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ENDOGENOUS MORPHINERGIC SIGNALING AND TUMOR GROWTH

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1. ABSTRACT

The mu_3 opiate receptor subtype has been characterized by various binding assays as opiate alkaloid selective (e.g. morphine) and opioid peptide (e.g. methionine enkephalin) insensitive. This opiate receptor subtype has been found on human, including cancer cell lines, and invertebrate tissues, demonstrating that it has been conserved during evolution. Furthermore, in numerous reports, this receptor is coupled to constitutive nitric oxide release. In this regard, for example, morphine immune down regulating activities parallels those actions formerly attributed to nitric oxide. We have now identified the mu_3 receptor at the molecular level and sequence analysis of the isolated cDNA suggests that it is a novel, alternatively spliced variant of the mu opiate receptor gene (MOR). Furthermore, using Northern blot, reverse transcription coupled to polymerase chain reaction (RT-PCR) and sequence analysis, we have demonstrated the expression of this new mu variant in human vascular tissue, mononuclear cells, polymorphonuclear cells, and human neuroblastoma cells. The presence of this mu splice variant, adds to the growing body of evidence supporting the hypothesis that morphine is an endogenous signaling molecule in neural, immune and vascular systems. In addition to their use in the treatment of pain, opioid peptides appear to be important in the growth regulation of normal and neoplastic tissue. This review will focus on the influence of opiate alkaloids, e.g., morphine, on tumor growth, with emphasis on immuno-regulatory and antiproliferative mechanisms.

2. INTRODUCTION

Opium has long been associated with human history (1). Sumerian ideograms (4,000 B.C.) refer to poppy as the "plant of joy" and Egyptian medical papyri used opium in many of their beneficial medical formulas. It is referred to in Homer's Iliad and Odyssey and in Virgil's Aeneid. The Persian physician Avicenna prescribed it for cough, anemia and diarrhea. Despite this positive medicinal link between man and opium, we all know it has its dark side in substance abuse, culminating in addiction. 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 antinociceptive drugs. This focus changed following the discovery (2) 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 system (3, 4). 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.

3. ENDOGENOUS MORPHINE PROCESSES

3.1. Mu_3 binding

Most of our knowledge of opioid receptors is derived from studies of these receptors in the nervous system. These receptors have been classified as delta, mu and kappa, and heterogeneity has been shown for each of these major subtypes (5-11). Amino acid sequences have been derived from cloning of certain delta, mu and kappa (neuronal) opioid receptor subtypes (12-15).

Table 1. Displacement of ^{3}H-dihydromorphine (DHM; nmol-L^{-1}) by opioid ligands in various human tissue membrane suspensions

LIGAND	Vein	Artery	Atria-E	Granulocytes	Monocytes
Delta-agonist					
DPDPE	>1000	>1000	>1000	1000	>1000
Mu-agonist					
Endomorphin 1	>1000	>1000	>1000	>1000	>1000
Endomorphin 2	>1000	>1000	>1000	>1000	>1000
Orphanin FQ (N)	>1000	>1000	>1000	>1000	>1000
DAMGO	>1000	>1000	>1000	>1000	>1000
Fentanyl	>1000	>1000	>1000	>1000	>1000
Dihydromorphine	19 ± 2.1	21 ± 3.0	24.3 + 3.1	18.4 + 2.9	20.6 + 3.8
Kappa-agonist					
Dynorphin 1-17	>1000	>1000	>1000	>1000	>1000
50-488h	>1000	>1000	>1000	>1000	>1000
U69-593	>1000	>1000	>1000	>1000	>1000
Antagonists					
Naltrexone	34 ± 5.1	37 ± 3.8	32.6 ± 3.5	3.7 ± 6.8	47.6 ± 9.3

One hundred per cent binding is defined as bound ^{3}H-DHM in the presence of 10 uM dextrorphan minus bound ^{3}H-DHM in the presence of 10 uM levorphanol. IC_{50} is defined as the concentration of drug which elicits half-maximal inhibition of specific binding. The mean SD for three experiments is given. DPDPE = (D-Pen^{2}, D-Pen^{5})-enkephalin; DAMGO = [Tyr-D-Ala^{2}, Gly-N-Me-Phe^{4}, Gly(ol)^{5})-enkephalin]

Another mu opiate receptor subtype has been demonstrated, namely mu_3 (16). The mu_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 (17). However, the opiate alkaloid binding that was present was naloxone sensitive, demonstrating its opiate receptor properties (18). 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 mu_3 receptor (16, 19-22). Binding sites for this novel morphine (opiate alkaloid-selective, opioid peptide-insensitive) receptor, designated mu_3, were first reported to be present in human peripheral blood monocytes and in invertebrate immunocytes and later on other cell types (16, 19, 20, 22-24). The newly discovered opioid peptides endomorphin-1, -2 and orphanin FQ also do not bind to this opiate receptor subtype (table 1) (25). 6-Glucuronide, not the 3-glucuronide metabolite of morphine, binds to the mu_3 receptor. The potent mu opiate agonist fentanyl does not bind to this receptor as well (20, 22, 23) (table 1).

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 (6). In this regard, the mu_3 receptor is also linked to G protein, based on guanine nucleotide effects on agonist binding to the receptor (20).

3.2. Nitric oxide (NO) and morphine

There has been in the literature an association of nitric oxide (NO) with morphine actions. Peripheral morphine analgesia involves NO-stimulated increases in intracellular cGMP (26). Nitric oxide has been associated with antinociception (27) as well as tolerance and dependence (28). In addition, the morphine-induced suppression of splenic lymphocyte proliferation has been shown to involve NO (29). Morphine and NO have been linked in gastrointestinal regulation (30, 31). 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 (18, 25, 32-39). These data suggest that the mu_3 receptor is coupled to constitutive NO release in these cells.

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 (18, 37, 39, 40). Additional information on opiate alkaloid signaling substances can be summarized as follows: Injection of vertebrate animals with morphine results in deficient macrophage function (41) and an alteration of T-cell activity (42). Morphine also antagonizes interleukin-1alpha or tumor necrosis factor-alpha-induced chemotaxis in human granulocytes and monocytes (43, 44).

3.3. Cloning of mu_3

In order to identify mu_3 at the molecular level, a human testis cDNA library was screened using a MOR-1 gene-specific probe and a 1338 base pair clone was identified (45). Sequencing of this clone and subsequent analysis (NCBI Blast software) shows that the clone exhibits 100% identity to mu_1 in the center and conserved region, but is truncated at the 5'-end (position 503 of mu_1 mRNA) (missing several hundred nucleotides). In addition, the 3'-end of the new clone contains the 3'-end of the mu_1 receptor, followed by a new fragment of 263 bases, and then

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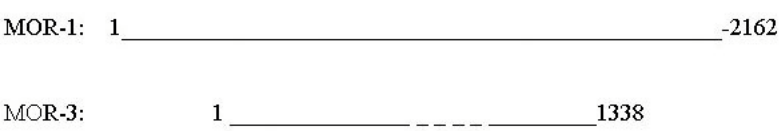

B

Figure 1. A: Mu3 cDNA sequence (1338 bp). The underlined sequence represents the novel 263 bp segment, and the bold letters represents *Homo sapiens* Oprm 3'UTR DNA sequence (nucleotide position 1625-1829 of Oprm). B: The diagram shows the mu3 cDNA sequence starting at position 503 of Oprm. The dotted lines represent the novel 263 bp mu3 sequence (between nucleotides 1376 and 1625 of Oprm), and position 1625-1829 is the Oprm 3'UTR DNA sequence that represents the 3'-end of mu3.

a 202 bp fragment of the 3'-end of the mu_1 gene untranslated region (45) . RT-PCR and subsequent sequence analysis revealed the presence of this opiate receptor subtype in human vascular endothelial cells, mononuclear cells and polymorphic nuclear cells (45) (see sequence in figure 1).

To determine if the cDNA clone we isolated was functional and had the biochemical properties expected of the mu_3 receptor, the cDNA was expressed in a heterologous system (Cos-1 cells). Following exposure to morphine, the transfected Cos-1 cells released NO in a naloxone antagonizable manner (45). Untransfected Cos-1 cells failed to produce any detectable NO upon addition of morphine (45). The addition of Met-enkephalin, DPDPE, or Leu-enkephalin did not stimulate NO release in the controls or transfected cells (45). 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 mu_3 opiate receptor (17, 46-48). In addition, we analyzed human heart endothelial tissue, human leukocytes, and Cos-1 transfected cells for expression of the mu_3 mRNA by Northern blot analysis (45). A band of the expected size of approximately 1.45 kb was observed in the various tissues (45). The selectivity of the mu_3 opiate receptor subtype, therefore, provides further evidence for the status of morphine as an endogenous signaling molecule (46).

3.4. Endogenous morphine

The demonstration of endogenous opiates, i.e., morphine, codeine, in various vertebrate tissues, including the nervous system (3, 40, 49-53), is quite important for establishing the significance of the mu_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 (52, 54-56). In invertebrates, specifically *Mytilus edulis*, the presence of morphine, morphine 6-glucuronide, morphine 3-glucuronide, codeine, THP and reticuline also have been reported (16, 46, 57-59). Endogenous opiate levels can be induced to change following stimulation (46). Morphine has also been found in human plasma (60, 61), suggesting a hormonal action with immune, vascular and gut tissues as targets (31, 62). Additionally, in recent studies sources of contamination for these compounds were examined, i.e., rat food, supplies, etc, and not found (56). Besides these biochemical studies, immunocytochemical localization of a morphine-like material was reported in neural and immune tissues (63-66) as well as in invertebrate tissues (16). Taken together, these reports suggest that animals appear to have the ability to synthesize opiate alkaloids.

In earlier reports 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 (18, 31, 40, 46, 66). 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.

4. MORPHINE AND TUMOR GROWTH

4.1. Effects of morphine on tumor growth

Opioid peptides, such as methionine enkephalin, have been implicated in a wide variety of pharmacological and physiological functions (18). In addition to their use in the treatment of pain, opioid peptides appear to be

important in the growth regulation of normal and neoplastic tissue. This literature regarding opioid peptides and tumor growth has been reviewed recently (67). This portion of the review will focus on the influence of endogenous and exogenous opiate alkaloids, e.g., morphine, on tumor growth, with emphasis on immunoregulatory and antiproliferative mechanisms. This has become important because morphinergic signaling appears to be an endogenous messenger system, as noted in the earlier discussion. Additionally, most of the reports that will be discussed examine morphine signaling from an exogenous perspective, leaving open the issue of how endogenous morphine impacts tumor growth. This article will focus on morphine's influence on the regulatory mechanisms involved in tumor growth.

Opiate alkaloids appear to represent one of the immune and vascular inhibitory/anti-inflammatory systems in an organism whereas opioid peptides appear to have proinflammatory capabilities (18, 46, 68). Thus, from an immune perspective, these signaling molecules are potential candidates as tumor growth modifiers.

4.2. Exogenous morphine affects tumor growth

Morphine is widely used as an analgesic to treat pain in a variety of patients, including those with cancer. There is evidence that morphine has extra-analgesic actions and significantly alters tumor growth. Ishikawa *et al* demonstrated that morphine (10 mg/kg) given daily for 10 days enhanced the growth of several different tumor cell lines *in vivo* (69). However, other studies suggest that the analgesic qualities of morphine contribute to the control of metastasis following surgery. Page *et al* demonstrated that pre- and postoperative administration of morphine significantly attenuated the metastatic-enhancing effects of surgery (70-72). In addition, intermittent bolus morphine administration to animals was associated with a reduction in the growth of tumor cells that gained access to the circulation during the surgical procedure (73). The authors proposed that the most likely explanation was a direct morphine effect on host resistance to metastatic tumor growth.

Hatzoglou *et al* demonstrated that morphine decreases the cell growth of human breast cancer cells *in vitro*, despite the lack of mu receptors in the cancer cells as determined by a limited screening with pharmacological agents (74). They raised the question whether this antiproliferative effect of morphine could be mediated through interaction with other receptor systems. In a further study they showed, that morphine may exert its antiproliferative effect on breast cancer cells through interaction with the somatostatin receptor SSTR2, suggesting a functional interaction of morphine with the inhibitory somatostatinergic system (75). Previous studies have demonstrated a direct inhibitory effect of somotostatin analogues on the growth of human cancer cells (76-78). The possible interaction of morphine with other receptor systems is supported by the findings of Maneckjee and Minna. They found that the inhibitory effects of morphine on the growth of lung cancer could be reversed by nicotine, suggesting an interaction between the opioid system and acetylcholine receptors (79). In addition, Zagon *et al* demonstrated in receptor binding studies that only a few tumors express mu opioid binding sites (80). Thus, given the knowledge at that time, it seems that mu opioid receptors do not play a significant role in opioid mediated regulation of tumor growth. However, new evidence from our group suggests that other physiologically active mu opioid receptor splice variants may be present and operational in immune and vascular tissues that had gone previously undetected (45, 48). Thus, morphine may be protective in many tissues in response to stress or injuries (46) and it is no surprise that in cases where there is tissue injury such as in wound healing it is involved in angiogenesis (81). In part, this effect may be due to rebound excitation, following morphine's immediate action, which is down regulating in nature (37, 40, 46). This rebound excitation allows tissues, i.e., immune, a chance to exhibit enhanced surveillance, following morphine stimulated immune suppression (82, 83). Clearly, this action has important organism survival value, which can also be surmised by the fact that this process is found in organisms that evolved 500 million years before man (82).

4.3. Morphine and apoptosis

The molecular mechanism by which morphine influences tumor growth *in vivo* and *in vitro* is not clear. Basically, this situation exists because of a lack of realization that morphine represents an endogenous signaling system (40, 46, 56). However, one hypothesis is that morphine promotes apoptosis in tumor cells. Maneckjee and Minna demonstrated that treatment of human lung cancer cells with 0.1-1.0 uM morphine or methadone resulted in morphological changes and cleavage of DNA into nucleosome-sized fragments characteristic of apoptosis (84). Sueoka *et al* demonstrated that morphine attenuated the growth of various cancer cell lines *in vitro* through inhibition of tumor necrosis factor (TNF)-alpha mRNA expression and TNF-alpha release (85). Transcription of TNF-alpha gene is in part regulated by the transcription factor, nuclear factor kappa B (NFkB) (86). In a further study, they demonstrated that the anticancer activity of morphine and the five times more potent morphine derivatives, (-)-3-Acetyl-6beta-acetylthio-*N*-cyclopropyl-methylnormorphine (KT-90) , (-)-6beta-acetylthiomorphine (KT 87) was mediated through apoptosis associated with inhibition of NFkB in human cancer cell lines (87). NFkB is a DNA binding protein that induces expression of genes for several inflammatory mediators such as TNF-alpha and augments transcription of various genes involved in cell proliferation. Recently it was demonstrated that inhibition of NFkB attenuates apoptosis resistance in lymphoid cells (88). Furthermore, we demonstrated that morphine can directly inhibit NFkB actions via the liberation of NO (89, 90), introducing another variable in the morphine signaling cascade that may explain morphine's antiproliferative and apoptotic actions (figure 2).

4.4. Morphine influences natural killer (NK) cell activity

The role of NK cells in both the metastasis enhancing effects of surgery and the attenuation of these effects by morphine was investigated by Page *et al* (71). Surgery induced a suppression of whole blood NK cytotoxic

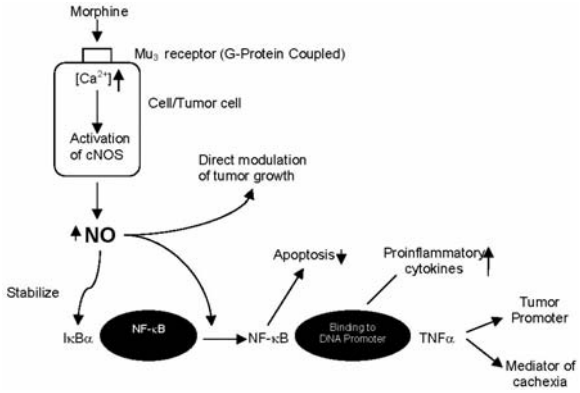

Figure 2. How morphine might alter tumor growth**.** Morphine stimulates intracellular Ca^{2+} transients that, in turn, activate constitutive nitric oxide synthase (cNOS) and liberate nitric oxide (NO). This modulation occurs via the Mu_3 opiate receptor subtype that is G protein regulated. NO may have a direct effect on tumor growth. Furthermore, NO inhibits disassociation of the IkBa inhibitor complex, NF-kB binding to the respective DNA promoter region and the subsequent expression of inflammatory cytokines, thereby stimulating apoptosis and downregulation of the carcinogenic effects of TNF-alpha.

cytotoxic activity and a decreased number of circulating Large Granular Lymphocytes (LGL)/NK cells measured 4 h postoperatively (71). The authors observed that in LGL/NK depleted animals, morphine had no impact on tumor cell retention. They suggested that LGL/NK cells play a critical role in morphine's antimetastatic effects. In addition, Provincialli *et al* reported that "chronic" morphine treatment in cancer patients was accompanied by a decrease in the *in vitro* NK cell cytotoxic activity (91) while the treatment significantly increased Lymphokine Activated Killer (LAK) cell activity when compared to healthy controls (91).

4.5. Morphine's influences on DNA synthesis

It has previously been demonstrated that mu-, delta-, and kappa- opioid agonists attenuate thymidine incorporation into DNA in glial (92) and developing neural cells (80, 93-96). Furthermore, Barg *et al* showed that morphine inhibited DNA synthesis through inhibition of thymidine incorporation in C6 rat glioma cells that express opioid receptors (97). Their results imply that inhibition of phosphoinositol signal transduction and CA^{2+} mobilization is responsible for reduction in thymidine incorporation (98). This finding is in accordance with previous reports demonstrating that opioid peptides and opiate alkaloids can inhibit phosphoinositol turnover (92, 99-101).

4.6. Endogenous morphine, nitric oxide and the regulation of tumor growth

Morphine has been shown to be involved in immunomodulation. Studies have demonstrated that morphine, not opioid peptides, via the mu_3 opiate receptor is coupled to constitutive NO release in endothelial and immunocompetent cells (22, 45, 102, 103). Recently, expression of this opiate receptor subtype was demonstrated in human specimens of cancer tissue (non-small-cell lung carcinoma) (104). The authors demonstrated that activation of the mu_3 opiate receptor by opiate alkaloids in tumor cells leads to a rapid and substantial release of NO (104). They suggested that the anti cancer effects of morphine were mediated by NO through the mu_3 receptor. They also speculated that increased NO production in lung carcinoma may indicate that tumors use endogenous opiates that bind to the mu_3 receptor and thereby down regulate the host immune response to tumor growth.

Based on the earlier discussion, concerning endogenous morphine and the mu_3 opiate receptor's coupling to cNOS-derived NO we surmise this NO, in turn, inhibits binding of the DNA transcription factor NFkB to DNA and thereby down regulates the expression of genes for several inflammatory cytokines (46, 89, 90, 104, 105) (figure 2). Furthermore, NO stabilizes the NFkB inhibitor, Inhibitory kappa B alpha (IkBa), which prevents its degradation. In addition, inhibition of NFkB attenuates apoptosis resistance as described previously and with that promotes down regulation of tumor growth (figure 2).

Regarding the effect of NO on tumor growth regulation, previous studies have shown that NO produced by immune and endothelial cells is tumoricidal possibly by inducing apoptosis (106). Other studies have demonstrated that up regulation of the expression of inducible NO synthase (iNOS) in hepatic metastases and metastatic melanoma cells is associated with apoptosis, suppression of tumorigenicity, and abrogation of metastasis (107, 108). However, other studies have demonstrated tumor promoting effects of NO, and overall, NO seems to play a variety of contradictory roles in tumor growth regulation (109, 110). Contradicting results have also been obtained regarding the functional interaction between the opioidergic and the NO system. Kampa *et al* demonstrated that opioid agonists, active on kappa-opioid receptors decrease NO_2-/NO_3- release and NOS activity *in vitro* (111). Based on their results and reports demonstrating NO involvement in tumor progression and metastasis, the authors suggested opiates as a potential intervention in cancer treatment. Furthermore, ambiguity is introduced into this field because most research fails to take into account the rebound effect that occurs following exposure of morphine to these cells (37, 38, 82, 83, 112). Here we've surmised, since morphine is endogenous, that following immediate morphine exposure processes are down regulated, then after some time (hours), their activity returns but to a higher level for a short period of time. This secondary immune and vascular enhancement of activity, we surmise, is designed to take care of any emergent problems that occurred during the period of down regulation, offering yet another mechanism that enhances the survival of such endowed organisms (37, 38, 82, 83, 112).

Recently, Gobert *et al* demonstrated that the vigorous host response, i.e., up regulation of iNOS, to the human gastric pathogen *Helicobacter pylori* failed to eradicate the organism (113). This was due to bacterial arginase down-regulating eukaryotic NO production. We surmise a similar process may be occurring with tumors,

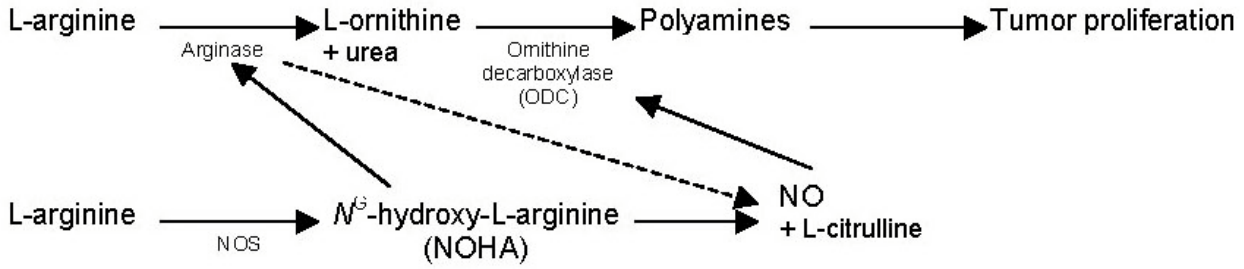

Figure 3. The mechanisms by which L-arginine metabolites influence cell proliferation. L-arginine is the common substrate for two enzymes, arginase and nitric oxide synthase (NOS). Arginase converts L-arginine to L-ornithine. L-ornithine can be subsequently used by ODC to form polyamines, which are essential for cell proliferation. NO is a potent inhibitor of ODC, suggesting that the antiproliferative role of NO is attributed to inhibition of polyamine formation. L-arginine is also metabolized by NOS to L-citrulline and NO via an intermediate, NOHA. NOHA interferes with cell proliferation by inhibition of arginase and arginine-derived polyamines. Arginase promotes polyamine formation and suppresses tumor cytotoxicity by reducing NO production.

explaining the contradictory results. That is, certain tumors have a process to neutralize NO tumoricidal actions. In this regard the generation of NO through activation of NOS has been shown to be antiproliferative (114). However, the mechanism by which increases in NOS activity and the production of NO causing cytostasis is not clear. The intermediate *N*-omega-hydroxy-L-arginine (NOHA) in the oxidation of arginine is a strong inhibitor of the enzyme arginase. One of the products of arginase catalyzing L-arginine is L-ornithine. L-ornithine can be subsequently used by the enzyme ornithine decarboxylase (ODC) to form polyamines, which are essential components of cell proliferation (figure 3). Cells that have been activated with cytokines show a significant increase in NOHA and NO or its oxidized metabolites (114)**.** Therefore, NOHA may be playing a role as a biological inhibitor of endogenous arginase activity, thereby promoting down regulation of tumor cell proliferation (figure 3). This may explain the mechanism by which activation of NOS is involved in cytostais. In addition it has been demonstrated that NO is a potent inhibitor of ODC, thereby suggesting that the antiproliferative action of NO is attributed to inhibition of polyamine formation (114) (figure 3)**.**

5. PERSPECTIVES

Few breast cancer cells lines were shown to have high arginase activity and very low NOS activity (115)**.** Nitric oxide derived from macrophages is known to have tumoricidal activity and polyamines may promote the growth of tumor cells (116). Therefore, it appears that arginase may be playing a role in promoting tumor growth by inhibiting the production of NO. The significance of this phenomenon is enhanced by our early discussion concerning morphine's ability to stimulate cNOS derived NO release, since this action may not be observed in the presence of arginase. In this regard, a biomedical strategy recognizing this process may be designed, strengthening a role for opiate as a new tumoricidal agent.

6. ACKNOWLEDGEMENTS

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Abbreviations: cGMP: cyclic guanosine monophosphate; CTOP: D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; DPDPE: [D-Pen^2,D-Pen^5]enkephalin; LAK: lymphokine activated killer; LGL: large granular lymphocytes; NK: natural killer; IkBa: inhibitory kappa B alpha; NFkB: nuclear factor kappa B; NO: nitric oxide; NOS: nitric oxide synthase; cNOS: constitutive nitric oxide synthase; iNOS: inducible nitric oxide synthase; THP: tetrahydropapoverine; TNF: tumor necrosis factor; ODC: ornithine decarboxylase; NOHA: *N*-omega-hydroxy-L-arginine

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