNOS) and inducible NOS (iNOS) [111,123]. We can thus speak of NO as a double-edged sword, possibly explaining some of the contradictory results in cancer research related to NO and morphine regulation, thereby also acknowledging the fact that certain tumors have the ability to neutralize NO tumoricidal actions.

Taken together, NO may be tumoricidal, e.g., by apoptosis induction [124–126]. However, tumor promoting effects of NO have also been described, underlining the contradictory role of NO in cancer regulation [127–129]. Here, we offer rebound effects – that occur in morphine–NO coupling (as described) – as a possible explanation [28–30,103,130], and these effects should be taken into account in future research, which is essentially needed in this field.

9. Detection and functional characterization of a novel opiate-alkaloid selective μ_3 –like receptor subtype in human umbilical cord stem cells

A considerable body of published work has established a temporal profile of endogenous opioid peptide and opioid receptor gene expression in the development of mammalian brain [131–133]. Recently, similar analyses have demonstrated programmed expression of opioid peptide and opioid receptor genes in cultured neural progenitor cells at various stages of differentiation [134,135]. Human multilineage progenitor cells (MLPC) derived from post-partum umbilical cord blood have recently been established as a high resolution model [136–142] for studying biochemical and molecular mechanisms underlying differentiation of multi-potent progenitor cells into clonal cell lines (e.g., adipocytes, osteoblasts, myocytes, vascular endothelial cells, neurons, astrocytes, and oligodendrocytes). Because MLPC are non-transformed, and non-immortalized, their potential for both proliferation and differentiation into phenotypically distinct clonal lines is temporally defined by the complex chemical profile of their respective micro-environments [143]. Previous studies of opioid regulation of hematopoiesis in adult animals from other groups [144–146] have provided initial insights into the potential role of opioid processes in MLPC maturation.

In a recent study from our laboratory it was demonstrated that a μ_3-like opiate receptor subtype on MLPC is present [147] (Fig. 2). The functional role and potential biological importance of expressed μ_3 opiate receptors are supported by independent lines of pharmacological evidence demonstrating that morphine-evoked release of NO from MLPC is inhibited either by a selective receptor antagonist or by NG-Nitro-L-arginine methyl ester hydrochloride (L-NAME), a competitive inhibitor of cNOS. Importantly, a saturating concentration of the prototype opioid peptide methionine enkephalin, capable of activating traditional μ_1, delta and kappa opioid receptors, was observed to be without effect on evoked release of NO from human MLPC.

Complementary microarray analysis of extracted RNA indicated that traditional μ_1, delta, and kappa opioid receptor gene expression is not detected in both undifferentiated and differentiated MLPC, and other prominent classes of neural receptors such as the tachykinin and 5-HT2B are only weakly expressed following differentiation [147]. Incomplete expression of an active DA system is also indicated by the lack of expression of DA1 and DA3 receptors and the vesicular DA transporter [147]. The strong expression of the cellular DA transporter suggests that peripherally synthesized DA may taken up and utilized for non-synaptic functions or for the synthesis of endogenous morphine [3,4]. Importantly, candidate genes involved in endogenous morphine biosynthesis including catechol-*O*-methyl transferase (COMT), CYP2D6, and phenylethanolamine *N*-methyltransferase (PNMT) are expressed in both undifferentiated and differentiated MLPC, suggesting that MLPC have the potential for morphine expression. This data also suggests that morphinergic signaling preceded that of catecholamines [148]. Taken together, for the first time, we show elements of an endogenous morphine signaling in progenitor stem cells, suggesting the presence and physiological significance of a μ_3-receptor during early development.

Functional expression of a μ_3-like opiate receptor by embryonic stem cells strongly indicates its role as a primordial or progenitor opiate signaling system that has adaptively conserved through evolution. Morphine-mediated NO production may therefore represent the original transductive pathway underlying the cellular actions of opioids. Nitric oxide signaling has been demonstrated in embryonic cells, adding and supporting the current findings [149,150]. NO inhibits subventricular zone-derived neural stem cells proliferation, which was found not to involve cyclic guanosine monophosphate (cGMP) synthesis [151]. These neurosphere cells expressed the neuronal and endothelial isoforms of NO and produced NO in culture. This study further suggests NO was acting in an autocrine/paracrine manner. Recent studies demonstrate that NO-cGMP signaling system exists in embryonic stem cells and may be involved in forming committed precursor cells [150]. Our laboratory has hypothesized that basal/tonal NO serves to limit micro-environmental noise, maintain cells in a state in inhibition, thus subserving essential processes of cellular preservation in states of biological readiness [9], and demonstrating the role of NO signaling components in differentiation events or physiological processes of human embryonic stem cells.

In sum, we have identified for the first time in human MLPC the expression of the μ_3 opiate receptor variant of the opiate receptor gene family. These progenitor cells not only expressed this gene, but also the protein produced from this transcript exhibits the biochemical characteristic of the μ_3 receptor by the production of NO in the presence of morphine. This finding is important in further understanding the role of endogenous opiates and opiate receptors in the developmental process, which may have a strong implication in the development of tolerance in pain and in drug addiction. Our data also provide compelling evidence in support of both the evolutionary primacy and primordial regulatory role of a μ_3-like opiate receptor/NO in embryogenesis.

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10. Working hypotheses and conclusions

Tumor progression involves disregulated cellular growth despite controlled release of inhibitory chemical mediators within the micro-environment and normal expression of transcriptional inhibitors. Thus, unconstrained proliferation may represent primitive cellular phenomena whereby regulation via chemical messengers is suspended or compromised or even in a cellular state of developmental aberrations associated with metabolic or transcriptional perturbations of cell cycle. This has been recently suggested by the finding of novel opiate-alkaloid selective receptor subtype in human MLPC [147], which has previously been identified and cloned from various animal tissues, including human, namely the μ_3 receptor [24]. Adding to the significance of morphinergic signaling are the findings of its presence in plant, invertebrate and vertebrate cells, which also have been shown to synthesize this messenger as well [8]. Interestingly, we and others have shown that some human cancerous tissue contain morphine [152–154]. Furthermore, in medullary histolytic reticulosis, which is exemplified by cells having hyperactivity, an absence of μ_3 receptors, suggests a perturbation of morphine-regulated cellular events [155]. Thus, it would appear that morphinergic signaling has inserted itself in many processes taking a long time to evolve during evolution [148], including those regulating the proliferation of cells across diverse phyla.

Morphine may decrease the incidence, development or spread of certain cancers, because it has been associated with down regulation of developmental processes and cell activity, including tumor cells, i.e., tumor growth retardation. Moreover, endogenously expressed morphine has been demonstrated to initiate a regulatory signaling cascade with anti-proliferative, apoptotic and anti-neoplastic properties, involving protective cNOS-dependent cellular pathways. Opiate alkaloids may have a profound effect on an animal's maturation processes via pharmacological actions at physiologically damaging concentrations, thereby exerting profound perturbation of essential processes mediated by endogenous morphine/NO-coupled signaling [147]. Importantly, mammalian and human cells have the ability to make morphine via a multienzyme mediated process, containing numerous feedback inhibitory steps [3,4], and pharmacological administration of high concentrations of exogenous morphine may one or all of these regulatory steps. Finally, cocaine, alcohol and nicotine may also alter endogenous morphine processes, which now may effect negative consequences on both embryonic and adult stem cell viability and differentiation [156–158].

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