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## Nitric oxide: emerging implications for headache mechanics

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**Abstract** The involvement of nitric oxide (NO) in the pathophysiology of primary headaches was suggested by several authors during the last decade. Migraine, cluster headache, tension headache, and cervicogenic headache have been extensively studied on the basis of NO donor headache pain. Different mechanisms seem to be involved in the generation of pain in these clearly different clinical head pain disorders. NO could control all the mechanisms leading to head pain. In migraine NO is correlated with endothelial activation, in cluster headache with a brainstem unravelling of the on/off regulatory clocks, in cervicogenic headache with a cytokine-dependent pain, and in tension-type headache with a sen-

sitization of pain pathways at the spinal/trigeminal level. The next natural frontier in the study of pain in primary headaches seems to be the functional study of the relationship between NO and the immune regulatory system.

**Key words** Nitric oxide • Migraine • Tension-type headache • Cervicogenic headache • Cluster headache

### What is nitric oxide and where does it come from?

Nitric oxide (NO) accounts for the activities originally ascribed to “endothelium-derived relaxing factor” first described by Palmer et al. in 1987 [1]. After its formation, NO diffuses into vascular smooth muscle where it activates soluble guanylate cyclase with the formation of cyclic GMP which, in turn, relaxes the muscle and dilates the blood vessels. Three genes encoding NO synthases (NOS, EC, 1.14.13.39) are expressed as enzymes in mammals [2] and a given monotypic cell population can express one, two, and perhaps all three of the isoforms.

Generally, but not invariably, expression of eNOS is constitutive, whereas expression of iNOS is inducible and independent of elevated intracellular  $Ca^{2+}$  [3]. However, it is now known that the level of gene expression of both eNOS and nNOS may also be induced under different physiological conditions (e.g., hemodynamic shear stress or nerve injury) and conversely that iNOS may function as a “constitutive” enzyme under physiological conditions in some cells [4].

Expression of iNOS protein requires transcriptional activation, which is mediated by a specific combination of cytokines. All three NOS use NADPH as an electron donor and employ five enzymes cofactors to catalyze a five-electron oxidation of arginine to NO with formation of citrulline.

NOS1 and NOS3 are responsible for NO production after both chemical stimulation, such as the neurotransmitter glutamate, and mechanical stimulation, such as pulsatile strain in an arterial wall. In contrast, NOS2 is engaged during inflammation and infection. NOS2 in mouse macrophages can produce NO for as long as 5 days when care is taken to replenish both the inductive stimuli and the L-arginine substrate [5, 6].

Differential tissue-specific splicing of nNOS mRNA generates structurally distinct protein molecules when the enzyme is expressed in neurones and skeletal muscle [7].

NO plays a major role as a non-adrenergic non-cholinergic (NANC) neurotransmitter in the autonomic nervous system. This NANC pathway plays an important role in producing relaxation of smooth muscle in the cerebral circulation. It has been demonstrated that the dysregulation of NOS activity in the autonomic nerves plays a major role in many pathophysiological conditions [8].

## NO and migraine

NO is an important mediator of vasodilatation in intra- and extra-cranial blood vessels and it is also an algogenic substance. This small and almost ubiquitous messenger molecule does not interact with specific receptors, but diffuses freely across membranes. It has been widely demonstrated that the intravenous injection of NO donors, such as glyceryl trinitrate (GTN), evokes a headache attack even in healthy subjects [9]. Moreover the *N*-acetylcysteine, which augments the effects of GTN in the heart by increasing the formation of NO or by enhancing the effects of NO itself, also augments the headache response to GTN and prolongs arterial dilatation of the superficial temporal artery, but not of the radial arteries [10].

NO acts as a neurotransmitter in the perivascular nerve and produces smooth muscle relaxation via increased production of cyclic GMP [11]. This vasodilator nerve function is impaired and physiologically antagonized by sumatriptan at the postjunctional site. Finally the dilatation of the middle cerebral artery induced by GTN was significantly greater in migraine patients than in normal controls [12].

Cortical spreading depression (CSD), which is presumed to underlie the migraine aura, induces a multiphase release of NO, characterized by an initial peak or a slow, smaller amplitude second peak when assayed by an NO-selective microelectrode [13].

A new experimental study reports that a potential novel anti-migraine agent, the SB-220453, inhibits NO release, following induction of CSD in anesthetized cats [14].

In the past NO donors have been largely used in a reliable trigger test to reproduce the migraine attack [15].

Furthermore, we have shown that pain crises induced in migraine patients by administering the NO donor were able to induce strong NO production, as detected by accumulation of NO-oxidized derivatives [16]. Therefore, according to Christiansen et al. [17], NO (exogenous and endogenous) may work as the switch molecule in both forms of migraine, being responsible for: (1) activation of cytoplasmic guanylate cyclase, (2) increase in cyclic GMP, (3) decrease in intracellular  $CA^{2+}$ , (4) vasodilatation of cerebral arteries, (5) peripheral or central sensitization, (6) direct cytotoxic effect. In addition, exogenous NO enhances endogenous NO release during CSD in an animal model [18], which may mimic the role played by NO during CSD in migraine with aura.

We have reported that during the pain crises induced in patients with migraine without aura by administering a NO donor, the monocyte expression of intercellular adhesion molecule 1 (ICAM-1) and its soluble isoform (s-ICAM), as well as serum level of interleukin-4 (IL-4), were reduced [19]. These values were also significantly lower during spontaneous migraine attacks. A possible explanation of these results is that NO may create a *disequilibrium* within Th1/Th2 type cytokines, with a consequent inhibition of IL-4 synthesis, that in turn may downregulate ICAM-1 expression and s-ICAM1. Therefore a complete picture of neuroimmunological events that occur at a cerebral vascular level during migraine attack will be provided by the ongoing studies of Th1/Th2 activation and related cytokine production. NO produced during migraine attack can in turn directly and/or indirectly contribute to systemic reactions, due to its ability to increase the release of powerful cytokines, including tumor necrosis factor (TNF) and interferon. These cytokines may be able to enhance the spread of nociceptive pain impulses [20]. Thus, NO can be considered not only as a potent algogenic molecule that works at the endothelial or CNS level, but also as an immunomodulating agent. Moreover, the administration of the NO synthesis inhibitor 546C88 significantly reduced both migraine pain and associated autonomic symptoms [21].

Several studies have also been performed on platelet function in migraine patients. The observation of an increase in *in vitro* agonist-induced aggregability and *in vivo* formation of platelet microaggregates supports the hypothesis that platelet hyperactivity is involved in this pathological condition [22, 23]. Platelets, when stimulated by collagen, produce NO, using L-arginine as substrate. It has been reported that in migraine without aura patients, an increased activity of the L-arginine/NO pathway is present, with subsequent increased production of NO and cyclic GMP [24].

Even during the ovarian cycle, changes in the activity of the L-arginine/NO pathway in collagen-stimulated platelets have been reported [25]. The modification in NO production in platelets could reflect a more-generalized increase in NO

synthesis [26], which could be involved in other pathophysiological alterations observed in migraine, such as regional cerebral blood flow changes [27].

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## NO and cluster headache

Cluster headache (CH) is a well-defined disorder in which patients suffer extremely painful headaches with clock-like regularity one to three times, or even more, a day for perhaps several months, followed by a period of remission. CH is considered a result of a hypothalamic derangement, with a peripheral neurovascular implication [28–32]. The weak changes in the cerebrovascular blood flow during the CH attacks do not justify a peripheral-independent pain. However there is strong evidence supporting a central hypothalamic mechanism (the periodicity of the attacks and the great autonomic involvement, which is often more severe on the painful side, suggest that the hypothalamus could be at the route of this disorder). Recently May et al. [28] reported a hypothalamic activation during a NO donor (nitroglycerin) induced CH attack. Since NO is able to activate the brainstem and the hypothalamic nuclei in rats [33], one could speculate that it may be directly involved in the elaboration of nociceptive signals in CH.

NO inducible routes could also be modulated by an immunology pathway through cytokines, as has been reported for IL-1 [34, 35]. At present, there is no evidence of constitutive NOS involvement in CH. We have recently demonstrated [36] that during cluster headache attacks there was an activation of the Th1 lymphocyte subset with a subsequent increase of serum Th1-type cytokines (interferon- $\gamma$  and IL-2). Although there is no evidence in CH of damage to the blood-brain barrier (BBB) caused by activated white cells, this phenomenon has been repeatedly observed in rodents [37, 38] and it represents a well-recognized mechanism of BBB damage in basic neuroscience [39]. Nevertheless, at present a role for this in CH is hypothetical.

It has been demonstrated in humans that these cytokines can activate central neurones, directly through an increase of vascular permeability and diapedesis of the white cells, or indirectly through an increase in the production of IL-1 at a hypothalamic neuronal level [40]. However, in spite of evidence of peripheral proinflammatory cytokine increases, the role in the pain phase during CH attacks remains unclear. NO interacts, at a ganglionic level, with substance P through a positive feedback [41]. The looping effect played by NO, which occurs during “inflammatory” conditions [42, 43], should be stressed.

Furthermore, it has recently been demonstrated that the blockade of the synthesis of NO by a NO synthesis inhibitor can abort acute CH attacks [44].

These data indicate that NO could represent an additional, important pain mediator in CH.

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## NO and cervicogenic headache

Cervicogenic headache (CEH) is a common and still controversial headache disorder characterized by unilateral head pain arising from the neck [45] at C2/C3 dermatome level. CEH pain radiates to the occipital area, to the vertex of the head (C2 level), to the peri-oculo-frontal-auricular-temporal area. Sometimes even the mandibular and neck region (C3 level) are involved [46]. The pain is often continuous, non-pulsating, and non-burning, with some radicular characteristics.

No clear evidence of inflammation has yet been demonstrated in CEH, although the administration of epidural corticosteroid seems to have a short-term clinical effectiveness [47, 48]. We recently observed increased levels of serum IL-1 $\beta$  and TNF- $\alpha$  in CEH both during periods of spontaneous fluctuating basal pain and during mechanically induced attacks [49].

The nature of the pain in CEH has not yet been defined. However, the enhanced production of cytokines could represent a specific signal from the immune system, resulting in the subsequent activation of the well-known links between immune-peptides and neuro-peptides, such as substance P and calcitonin-gene-related peptide (CGRP) [50]. Furthermore, we showed a more-pronounced activation of the NO pathway than that reported in migraine or in CH [43]. Additionally no difference in NO release was found between a spontaneous CEH attack and CEH pain elicited by administration of NO donor [43].

In the pathogenesis of other headache disorders, the activation of the NO-ergic vascular endothelial system is widely accepted [51]. The cerebral blood flow velocity has been shown to be unchanged during the CEH pain phase [52]. Therefore, the upregulated NO-ergic system that occurs during the CEH attack cannot be accounted for by a cerebrovascular dysfunction. In view of the incomplete knowledge of this clinical head pain syndrome, the peripheral immune cells could represent a preferential target for the large amount of NO, being responsible for the release of proinflammatory cytokines (IL-1 $\beta$  and TNF- $\alpha$ ) [53] at neck algogenic structures, such as nerve endings, osteotendinous structures, etc.

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## NO and tension-type headache

The tension-type headache has an immense social impact: it has a lifetime prevalence of up to 30% in the general population [54]. The clinical symptoms consist of aching or sen-

sations of tightness, pressure, or constriction, which vary widely in intensity, frequency, and duration. Increased pericranial muscle tension and tenderness may contribute to the development of tension headache [55], at least in some patients, but the pathogenesis has not been fully established.

However the clinical response to GTN infusion in patients with chronic tension headache seems to be similar to that in migraine (biphasic pain response), so that NO-related central sensitization has been claimed to be important in both migraine and tension-type headache [56]. As reported by Ashina et al. [56], a direct effect of NO on perivascular sensory afferents and/or NO-induced arterial dilatation are responsible for the immediate headache. The NO-induced enhancement of central sensitization at the spinal/trigeminal level is responsible for the delayed headache.

Moreover, in these patients increased muscle hardness and increased myofascial tenderness are present, and a decrease in muscle hardness, perhaps caused by a reduction of central sensitization, has been detected after treatment with L-NMMA [57]. This hypothesis of NOS inhibition as alternative analgesic therapy in patients with chronic tension-type headache has been recently confirmed [58].

### Why NO may cause pain in primary headaches?

Although it has been clearly demonstrated that NO could be involved in the pathogenesis of several types of headaches, we do not yet have a clear and definitive explanation of why NO may cause pain in migraine. One hypothesis is based on the vasodilator effect induced by NO; another is the sensitization of perivascular sensory nerves and, perhaps, a toxic effect of these nerves. The latter is based on NO reaction with  $O_2^-$  leading to the local synthesis of highly toxic compounds, such as peroxynitrite (ONOO<sup>-</sup>) and hydroxyl radicals (OH) [59].

It has also been postulated that NO-induced headache could be a direct action on central nociceptive pathways mediated via the *N*-methyl-D-aspartate receptor. Probably a second messenger is involved in the algogenic effect played

by NO. All the known models that readily produce vascular headache and/or migraine seem to stimulate the production of cyclic GMP. NO is a potent inducer of guanylate cyclase and has been demonstrated that the intravenously infusion of cyclic GMP induces headache. Certainly NO causes pain, as demonstrated by the analgesic effects of many pharmacological compounds inhibiting NOS activity, but further studies are necessary to better understand the biochemical basis of NO-induced pain [60].

### Conclusions

In conclusion, the proposed pivotal role of NO seems to be different in the various forms of primary headache. Migraine pain may originate at the endothelial level via NOS activation [61–65] in connection with activation of the cyclooxygenase pathway [66]. CH may involve, among other mechanisms, NOS activation in the brainstem and at the hypothalamic nuclei level [28, 32], with a peripheral NO derangement [67, 68]. CEH pain may be the result of a direct pain-producing activity originating from NO itself and proinflammatory cytokines [43]. Tension headache may be the result of a NO-mediated hypersensitization of second-order neurons due to prolonged nociceptive input from myofascial tissue [57].

Future study of NO in primary headaches should focus on the relationship between the immune compartment and NO itself, as hypothesized for natural killer cells [69] and more recently re-proposed for activated leukocytes [19, 70].

However, the role of the immune system in migraine and in other primary headache, although very intriguing, still remains to be fully clarified.

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