The role of nitric oxide in vascular headache

• Paul-Hugo M. van der Kuy and Joseph J.H.M. Lohman

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P.-H. M .van der Kuy (correspondence)**:** Department of Clinical Pharmacy and Toxicology, Maastricht Academic Hospital, Maastricht, The Netherlands
(e-mail: hkuy@kfls.azm.nl)
J. J.H.M. Lohman: Department of Clinical Pharmacy, Clinical Pharmacology and Toxicology, Maasland Hospital, Sittard, The Netherlands

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Abstract
Shortly after the invention of nitroglycerin (NTG), it was noticed that this substance is capable of inducing a violent headache. Only recently, it became known that this was due to the release of nitric oxide (NO) by NTG. As the molecular mechanism of migraine pain remains to be determined, NTG, being pro-drug for NO, has been used to study the aetiology and pathophysiology of migraine. Such studies with NTG- and also histamine-induced headaches, have led to propose that NO may be the causative molecule in migraine pain. The evidence supporting the role of NO in migraine is discussed, e.g. substances capable of inducing experimental vascular headache do so with NO as the common mediator, while drugs with antimigraine activity inhibit NO and the cascade of intracellular reactions triggered by NO. The importance of NO as a potential initiator of the migraine attack opens new directions for the pharmacological treatment of migraine and other vascular headaches.

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Nitroglycerin in historical perspective

Ascanio Sobrero (1812–1888) achieved the nitration of glycerol using a mixture of nitric and sulphuric acids: nitroglycerin (NTG) was synthesized[1]. In February 1847, Sobrero gave a famous lecture for the Academia delle Scienze di Torino, where he demonstrated his discovery by detonating a small amount of material. For reasons that are not recorded, he had tasted the compound and found that it was sweet, pungent and aromatic; but … "great precaution should be used, for a very minute quantity put upon the tongue produces a violent headache for several hours"[2].

Knowing of Sobrero's reports of headache, Hering, a homeopath, promptly repeated the observations in healthy volunteers, publishing his findings in 1849, writing: "… there is nothing known which in such small quantities and with such precision causes headache. Every substance with certainty of effect, ought also be considered as important to the physician"[3].

In 1858, NTG was first used successfully as a therapeutic substance in a 68-year-old woman with intense chest pain[4], now currently referred to as angina pectoris. Nevertheless, Brunton reported in 1876 that NTG gave him "such an awful headache" that he hesitated giving it to patients[5].

In the 20th century, workers in the explosives industry unwittingly "performed" experiments that gave a fascinating insight into the effects of inhaled nitrites. Severe headaches, dizziness and postural weakness were common occurrences during the first days of handling NTG[6]. However, tolerance to these symptoms then develops and workers can cope with their duties more easily. This tolerance is short-lived, so that workers who were not exposed to NTG over the weekend, suffered from a recurrence of malaise upon returning to work: Monday disease [6].

In order to overcome Monday disease, it became common practice for men to carry small pieces of NTG home, rubbing it on their skin or wearing clothes impregnated with the material over rest periods as a "prophylaxis against unpleasant reactions when they returned to work"[7].

Endogenous nitric oxide systhesis

As NO cannot be stored, released or inactivated by conventional regulatory mechanisms, biosynthetic regulation is more important for NO than for other mediators. Endogenous nitric oxide is synthesised on demand by NO synthase (NOS). Three different isoforms of NOS have been demonstrated in the brain [8]:

– *Neuronal NOS* (nNOS) has been localised in a limited percentage (1–2%) of the total neuronal population.
– *Endothelial NOS* (eNOS) in the brain is located in endothelial cells in the vasculature and in a small population of neurons, including the hippocampus and dentate gyrus.
– *Inducible NOS* (iNOS) is not expressed in the healthy brain. However, it has been observed in microglia and astrocytes following several pathologic insults.

The regulation of the NO synthase is not fully understood. It is clear that iNOS can be induced by stimulation with cytokines or endotoxins and that eNOS and nNOS are stimulated by an increase in intracellular calcium[9]. Stimulation of several specific membrane-bound receptors, by for example, glutamate (NMDA-receptor (N-methyl-D-asparate)), bradykinin (B_2-kinin receptor), 5-HT (5-HT_{2c} receptor), acetylcholine (muscarinic M_1 or M_3 receptor), histamine (H_1 receptor), endothelin-1 (ET_{1b} receptor) and substance P increases eNOS and nNOS activity[10].

Pharmacological effects

NTG may be regarded as a pro-drug for nitric oxide, since its biological effects are due to the formation of NO[11]. NTG is a lipid soluble molecule and hence easily crosses biological membranes, including the blood-brain barrier. Nitric oxide itself cannot be used experi-

mentally because of its rapid inactivation. Therefore, NTG has been used instead to study the effects of NO.

The mechanism underlying relaxation of vascular smooth muscles induced by NTG has been studied for the most part *in vitro* on large peripheral vessels such as the aorta, pulmonary artery and vein. The classical view is that nitrates act directly on vascular smooth muscles to generate NO, either spontaneously, or through interactions with tissue components. NO then activates soluble guanylate cyclase and thus increases cGMP and cGMP-dependent protein kinase with resultant smooth muscle relaxation[12].

An indirect mechanism underlying NTG-mediated vasodilatation was observed in the cerebral microcirculation[13]. The organic nitrate acts on sensory fibres that innervate pial vessels to release calcitonin gene-related peptide (CGRP). CGRP is a 37 amino acid-containing peptide and is one of the most potent vasodilating substances. Released CGRP then diffuses to the vascular smooth muscles, where it activates soluble guanylate cyclase to cause vasodilatation. A competitive receptor antagonist of CGRP inhibited the NTG-induced vasodilating effect upon pial arterioles[13].

The NO hypothesis of migraine and other vascular headaches

The molecular mechanisms of migraine pain remain to be determined. Several substances, including substance P, CGRP, neuropeptide Y, other vasoactive polypeptides and 5-hydroxytryptamine (5-HT) have been proposed as causative molecules in migraine pain. However, none of these can satisfactorily explain the headache pain in migraine [14]. Olesen et al. have proposed nitric oxide as a candidate for the causative molecule in migraine and they presented the following arguments to support their hypothesis[14]:

1. Activation of the NO-cGMP pathway causes migraine attacks in migraineurs, cluster headache attacks in cluster headache sufferers during cluster periods and non-specific vascular headaches in others.
2. Drugs that are effective in the treatment of migraine and other vascular headaches, and which are not general analgesics, exert their activity by inhibiting one or more steps in the NO-cGMP pathway or by antagonising the effects of products of this pathway.
3. Substances that can cause an attack of migraine or an attack of other vascular headaches do so by stimulating one or more steps in the NO-cGMP pathway, or by exerting effects that are agonistic to those of one or more steps in this pathway.

Why other hypotheses failed

Migraine attacks are associated with intra- and extracranial arterial dilatation. The site of the observation of the pain (nociception) is believed to be the perivascular space, where sensory nerve endings are stimulated. The nature of this stimulus is still to be unravelled, but a leading hypothesis involves the liberation of neuropeptides from trigeminal nerve endings. Most likely candidates for this role are substance P and CGRP to set up a state of inflammation. However, neurogenic inflammation has not yet been identified in cranial blood vessels during a migraine attack and no change in the concentration of substance P was observed in external or internal jugular venous blood[15]. The concentrations of both neuropeptide Y and vasoactive polypeptides (VIP), which are located in sympathetic and parasympathetic nerve fibres supplying the intra- and extracranial blood vessels, also remain unchanged during a migraine attack. Only one peptide is known to be released during migraine: CGRP. However, CGRP is only increased in the external and not in the internal jugular venous blood[15] and CGRP neither causes nor potentiates pain when it is infused intravenously or injected into the superficial temporal muscle[16].

Migraine patients have a systemic disturbance of 5-HT metabolism, associated with low 5-HT plasma levels between attacks and increased levels and release of platelet 5-HT during attacks[17]. Contrary to earlier belief, the ictal rise of plasma 5-HT is not the cause of migraine, but probably represents a self-defence mechanism. When 5-HT is injected intravenously or locally into the superficial temporal muscle it does not cause pain[18]. On the contrary, injection of 5-HT can abort migraine attacks, but at the cost of significant side effects.

Olesen et al. concluded that none of the above-mentioned peptides or monoamines were likely to cause the nociception responsible for migraine pain. Only NO remains as a likely candidate[14].

The experimental headache: nitroglycerin

According to clinical experience, migraine attacks may be provoked by many agents, but during controlled conditions these agents often fail to induce migraine. The property of NTG to induce headache is reproducible. Other positive properties of NTG are its safety, tolerability and controllability (due to its short half-life)[19]. The NTG-model of migraine can be studied in both healthy subjects and migraine patients.

Healthy subjects

The headache induced by NTG in healthy subjects with no history of migraine is dose dependent and shows a low day-to-day variability in both headache intensity and characteristics[19]. Nine out of ten healthy volunteers experienced headache during a NTG infusion on two different occasions. The non-responding subject was the same on both days. The intravenous infusions were given for 10 minutes followed by a washout period of 10–30 min. Doses increased from 0.25 $\mu g.kg^{-1}\cdot min^{-1}$ to 2.0 $\mu g\cdot kg^{-1}\cdot min^{-1}$. Maximal headache scores occurred within two to five minutes and declined rapidly after termination of the NTG infusion (see Figure 1). In all subjects the headache was bilateral. The headache was pulsating in seven and pressing in two subjects. At 0.5 $\mu g.kg^{-1}\cdot min^{-1}$ a reproducible ceiling effect in headache score was seen[19]. This maximal effect was not due to the development of tolerance, as headache intensity showed no attenuation during 7 h of infusion of NTG[20]. It can be concluded that in non-migraineurs the headache has some of the features of a migraine attack, but differs by being milder and without nausea, photo- and phonophobia.

Migraine patients

Migraineurs experience a headache response to NTG

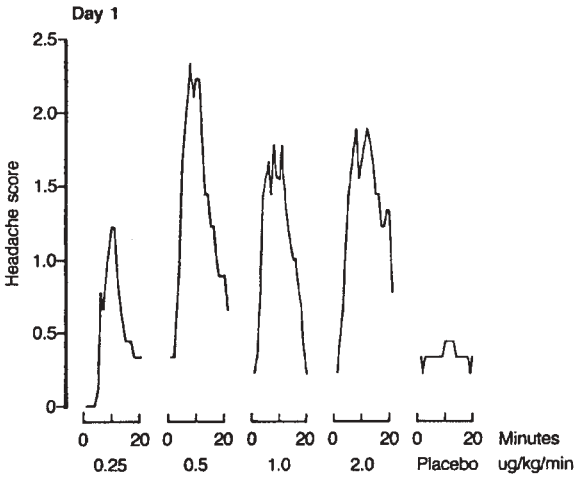

Figure 1 *Mean headache scores (0–10 scale) during and after four doses of intravenous nitroglycerin in normal headache free subjects on day 2 of two separate study days. Nitroglycerin was infused during 10 min and during this period a rapid increase in headache was seen. This was followed by a 10-min wash out period, which resulted in a rapid decrease in headache. There was a relatively low day-to-day variation and a ceiling effect at approximately 0.5 μg.kg^{-1}.min^{-1}. Reproduced with permission from Iversen et al.[19] (© Elsevier Science).*

infusion that differs from healthy subjects (see Figure 2)[21].

The intensity of the headache during infusion was more severe in migraineurs than in both healthy controls and in tension-type headache patients[22]. This could reflect a greater general sensitivity to pain or it could be due to an increased sensitivity to NO.

At the end of the infusion, the headache disappeared rapidly in healthy control subjects. The migraine sufferers experienced either no relief of headache or an initial relief followed by a subsequent worsening[22].

In another study, the post-infusion migraine attack in migraineurs was very similar to spontaneous attacks in the same subjects[23]. Accompanying symptoms, such as vomiting, nausea and photo- or phonophobia, were absent during the infusion, but were present during the headache after the NTG infusion.

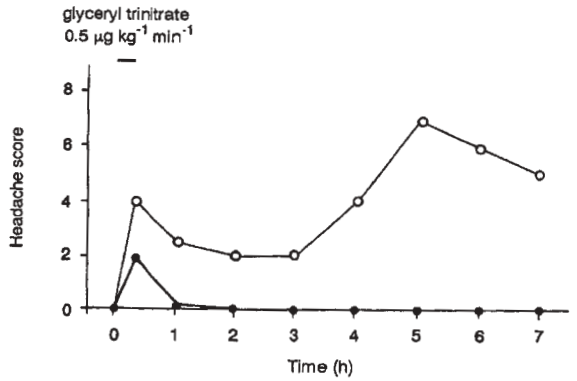

Figure 2 *Comparison of the mean headache scores (0–10 scale) in response to nitroglycerin (0.5 μg·kg^{1}·min^{-1} for 20 min) in migraineurs (open circles) and non-migraineurs (closed circles). Reproduced with permission from Olesen et al.[21] (© Elsevier Science).*

Eight of ten patients developed a regular migraine attack fulfilling IHS-criteria, with a headache peaking 5.5 h after NTG infusion[23].

Pre-treatment of migraine patients with the histamine H_1-blocker mepyramine failed to prevent or even reduce both immediate NTG-induced headache and the delayed NTG-induced migraine[24].

Sumatriptan reduced both the NTG-induced immediate headache and the delayed migraine. Parallel with changes in headache, an effect was seen on the dilatation of the temporal artery[25]. The relation between headache and arterial dilatation is, however, not clear-cut. Not all vasodilatators cause headache. For example CGRP and papaverine, both acting independently of the NO-system, do not induce headache[26]. It remains to be elucidated whether sumatriptan interacts with the NO-system or whether it works via other mechanisms. A recent experimental study suggests that sumatriptan possesses inhibitory actions on NO synthase in guinea pig cerebral vessels[27].

N-acetylcysteine is known to augment NTG responses in the heart, either by enhancing the effect to NO itself, or by increasing the formation of NO. N-acetylcysteine also appears to augment the headache response to NTG[28].

NTG not only induces migraine attacks in migraineurs, but also generates cluster headaches in patients during a cluster period[29–31]. The cluster headache occurs with a latency of only 30–50 min after administration.

The observation that migraine attacks develop several hours after an NO challenge suggests that NO may be involved in the early phase of spontaneous migraine attacks.

The experimental headache: histamine

A second, less extensively studied, model to study migraine is called the histamine-model. A headache response is obtained by continuous infusion of various amounts of histamine (0.16, 0.33 and 0.66 μg·kg^{1}·min^{-1}). An immediate headache response was seen in both groups of patients with tension-type headache and with migraine, but the headache was more severe and more pulsating in the latter group[32]. No headache was reported by healthy volunteers; only a mild pressing sensation in some subjects. Interestingly, headache greatly diminished within two minutes after administration of the H_1-blocker mepyramine. If cimetidine was administered only a slight decrease in headache score was seen[32].

Comparable to the NTG-model, migraine patients develop a delayed migraine attack approximately 5 h after histamine infusion[33]. Pre-treatment with mepyramine (0.5 mg·kg^{-1}) prevented the occurrence of delayed migraine attacks in all migraine patients[33].

As the headaches induced by NTG and histamine are virtually identical, each with an immediate headache during infusion followed by a recurrence of the headache several hours later, resembling a typical migraine attack, it is very likely that both models have a common mediator. Nitric oxide was suggested by Olesen et al. as this common mediator[14].

Naturally occurring headaches

Apart from migraine, other headaches may partly share the same mechanism leading to pain.

Meningitis and encephalitis, which cause severe headache, increase the formation of cytokines. Cytokines are known to stimulate macrophage inducible NO synthase and, consequently, the production of NO[34].

Hypoxia causes headache as well, which is illustrated by high-altitude headache. It has been shown that persons living at high altitude showed a much larger prevalence of migraine[35]. Hypoxia enhances NO in the blood vessels of several species[36,37]. Hypoxic vascular headache and hypoxia induced-migraine therefore may involve the spontaneous production of increasing NO concentration with prolonged action. Hyperoxia causes a more rapid inactivation of NO and thereby shortens and diminishes its effect[38]. Pure oxygen has been used successfully in the treatment of cluster headache[39]. The therapeutic effect of oxygen has been studied less extensively in migraine. The results of these studies will be discussed below.

Vascular headaches are also seen after cerebrovascular ischemia. During platelet aggregation and thrombus formation a number of substances are released, such as 5-HT, ADP, ATP, platelet activating factor (PAF), thrombin and prostacyclin. Several of these compounds are known to stimulate the formation of NO[40]. Prostacyclin has even been shown to cause vascular headache in migraine patients[41], which might be explained by its action on an endothelial receptor-mediated liberation of NO[38].

Compounds evoking migraine

Apart from NTG and histamine, other compounds are also reported for their migraine-initiating power. Administration of meta-chlorphenylpiperazine (mCPP) appeared to induce a migraine-like headache. Unfortunately, mCPP was studied in only two clinical trials. The first trial was not even designed to study the headache pattern[42]. In a second, double blind trial headache was reported only by 50% of the migraine patients and by 40% of the normal control subjects[43]. However, a smaller dose of mCPP was used. At the molecular level, mCPP interacts with the 5-HT_{2c} receptor, which in some tissues is coupled to activation of NO-synthase and consequently the formation of NO[44].

Reserpine has been given intramuscularly or intravenously to induce headache. The development of the migraine-like headache seems roughly to parallel the depletion of serotonin, as caused by the reserpine, with its lowest value attained 5–7 hours after administration [45]. Serotonin acts on the 5-HT_{2c}-receptor, which is thought to lead to activation of NO synthase and, consequently, the formation of NO [44].

Compounds counteracting migraine associated to NO

Sumatriptan, worldwide the most used migraine-specific compound to treat acute migraine attacks, is also effective in attenuating both immediate NTG induced headache and the delayed NTG induced migraine[46]. It is believed that the main action of triptans in migraine is to constrict dilated cranial extracerebral blood vessels via 5-HT_{1B} receptors[47]. However, the mechanism of action of sumatriptan in migraine has not yet been completely established. NO antagonism may turn out to be an important effect[14].

Calcium entry blockers have a prophylactic effect in migraine. They block voltage-dependent Ca^{2+} channels, thereby reducing the concentration of free cytosolic calcium. As constitutive NOS is calcium dependent, this may lead to a decreased activity of NOS[14].

Pizotifen and methysergide, both used in the prevention of migraine attacks, are 5-HT_2 antagonists which do not discriminate between 5-HT_{2b} and 5-HT_{2c}-receptors. Stimulation of the latter receptor liberates endothelial NO[48]. Therefore, blockade of this receptor by pizotifen or methysergide reduces endothelial NO production, which may lead to therapeutic activity of these substances.

Some β-antagonists, such as propranolol, are effective in the prevention of migraine, but others appear to be ineffective in migraine prevention[48]. If the prophylactic effect was due to blockade of the β-receptor stimulated NO production, a similar therapeutic effect was expected for the whole group of β -antagonists. Propranolol also antagonises endothelial 5-HT_{2c} receptors[49], which, as discussed above, leads to reduction of endothelial NO production. Pindolol, which has been shown to be ineffective in migraine, lacks affinity for the 5-HT_{2c} receptor.

Oxygen therapy, which is widely accepted for the treatment of cluster headache, has also been studied in migraine. In a randomised controlled trial hyperbaric treatment (100% oxygen, pressure) reduced subjective migraine pain more than in the control group (100% oxygen, no pressure)[50]. In 1940 a series of 97 patients with headaches who were treated with undiluted oxygen (6–8 l/min) was reported. In patients with classical migraine 42% obtained complete relief and 44% obtained partial relief of symptoms. In the group of patients with headache other than migraine these percentages were respectively 16% and 24%[51].

Recently, it was concluded from a questionnaire covering 400 patients on anticoagulant therapy that acenocoumarol improved migraine in 63% of the patients versus 38% in patients with non-migrainous headache. Coumarin derivatives suppress enhanced NO and could therefore be a useful pharmacological tool for therapeutic interventions in NO-mediated pathophysiological events, including migraine [52].

Indomethacin pretreatment markedly reduced NTG-induced Fos expression, a marker for neuronal activation in, among others, the nucleus trigeminalis caudalis and the locus coeruleus. Indomethacin is a well-known potent cyclo-oxygenase inhibitor. Its inhibitory effect upon neuronal activation suggests that NTG may directly activate the nociceptive neurons by means of prostaglandin-mediated mechanisms. As mentioned above, prostacyclin has been shown to cause vascular headache in migraine patients by liberation of NO[41].

The efficacy of a NO-synthase inhibitor was demonstrated for both migraine and chronic tension-type headache. In a double blind study design patients randomly received l-N^Gmethylarginine hydrochloride (546C88) or placebo intravenously during a spontaneous migraine attack. Two hours after infusion 10 of 15 actively treated patients experienced headache relief, compared to 2 of 14 placebo-treated patients. Symptoms such as photo- and phonophobia were also significantly reduced[53].

In a randomised double blind cross-over trial in 16 patients with chronic tension type headache, patients received L-NMMA (N(G)-monomethyl-L-arginine hydrochloride) or placebo intravenously. L-NMMA reduced pain intensity on a visual analogue scale significantly more than placebo; 2h after the start of the treatment, the mean pain score was decreased from 49 to 33 after active treatment and from 44 to 40 after placebo[54].

Chlorpromazine can be used for non-specific NOS inhibition[10]. From a recent trial it was concluded that chlorpromazine and sumatriptan were both effective for the relief of severe migraine[55]. In a randomised, prospective trial, patients in the emergency department were given metoclopramide 10 mg and normal saline. Randomised by date, they received either chlorpromazine 12.5 mg intravenously, repeated at 25 minutes and 45 minutes if needed (*n*=23), or sumatriptan 6 mg intramuscularly (*n*=20). Relief of pain, as judged by the patient, occurred in 19 of 20 patients treated with sumatriptan and in 22 of 23 patients treated with the chlorpromazine[55].

Apart from NO-synthase inhibitors, the effect of NO may also be counteracted by NO-scavengers. Hydroxocobalamin was shown to be a NO-scavenger[56]. In an open study, intranasal hydroxocobalamin was administered daily as a prophylactic agent in migraine patients during a period of three months following a baseline period. A reduction of 50% or more was seen in 53% of the patients and an overall reduction in migraine frequency of 42% was noted[57]. However, a double blind placebo-controlled trial is needed to confirm these results.

Conclusion

Almost 150 years after the first description of headache after ingestion of NTG, it becomes more likely that NO plays an important role in migraine and may even be the most likely candidate for the initiating molecule that triggers the cascade.

Since the first formulation of the NO hypothesis by Olesen[14], more data have become available which support the theory. With increasing knowledge of NO and its physiological and patho-physiological actions, tools for further elucidation of this hypothesis rapidly will become available.

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