Nitric oxide and pain

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Review article

Nitric oxide and pain

"Something old, something new"

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Abstract

Challenges have emerged following the revival of nitric oxide (NO) from "something old", a simple gas derived from nitrogen and oxygen with a role in the early stages of evolution, into "something new", an endogenously formed biological mediator regulating a wide variety of physiological functions. Although pain is a common sensation, it encompasses multiple neurobiologic components of which NO is only one. In pain research, the study of NO is complicated by convoluted problems related mostly to the effects of NO, which are pro- or antinociceptive depending on the circumstances. This dual function reflects the two faces of the NO molecule described in physiology. This review covers current information about NO and its implications in pain mechanisms. In addition, it follows the pain pathways, demonstrating the role of NO in peripheral nociceptive transmission as well in central sensitization. This knowledge may provide the scientific basis for developing new drugs that are indicated for different types of pain, drugs that may be related to the chemical links of NO. A comprehensive approach to understanding the effects of NO will help clinicians identify novel agents that combine the pharmacological profile of native drugs with a controllable manner of NO release. Inhibitors of NO synthesis may have analgesic effects and would be of interest for treating inflammatory and neuropathic pain. Many specific NO inhibitors have been described and the most promising substances have been studied in animals. Unfortunately, only a few of these compounds have reached the stage of clinical pain trials.

Summary:

1. Introduction

The revival of nitric oxide (NO) indicates that "something simple and something old" may now be "new" again. It should be remembered that NO is a simple gas (1) derived from nitrogen and oxygen, and it played a crucial role in the early stages of evolution (2). NO may have constituted a critical defence mechanism for primitive microorganisms by counteracting oxidative destruction and giving them an evolutionary advantage (2). Not only is NO an ancient and widely used regulator of the life history of different species of eukaryotes and solitary ascidians (3), but the presence of NO was observed in plants much earlier than in animals (4). Discovered in the 17th century by Jan B. Helmont (5) and studied under the name "phlogisticated nitrous air" by Joseph Priestley (6), NO was regarded for many years as an environmental pollutant (7). It became "something new" in 1992 when it received intense media coverage due to its stature as a biological messenger (8) and became the molecule of the year (9). The 1998 Nobel Prize in Physiology or Medicine was awarded for "the first discovery that a gas can act as a signal molecule", emphasising that NO is a biological mediator produced by mammalian cells (10). The fascinating history of NO thereafter continued, with extensive research showing that NO plays an important role in most human organ systems, and in neurotransmission, immune defence, regulation of death cells and cell motility (10).

It was reported that NO modulates spinal and sensory neuron excitability that contributes to different pain states. However, investigating the implications of this molecule in nociception remains a challenging task. The aim of the present review is to summarise the contributions of NO to pain mechanisms, both in peripheral and central nociceptive transmission, and to elucidate therapeutic implications of its action.

2. Nitric oxide biosynthesis and pain

2.1. Basic physiology

In biological systems, NO is a molecular gas generated from L-arginine (11) and molecular oxygen by the activity of nitric oxide synthase (NOS). L-citrulline results from this reaction and is shunted into a metabolic pathway that regenerates free L-arginine (Fig.1). Furchgott first identified NO synthases implicated in NO generation in experiments on the aortas of rabbits (12). The different forms of NO synthase have been identified and named based upon the cells from which they were first isolated: neuronal NOS (nNOS, type 1 NOS) (13); inducible NOS, induced in macrophages upon stimulation with bacterial endotoxines and cytokines (iNOS, type 2 NOS) (14); endothelial NOS (eNOS, type 3 NOS) (15); and in some tissues they may exist in mitochondria (mNOS) (16). The NOS enzymes differ not only in their localization, but also in their function. The two calcium dependent constitutive NOSs-eNOS and nNOS, generate small amounts of NO, while iNOS production that is independent of intracellular calcium concentrations generates NO levels able to damage cells and microorganisms (17). These enzymes are distinct proteins encoded by genes on disparate chromosomes, but all of them have the same genomic structure and all of them facilitate the addition of the guanidino nitrogen of the amino acid arginine to molecular oxygen, producing NO and water (Fig.1) (15). The enzyme consists of an oxygenase domain and a reductase domain, each with its own catalytic activity (15) (Fig.2).

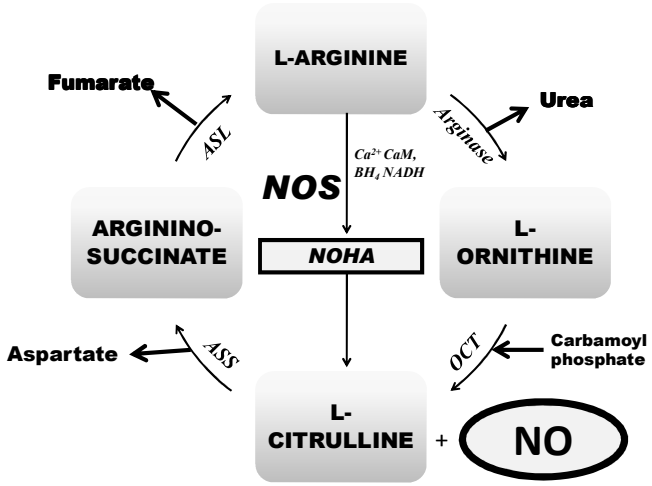

Figure 1 Production of nitric oxide. ***Nitric oxide synthases (NOS) catalyze an oxidation of one N$^{\omega}$-atom of the guanidine group of L-arginine to form NO (nitric oxide) and L-citrulline, with the intermediate N$^{\omega}$-hydroxy-L-arginine (NOHA). ASS, argininosuccinate synthase; ASL, argininosuccinate lyase; OTC, ornithine carbamoyltransferase.***

2.2. Nitric oxide synthases (NOSs) in relation to pain

Knowledge about the role of NO in different types of pain has been derived primarily from animal experiments examining the expression of the NOS isoforms in relation to a nociceptive stimulus. The idea that nNOS contributes to the role of NO in nociception is supported by the observation that its expression is rapidly upregulated in the dorsal horn neurons of the spinal cord after a peripheral noxious stimulation (18, 19). Genetic knockout and pharmacologic inhibition of neuronal nNOS attenuate nerve injury-induced mechanical hypersensitivity in mice, suggesting that nNOS may participate in the development and maintenance of mechanical hypersensitivity after nerve injury (20).

In mice lacking iNOS, there were fewer and smaller regenerating myelinated fibres and slowed reinnervation of muscle endplates distal to the injury zone, demonstrating that iNOS is a critical factor in the repair of injured tissue (21). Inducible NOS is upregulated in the inflamed tissue (22) and is involved in the development of hypersensitivity to pain in inflammatory and neuropathic pain models (23). These findings lead to the conclusion that

administration of iNOS inhibitors may have potential in the treatment of inflammatory and neuropathic pain syndromes (24).

NO generated by eNOS may modulate leukocyte adherence, a key factor in acute tissue inflammation (25). It participates in the regulation of vascular tone, vascular remodelling, angiogenesis (26, 27) and neurogenesis (28).

2.3. Potential role of tetrahydrobiopterin (BH4) in pain

Various cofactors, including tetrahydrobiopterin (BH4), flavin mononucleotide (FMN), flavin adenine dinucleotid (FAD), reduced nicotine adenine dinucleotide phosphate (NADPH), and calmoduline/Ca^{2+}, are necessary for the formation of NO if the synthase isoform is the constitutive NOS I or NOS III (29, 30) (Fig.2).

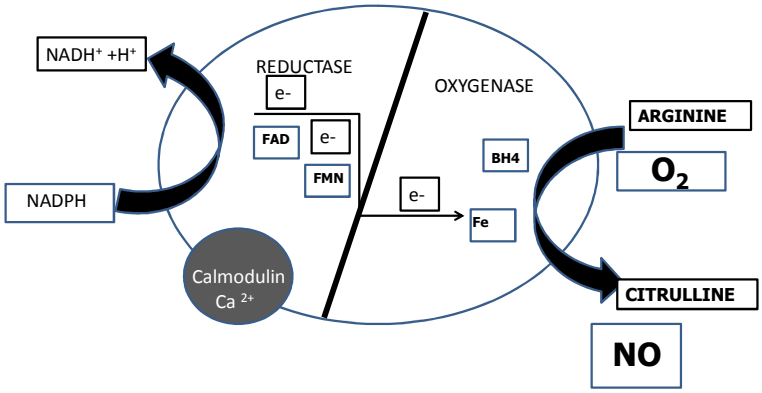

Figure 2 NOS cofactors. ***NOS has a reductase domain and an oxygenase domain. Electrons (e⁻) are donated by NADPH (nikotinamid-adenin-dinukleotid) to the reductase domain of the enzyme and proceed via FAD (flavin-adenin-dinukleotid) and FMN (flavin mononucleotide) carriers to the oxygenase domain. There they interact with the heme iron and BH4 at the active site to catalyze the reaction of oxygen with L-arginine, generating citrulline and NO. Electron flow through the reductase domain requires the presence of bound Ca2+/CaM (modified after Alderton) (30).***

The presence of BH_4 is important in dopamine formation, and in several neurotransmitters and NO formation, and any deficiency leads to the formation of other reactive oxygen species (ROS) (15). The role of BH4 as an intrinsic regulator in neuropathic and inflammatory pain was confirmed by increasing BH4 synthesis in the rat sensory neurons in response to both axonal injury and peripheral inflammation, and also by the observation that blocking the BH4 synthesis reduced neuropathic and inflammatory pain (31). The enzyme implicated in BH4 synthesis, GTP cyclohydrolase (GCH1), is a key modulator of peripheral neuropathic and inflammatory pain (32) (Fig.3). In animal models of both neuropathic pain and inflammatory pain, injecting GTP cyclohydrolase inhibitor, called 2, 4-diamino-6-hydroxypyrimidine (DAHP), alleviated hypersensitivity to pain and reduced the levels of NO (31, 32). Furthermore, the animal models were correlated to clinical pain when a "pain-protective" haplotype of the GCH1 gene was identified (31). This haplotype that reduces BH4 upregulation was protective against persistent neuropathic pain, and it decreased pain sensitivity in low back pain patients following herniated disc surgery and also in normal volunteers in an experimental pain model (31).

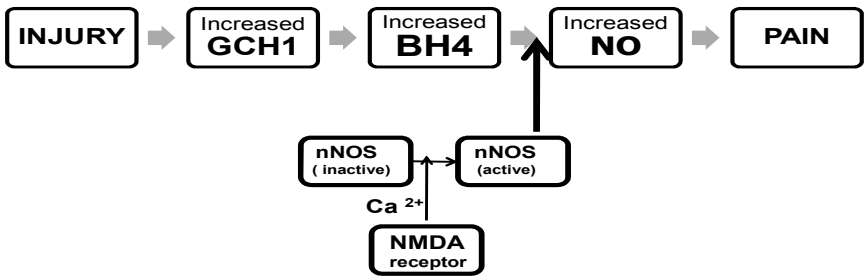

Figure 3 Implications of BH4 and GTP cyclohydrolase in pain *(modified after Pasternak)*

(33). Noxious stimuli induce the upregulation of GTP cyclohydrolase (GCH1). BH4 modulates several

enzymes, including neuronal nitric oxide synthase (nNOS), which generates nitric oxide (NO) that is under the control of NMDA (N-methyl-D-aspartate) receptors through their regulation of intracellular calcium.

In the absence of substrate and cofactors, NOS no longer produces NO, but transfers the free electrons and produces free oxygen radicals (34).

3. Reactive oxidative species (oxygen/nitrogen) and pain

Reactive oxidative species are highly reactive chemicals that contain only oxygen atoms (reactive oxygen species-ROS) or both nitrogen and oxygen (reactive nitrogen species-RNS). ROS react easily with other molecules, resulting in potentially damaging modifications (35). The main members of reactive oxygen species are superoxide (O_2^-), H_2O_2 and the hydroxyl radical (OH^-). RNS are a group of chemically reactive molecules derived from nitric oxide (NO) that may react with other radicals to give species of greater reactivity and toxicity such as peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3) (36). These reactions account for the **indirect effects** of NO (35) observed under pathophysiologic circumstances when NO flux becomes enhanced, mainly as a consequence of iNOS expression. The interaction between nitric oxide and superoxide is responsible for the formation of peroxynitrite (37), which is a potent cytotoxic and pro-inflammatory molecule (38). Both superoxide and peroxynitrite are mediators of pain that accompanies inflammation (39, 40, 41). Furthermore, ROS contribute to hyperalgesia (clinically defined as an augmented sensitivity to painful stimuli) due to nerve injury (42, 43, 44) and to delayed recovery of injured nerves (45). Thus, peroxynitrite formed after partial nerve injury contributes to the initiation of hyperalgesia and Wallerian degeneration (46), damage that is alleviated by ROS scavengers (42). In spite of many published articles, investigation of the involvement of ROS/RNS in pain is limited to animal studies describing the effects of antioxidants in attenuation of neuropathic or inflammatory pain (47, 48). “Spin-trap reagents” are the most potent ROS scavengers, with phenyl-N-tert-butylnitrone (PBN) being especially noteworthy (47). PBN has been shown to possess a neuroprotective action in several animal models of

brain ischaemia (49) not only because of its capacity to scavenge free radicals, but also due to the inhibition of inducible nitric oxide synthase (iNOS) expression, as well as to the inhibition of cytokine production (50). In animal models PBN relieves mechanical allodynia and inhibits development of neuropathic pain-like behavior (51). The site of action of PBN is considered to be spinal rather then peripheral (42). The results suggest that ROS scavengers can be developed as powerful analgesic drugs for neuropathic pain (42). N-*t*-butyl hydroxylamine (NtBHA) is one of the breakdown products of PBN and is the only spin-trap reagent that has been used in humans as a neuroprotective agent in acute ischaemic stroke (52). However, it is still not clear whether antioxidant therapy is effective in human pain, but results obtained from small clinical trials indicate that treatment with nutritional antioxidants (vitamin C, vitamin E, and beta-caroten) may reduce pain in patients suffering from chronic pancreatitis (53, 54) and fibromyalgia (55, 56).

4. The NO-cyclic guanosine monophosphate (cGMP) pathway

4.1. Basic physiology

The stimulation of cyclic guanosine monophosphate cGMP-dependent kinases is one of the numerous **direct biological actions** of NO (57). NO activates the guanylyl cyclase and increases the synthesis of cyclic guanosine monophosphate (58). The major target of NO is NO-sensitive guanylyl cyclase (GC) or soluble guanylyl cyclase (sGC). Activation of guanylyl cyclase results in conversion of guanosine triphosphate to the second messenger cGMP (59). The NO/cGMP signalling cascade (Fig.4) is important in the cardiovascular and nervous systems, where it controls smooth muscle relaxation and modulation of synaptic transmission (60).

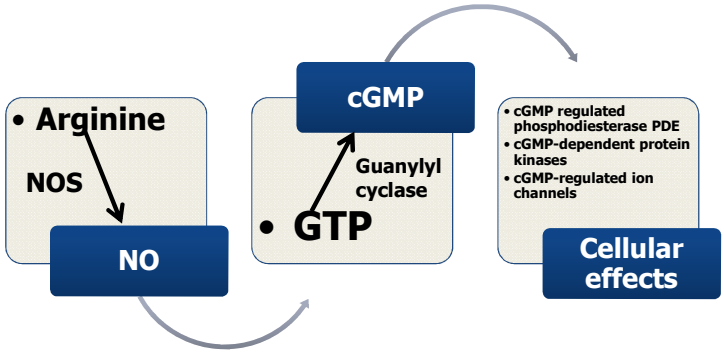

Figure 4 NO/cGMP signaling cascade. ***NO endogenously produced by NO synthases activates NO-sensitive GC and leads to increased synthesis of cGMP. This intracellular messenger in turn modulates the activity of cGMP-dependent kinases, cGMP-gated ion channels, and cGMP-regulated phosphodiesterases.***

The second messenger cGMP has several targets, including cGMP-dependent protein kinase I (PKG-I) and PKG-II, activation of cGMP- regulated phosphodiesterase and cGMP-regulated ion channels (60). PKG-I is expressed in the spinal cord and is involved in the facilitation of synaptic transmission of nociceptive stimuli in the spinal cord (61, 62) and also in substance P synthesis (32).

4.2. The NO-cGMP pathway in pain

The NO-cGMP signalling pathway is present in neurons of the spinal cord and contributes to the development of hyperalgesia in animal models of pain (63, 64, 65) through PKG-I activation (66). It was recently demonstrated that during spinal nociceptive processing, cGMP produced by NO-GC may activate signalling pathways different from those activated by cGMP-dependent protein kinase I (cGKI) (67). Different studies have demonstrated the implications of the NO-cGMP pathway in the analgesic effect of several drugs that are indicated for the treatment of neuropathic pain, such as tramadol (68), spinal-administered clonidine (69), gabapentin (70, 71), and also in the antinociceptive effect of anti-inflammatory

drugs such as indomethacin (72), and in the hyperalgesia evoked by intrathecal high dose morphine (73,74). The NO-cGMP pathway interacts with other signalling pathways (75) such as the cholinergic, adrenergic, purinergic and peptidergic pathways in the peripheral nerve system, and also with endocannabinoids (76). In pain research, the significance of these interactions of the NO-cGMP pathway with other signalling pathways is only speculative, because they are still poorly understood.

5. The link between NO and NMDA

The *N*-methyl-D-aspartate (NMDA) receptor is activated via a voltage-gated ion channel that, once activated, allows Ca^{2+} to enter the neuron. It is the increase of intracellular Ca^{2+} that triggers the cascade of events that includes activation of a constitutive form of NOS, followed by increased production of NO (77). Many of the effects of NMDA activation are mediated via production of NO. The NO diffuses to adjacent neurons and glia, where it activates soluble guanylate cyclase (sGC), which in turn increases the intracellular content of cyclic guanylate mono phosphate (cGMP) (78). After tissue injury, sensitization of nociceptors and changes in the excitability of spinal neurons occur, which could underlie development of primary hyperalgesia and secondary hyperalgesia. The spinal NMDA receptor is associated with secondary hyperalgesia (79).

6. NO and peripheral nociception

The role of nitric oxide and cyclic GMP in the periphery has been controversial. Several studies have demonstrated that NO may promote both pro- and antinociceptive effects (80). Intracutaneous and paravascular injections of NO in humans can directly evoke pain in a dose-related manner (81, 82). NO-donors led to hypersensitivity in rats (83) and to antinociception in humans (84, 85). Thus, transdermal NO-donor glyceryl trinitrate was found to act as an antinociceptive when used to treat shoulder pain syndrome due to supraspinatus tendinitis (84) and elbow tendinosis (85). Topical application of nitric oxide using

transdermal glyceryl trinitrate patches can increase blood supply to the region due to local vasodilatation, which in turn increases the clearance of local inflammatory mediators or bioactive proteins such as substance P (85). It may also stimulate wound fibroblasts and increase fibroblast synthesis, as seen in the healing of tendons (84, 85). Furthermore, the arginine/NO/cGMP pathway is antinociceptive in subcutaneous tissues, while it is pronociceptive in intradermal tissues (86). In inflammatory pain, the action of NO depends on the stages of inflammation (80); it is protective during the initial hours after inflammation (80) and cytotoxic later (87). These studies suggest that NO and cGMP signalling are able to induce pro- or antinociception depending of the different concentrations of NO produced locally (87), and with a dose-response relationship (88), depending on the location (89) and also on the stage of pathological processes (80).

7. Effects of NO in central nervous system

A peripheral nerve lesion gives rise to structural changes in the spinal cord, probably related to the fact that NO acts as a modulator of dorsal horn spinal cord nociceptive pathways (90). NO is suggested to act as a "retrograde transmitter" because it can easily pass through neuronal membranes. Activation of a receptor results in the production of NO in a post-synaptic neuron from which it rapidly diffuses to enter the presynaptic neuron, where it modulates excitability and enhances synaptic connection (91). Our laboratory is engaged to examine changes in NOS expressions using immunohistochemistry and to relate these changes with morphological alterations in the spinal cord using light microscopy (92). Thus, in our model of chronic neuropathic pain in rats, induced by lesion or ligation of peripheral spinal nerves that induced hyperalgesia behaviour during 4 to 8 weeks after nerve lesion, we reported the role of NO in nociceptive signalling based on the upregulation of neuronal NOS in the superficial dorsal horn and intermediolateral cell column (92). This finding lead to the notion that NO regulates sensory transduction at the spinal cord level. Furthermore, the

mechanisms underlying pain hypersensitivity have been associated with release of NO within the spinal cord (93).The involvement of NO in nociception is supported by experiments in which inhibitors of NOS (L-NAME) were used to reduce NO production, which in turn reduced NOS upregulation and structural changes in the spinal cord (94). NO donors administered intrathecally have been shown to elicit a nociceptive behavioural response (95), whereas NOS inhibitors provide analgesia (96). Our group demonstrated that a peripheral nerve lesion in a rat model of chronic neuropathic pain induces selective alterations in the blood-spinal cord barrier (BSCB) permeability in a time-related manner and activates microglia (97). We assessed the integrity of the BSCB using immunohistochemistry for endogenous albumin that represents the breakdown of the BSCB following nerve lesion and also the state of glial activation was examined using antibodies to glial fibrillary acidic protein (GFAP), a specific marker of astrocytes (98). We hypothesise that neurochemical alteration would lead to morphological changes in the spinal cord endothelial cells and the astrocytes and contributes to the pathobiology of the neuropathic pain (90, 92, 99).There are reasons to believe that nerve lesion is associated with upregulation of nitric oxide synthase (NOS) is instrumental in morphological alterations in the spinal cord in animal models (90, 92, 100).

The brain expresses all three identified nitric oxide synthase isoforms, and NOS activity is higher than in any other tissue (101). In the brain, NO has been proposed to be involved in synaptic plasticity, or to act as a neurotoxin when produced in excess (102).

8. Cyclo-oxygenase (COX) and NO

Cyclo-oxygenase (COX) converts arachidonic acid into prostaglandins, prostacyclin and thromboxane A_2. The link between COX and NO pathways was observed in 1993 by Salvemini and co-workers when they demonstrated that the enhanced release of prostaglandines, which follows inflammatory mechanisms, was nearly entirely driven by NO (103). NO is implicated in the regulation of cyclo-oxygenase (104) activity and activates cyclo-oxygenase, followed by an increase in prostaglandin synthesis (105). Nitric oxide

mediates several of its beneficial effects, such as maintenance of blood vessel tone, inhibition of platelet aggregation and cytoprotection, through the activation of cyclo-oxygenase (106). The discovery of the reciprocal interaction between NO/COX has opened up the possibility of designing new drugs, with a better toxicological and safety profile, containing NO donors conjugated with COX inhibitors.

9. Potential applications of nitric oxide in pain treatment

Pharmacological modulation of NO levels and NO biosynthesis may be a therapeutic strategy for treating different conditions, including pain (107, 108).

9.1. NO-releasing drugs

9.1.1. Cyclo-oxygenase-inhibiting nitric oxide donating drugs (CINODs)

Cyclo-oxygenase-inhibiting nitric oxide donating drugs (CINODs) are also known under the name nitric-oxide releasing non-steroidal anti-inflammatory drugs (NO-NSAID) because they combine the pharmacological profile of NSAID drugs with a controllable manner of NO release (107). The release of NO together with NSAID prevents the major side effects of NSAIDs that appear after inhibition of cyclo-oxygenase and suppression of prostaglandin synthesis (109). CINODs are synthesized by the ester linkage of an NO-releasing moiety to conventional non-steroidal anti-inflammatory drugs such as aspirin (NO-aspirin), flurbiprofen (NO-flurbiprofen), naproxen (NO-naproxen), diclofenac (NO-diclofenac) and ibuprofen (NO-ibuprofen) (110). NO-naproxen is the first drug in the new class of analgesic and anti-inflammatory CINOD compounds (111,112) that have been evaluated clinically in treating the signs and symptoms of osteoarthritis of the knee (113,114).

a. CINODs and the gastrointestinal tract

Gastrointestinal disturbances are a side effect of NSAIDs, and have been attributed to inhibition of gastric COX-1 activity leading to loss of citoprotective prostaglandin (PGI_2 and PGE_2) formation. CINODs exploit the functional role of NO in gastric protection, leading to generation of anti-inflammatory drugs that are associated with less gastric toxicity than the

parent NSAID (Fig. 5), and with fewer erosions and a lower incidence of gastric and duodenal ulcers (110, 112). The mechanism of gastrointestinal protection is related to NO-mediated mucosal vasodilatation, which increases gastric mucosal blood flow and is also related to the inhibition of leukocyte adhesion in the gastric microcirculation (112).

b. CINODs exhibit enhanced anti-inflammatory activity (Fig.5)

NSAIDs exhibit anti-inflammatory action, but the mechanisms of enhanced anti-inflammatory activity of CINODs are probably related to the inhibition of caspase activity, thereby reducing cytokine formation (115). In contrast to conventional NSAIDs, as well as selective COX-2 inhibitors, CINODs inhibit the generation of T helper 1 (Th1)-type cytokines *in vitro* and interrupt Th1-type responses in animal models of inflammation (116). Caspases are a group of proteases implicated in apoptosis, but also in cytokine maturation and cell growth and differentiation (117). Among the caspases, those that most resemble caspase-1 are involved in mediating cytokine release and play a major role in inflammation, and those resembling members of the caspase-3 family are involved in apoptosis (117). Low levels of NO inactivate caspase 1 and 3, and thus the enhanced anti-inflammatory effects of CINODs are NO-dependent (110).

c. CINODs in pain and hyperalgesia (Fig.5)

It is well known that NSAIDs induce analgesia by inhibition of prostaglandin production. Based on previous reports, it might be expected that NO donor drugs would promote pain perception. On the contrary, however, NO donors acting at peripheral sites have been demonstrated to reduce pain perception, although the rationale for the enhanced anti-nociceptive activity of CINODSs is not clear (110).

d. CINODs have increased anti-thrombotic potency (106, 110), which is related to the effects of NSAIDs, as well as to a greater degree of inhibition of platelet function and vasodilatator

activity by NO that counteracts vasoconstriction caused by platelet-derived mediators such as TXA_2.

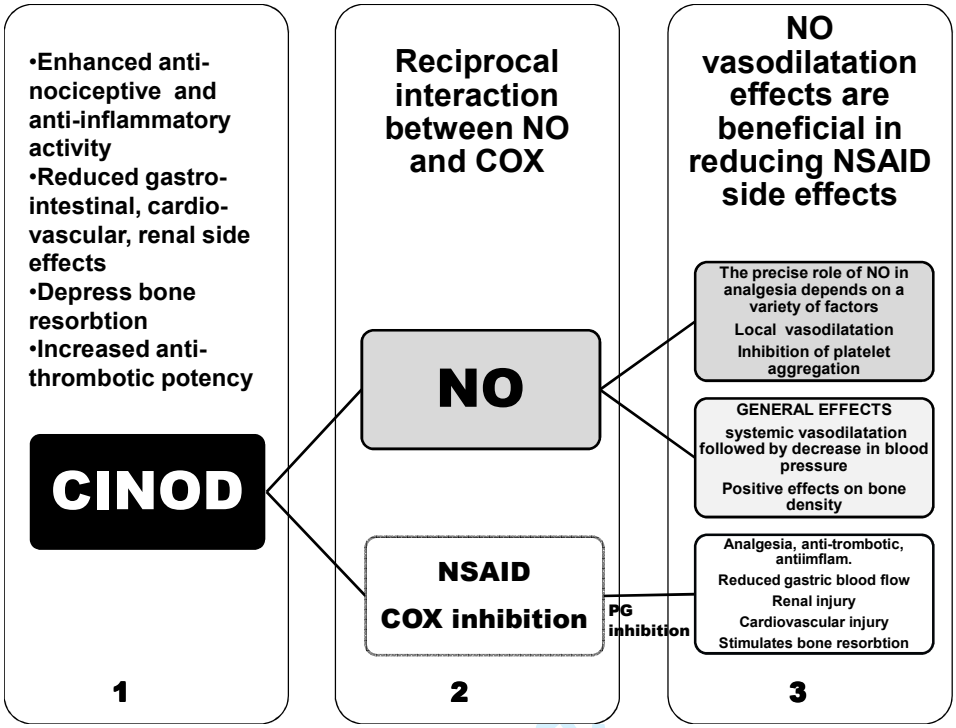

Figure 5. Cyclo-oxygenase inhibiting NO donating drugs (CINODs). *Column1: the effects of CINODs. Column 2: interaction between nitric oxide (NO) and COX (cyclo-oxygenase) pathway. NSAID (non-steroidal anti-inflammatory drugs) inhibit COX1 and COX2 enzymes, followed by prostaglandin (PG) inhibition. Column 3: NO and NSAID effects.*

9.1.2. NO-donating drugs (NODD)

NO-donating drugs (NODD) represent second-generation compounds with an ester-linked NO moiety, unrelated to NSAIDS. They have reduced adverse effects and have an improved profile of pharmacological activity in terms of enhanced therapeutic efficacy (106). Several compounds have been developed that fit these criteria, examples of which include nitroparacetamol (NO-paracetamol), nitroprednisolone (NO-prednisolone) and nitromesalamine (NO-mesalamine) (106). These drugs have not been developed for clinical use, and further research is necessary to investigate these compounds in humans.

NO-prednisolone has been shown to be more potent than prednisolone alone in a rat model of cholagen induced arthritis (118).

NO-paracetamol was found to be devoid of the hepatotoxic effects of co-administered paracetamol (119) and it is safe in mice with pre-existing liver disease. It even protects against the hepatotoxic effect of co-administered paracetamol (120). One of the first compounds developed was NCX-701, or nitroparacetamol. It has been shown to be effective in acute nociception as well as in neuropathic pain, situations in which paracetamol and other COX inhibitors have no effect. In addition, NCX-701 is more potent and, in some circumstances, more effective than its parent compound in different models of inflammatory pain (121). The augmented biological activity of this compound has been assumed to be due to the NO release from nitroparacetamol (122). The NO donating derivate of gabapentin alleviates neuropathic pain-like behaviour after spinal cord and peripheral nerve injury (123).

9.1.3. NO-donors (nitroglycerine, isosorbid dinitrate)

These are the classic nitric oxide donors which include *nitroglycerine (NTG)*, used for pain relief in coronary artery disease. The physiological effect of nitroglycerine as a potent vasodilator results after constitutive nitric oxide synthase (NOS) activation and synthesis of NO, which is responsible for vasodilatation (124). It seems worthwhile to mention the effects of transdermal nitroglycerine in analgesia, including enhancing the antinociception from spinal administration of either sufentanil following orthopaedic surgery (125) or neostigmine (126) in acute postoperative pain, and acting as coadjuvant in opiate therapy for the control of cancer pain (127). Impaired neuronal NO generation in diabetic rats induces hyperalgesia (128) and impaired endoneurial blood flow (129). Impaired NO generation may play a role in diabetic neuropathic pain as it was found that *isosorbide dinitrate spray (ISDN)*, a NO donor with local vasodilating properties, relieved pain in a small number of patients with diabetic neuropathic pain (130). It was speculated that alleviation of pain with ISDN spray follows NO-induced vasodilation, which improves microvascular blood flow and may induce angiogenesis of the vasa nervorum (130).

9.2. NO- inhibitors and pain

Chronic pain patients show a significant increase in plasma levels of NO in comparison with healthy individuals (131). Consequently, development of nitric oxide synthase (NOS) inhibitors could constitute future therapy for patients with chronic pain syndromes.

In animals there are well documented studies where NOS inhibitors suppress experimental nociception resulting from intraplantar formalin injection (132), suppress carrageenan-induced mechanical and thermal hyperalgesia (133) and inhibit NMDA hyperalgesia (134).

Inhibition of NO could be achieved by targeting the differential cofactors (tetrahydrobiopterin) (31, 32), or the differential substrate requirements for expressing various isoforms of NOS (L-arginine uptake blockers or arginase) (135). An important measure in treating different types of pain would be to achieve selective inhibition of NOS isoforms, particularly iNOS and nNOS; in other words, to develop pharmacological inhibitors with isoform specificity (106).

Finally, a combination of NOS inhibitors and COX-inhibitors should be mentioned. A significant decrease of neuropathic-like behaviour was demonstrated in animal models that received co-administered cyclooxygenase-2 (COX-2) inhibitors (meloxicam and rofecoxib) and an inducible nitric oxide synthase (iNOS) inhibitor (aminoguanidine hydrochloride) as compared with the groups treated with an iNOS inhibitor or COX-2 inhibitors alone (136). These results are controversial, because the effects of COX-2 inhibitors are not considered efficacious in neuropathic pain. It was previously reported that iNOS and COX-2 pathways interact closely and that iNOS stimulates COX-2 activity (103), and thus it is speculated that this may be why the combination of NOS inhibitors and COX-2 inhibitors was effective in the treatment of neuropathic pain (136).

As described before with other combinations of drugs that modulate NO levels (CINODs), the efficacy of the combination of iNOS and COX-2 inhibitors may be due to an added advantage, such as potentiated action over and above that of each of the compounds alone.

9.2.1. N^G-monomethyl-L-arginine hydrochlorides (L-NMMA) and **methylene blue (MB)** are the only inhibitors of NOS that have been thoroughly tested in humans. L-NMMA inhibits all three types of NOS (endothelial NOS, neuronal NOS, and inducible NOS) (137). L-NMMA is effective in the treatment of chronic tension-type headache (138, 139) and has also been tested in the treatment of neuropathic pain in humans (140). The mechanism responsible for headache has been studied using the NO-donor (glycerol trinitrate) model for experimental headache in humans (138). The L-NMMA inhibitor of NOS has an analgesic effect in different types of headache such as migraine and cluster-type headache (139).

9.2.2. Inhibitors of Soluble Guanylyl/Guanylate Cyclase

Methylene blue has direct inhibitory effects on nitric oxide synthases, both constitutive and inducible (141, 142), and blocks accumulation of cyclic guanosine monophosphate (cGMP) by inhibiting the enzyme guanylate cyclase (141). Data suggest that MB is a more specific and potent inhibitor of NOS than guanylyl cyclase, because direct NO-donating compounds in the presence of MB can still partially activate c-GMP signalling pathways (141, 142). It was recently demonstrated that MB reduces chronic discogenic low back pain (143) as well as the pain after lateral sphincterotomy (144). The underlying mechanisms of MB treatment in the above clinical pain conditions are not completely understood. However, MB is known to inhibit the formation of free oxygen radicals and superoxides (reactive oxygen species) by competing with molecular oxygen for the transfer of electrons by xanthine oxidase (145). MB acts as a neuroprotective agent after an experimental global ischemia-reperfusion incident in experimental animal models (146). Furthermore, a number of observational studies and case

reports have evaluated the use of MB in both the prevention and treatment of ifosfamide-induced neurotoxicity (147).

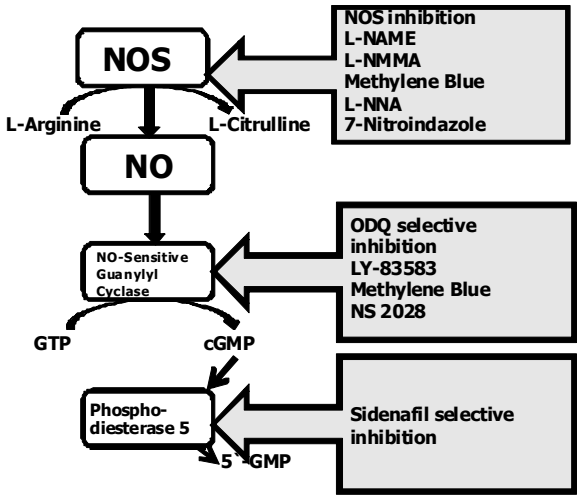

Figure 6. NO-cGMP pathway and inhibitors. ***The scheme shows the blockers used and their target enzymes. Nitric oxide synthase catalyses the formation of NO from L-arginine, NO activates the soluble guanylyl cyclase which in turn catalyses the formation of cyclic GMP from GTP. At the end of the cascade, the cGMP specific phosphodiesterase type 5 hydrolyses cGMP to 5`-cGMP. Inhibitors: L-NAME (NG-nitro-L-arginine methyl ester, a blocker of the nitric oxide synthase); ODQ, 1H-[1, 2, 4]oxadiazolo[4,3-a]quinoxalin-1-one, a selective blocker of NO-sensitive guanylyl cyclase; Sidenafil, a selective blocker of the cGMP specific phosphodiesterase type 5 (leads to an increase in cGMP level). NO (nitric oxide); GMP, guanosine monophosphate; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate.***

In conclusion, we believe that the inhibitors of the NO-cGMP pathway may be a tool for treatment of pain, and we hope that a specific therapeutic strategy will be developed in the near future.

10. Concluding remarks

This review has attempted to update the accumulated evidence concerning the mechanisms of NO action and the importance of NO in pain. The subject is controversial, in part because nitric oxide has an ambivalent character: it can have both harmful and beneficial effects in pain, with mechanisms that appear to contradict one another. Thus, the interpretation of data

requires caution and the beneficial and deleterious consequences of NO must be determined in order to attain effective therapeutic interventions in the treatment of pain. Also, the NO pathway interacts with other transmitter pathways as it is the reciprocal interaction between NO/COX pathways. If COX-inhibiting NO donating drugs and donating NO drugs are incorporated in the treatment of pain in humans, they will probably replace established drugs currently used in treating pain. An effective NO inhibitor is likely to provide a new tool for treating the pain associated with central sensitization. Unfortunately, only a few of these compounds have reached the stage of clinical pain trials.

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