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REVIEW

Headache-type adverse effects of NO donors: vasodilation and beyond

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Although nitrate therapy, used in the treatment of cardiovascular disorders, is frequently associated with side-effects, mainly headaches, the summaries of product characteristics of nitrate-containing medicines do not report detailed description of headaches and even do not highlight the possibility of nitrate-induced migraine. Two different types of nitrate-induced headaches have been described: (i) immediate headaches that develop within the first hour of the application, are mild or medium severity without characteristic symptoms for migraine, and ease spontaneously; and (ii) delayed, moderate or severe migraine-type headaches (occurring mainly in subjects with personal or family history of migraine), that develop 3–6 h after the intake of nitrates, with debilitating, long-lasting symptoms including nausea, vomiting, photo- and/or phono-phobia. These two types of headaches are remarkably different, not only in their timing and symptoms, but also in the persons who are at risk. Recent studies provide evidence that the two headache types are caused by different mechanisms: immediate headaches are connected to vasodilation caused by nitric oxide (NO) release, while migraines are triggered by other actions such as the release of calcitonin gene-related peptide or glutamate, or changes in ion channel function mediated by cyclic guanosine monophosphate or S-nitrosylation. Migraines usually need anti-attack medication, such as triptans, but these drugs are contraindicated in most medical conditions that are treated using nitrates. In conclusion, these data recommend the correction of summaries of nitrate product characteristics, and also suggest a need to develop new types of anti-migraine drugs, effective in migraine attacks, that could be used in patients with risk for angina pectoris.
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Abbreviations: 5-HT, serotonin; 5-ISMN, 5-isosorbide-mononitrate; ATP1A2, gene encoding the α_2 subunit of sodium–potassium pump ATPases; BK channels, calcium-activated potassium channels; CACNA1A, gene encoding the α_{1A} subunit of Cav2.1 (P/Q-type) voltage-gated neuronal calcium channels; CB1, cannabinoid receptor 1; cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; CNGCs, cyclic nucleotide gated channels; CSD, cortical spreading depression; EFNS, European Federation of Neurological Societies; FHM, familial hemiplegic migraine; GABA, gamma-amino-butyric acid; hKv1.5, ultrarapid delayed rectifier potassium channel; HVA, high voltage activated; IHS, International Headache Society; IKr, rapid component of delayed outward potassium current; IKs, slow component of delayed outward potassium current; IKur, ultrarapid delayed rectifier potassium current; Ito, transient outward current; K_{ATP} channel, ATP-dependent potassium channel; Kv3.1 and Kv3.2, neuronal voltage-gated potassium channels; Kv4.3, transient outward potassium channel; Kv7(KCNQ), Kv7 subfamily of voltage-gated potassium channels (also called KCNQ or M-channels); Na^+/K^+ ATPase, sodium/potassium ATPase; NO, nitric oxide; NOS, nitric oxide synthase; PKG, protein kinase G; SCNA1A, gene encoding the α_1 subunit of neuronal voltage-gated sodium (Nav1.1) channels; SmPC, summary of product characteristics; TTX, tetrodotoxin

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Nitrate compounds have been used in clinical practice for decades; their application for angina pectoris started in the 19th century. The first medicines included amyl nitrite (Brunton, 1867), glycerol trinitrate (nitroglycerin) (Ebright, 1914), erythritol-tetranitrate and penta-erythrol-tetranitrate (Weitzman, 1953). Other nitrates have also been introduced

Table 1 Different nitrate preparations and the frequency of headache

Medicine and its application	*The frequency of headache in SmPC (EMC, 2010; Pharmindex, 2008)*	*The frequency of headache in Micromedex database (Micromedex, Updated periodically)*
Nitroglycerin aerosol	Not properly specified, >10%	Any formulation: >60%
Nitroglycerin sublingual pill	Not properly specified, >10%	Migraine and cluster are mentioned
Nitroglycerin retard tablet	Not properly specified, >10%	
Nitroglycerin transdermal patch	very common >10%	
Isosorbid-mononitrate retard tablet	very common >10%	Between 25–40%
Isosorbid-dinitrate tablet	In the UK SmPC not specified Not marketed in Hungary.	Most frequent (>10%)
Sodium Nitroprusside	Not marketed in the UK and Hungary	Not specified
Pentaerythrol-tetranitrate tablet	Frequency is not specified. Not marketed in the UK	Frequency is not specified
Molsidomine tablet	Frequency is not specified. Not marketed in the UK	Between 10% and 25%

into medicine such as sodium nitroprusside for hypertension emergency (Page *et al.*, 1955), isosorbide dinitrate (Albert, 1961), isosorbide mononitrate (Abshagen and Sporl-Radun, 1981; Uberbacher *et al.*, 1983) and molsidomine (Jansen and Klepzig, 1976). Currently their most common indication is for angina pectoris but nitrates can also be used to treat hypertensive crisis and occasionally congestive heart failure (Bussmann and Kaltenbach, 1976; Dupuis, 1994; Elkayam *et al.*, 2008). The main basic mechanism of action of nitrates which is beneficial in the above mentioned pathological conditions is to reduce preload and afterload by vasodilation – vascular smooth muscle relaxation; thus nitrates decrease the strain on the heart and the cardiac oxygen demand. Nitrates are believed to exert their vasodilatory effect by releasing nitric oxide (NO), also known as endothelium-derived relaxing factor (Moncada *et al.*, 1988; Esplugues, 2002).

Unfortunately, adverse effects such as headaches, postural hypotension and reflex tachycardia are frequently associated with nitrate therapy, regardless of the means of administration – be it sublingual, intravenous, oral or even transdermal (Thadani and Ripley, 2007). As cardiovascular diseases are very common in the human population and many patients receive nitrates, side-effects may be encountered by a wide variety of physicians but predominantly by general practitioners, cardiologists and emergency physicians. Yet detailed descriptions of the side-effects still do not differentiate between a common headache (due to vasodilation) and migraine which is a headache syndrome with specific symptoms and time-course that makes it distinctive from a common headache. Even the most thorough reviews do not provide a detailed discussion of nitrate-induced migraine (Thadani and Rodgers, 2006; Thadani and Ripley, 2007). Surprisingly, the possibility of migraine is not noted in the summaries of product characteristics (SmPC) of nitrate-containing medicinal products either (Table 1.).

A headache often develops after the administration of any nitrate. Its incidence, according to clinical studies, varies between 20% and 82% (Thadani and Rodgers, 2006; Elkayam *et al.*, 2008). Approximately 10% of the exposed patients cannot tolerate the nitrate therapy because of unbearable headaches (Thadani and Rodgers, 2006), although it is unclear whether or not this intolerability is due to migraine. Usually the headache disappears 1–1.5 h after the administration of the nitrate and the symptoms do not resemble those of migraine without aura. However, other patients experience real migraine attacks several hours after taking the medicine (Bellantonio *et al.*, 1997; Juhasz *et al.*, 2004).

Our aim is to review the different types of nitrate-induced headaches and to discuss the possible mechanisms underlying these symptoms. We intend to point out that cardiovascular patients with headache syndrome, especially with migraine without aura, have a much greater risk of experiencing severe headache-type side-effects compared with non-migraineurs during nitrate therapy. We will discuss how to predict possible severe headache-type side-effects and consider treatments recommended by international guidelines.

The drug/molecular target nomenclature applied in the manuscript conforms to British Journal of Pharmacology's Guide to Receptors and Channels (Alexander *et al.*, 2008).

Headaches are not uniform

In order to differentiate correctly between the specific types of headaches, it is important to mention the second version of the International Classification of Headache Disorders (Headache Classification Committee of the International Headache Society, 2004) that provides guidance for diagnosing headaches. Table 2 summarizes the lifetime prevalence, the characteristics, the duration and the related subtypes of the different primary headache groups. Syndromes of primary headaches are those headaches that are not attributed to other medical disorders or conditions based on medical history, physical, neurological and other examinations. To assign a particular headache diagnosis the patient must, in most cases, experience a minimum number of attacks.

Tension-type headache is the most prevalent in the population (Stovner *et al.*, 2007) and if it is not frequent or chronic, medical consultation is rare (Goadsby, 2006b). It is often related to psychological stress or muscle tenderness and simple analgesics are the most effective class of drugs for acute treatment (Loder and Rizzoli, 2008).

Migraine is a common and disabling disorder. The main characteristics which separate migraine from the occasional tension-type headache are the associated features (Table 2.) that eventually lead to significant limitations in patients' lives (Goadsby, 2006b; Lipton and Bigal, 2007). Another important feature of migraine is its hereditary nature; several family clustering and twin studies suggest that genetic factors play an important role in the pathogenesis of migraine, with a heritability of around 50% (Wessman *et al.*, 2007). Migraine

Table 2 Summary of primary headaches based on the second version of the International Classification of Headache Disorders (Headache Classification Committee of the International Headache Society, 2004)

	Tension	*Migraine*	*Cluster*
Lifetime population prevalence	Male: 42% Female: 49% (Stovner *et al.*, 2007)	Male: 10% Female: 22% (Stovner *et al.*, 2007)	0.12%, more prevalent in males (Fischera *et al.*, 2008)
Headache characteristics	At least two of: 1. Bilateral 2. Pressing or tightening 3. Mild or moderate 4. NOT aggravated by routine physical activity	At least two of: 1. Unilateral 2. Pulsating 3. Moderate or severe 4. Aggravation by routine physical activity	1. Strictly unilateral (periorbital, temporal) 2. Severe or very severe
Associated symptoms	Photophobia or phonophobia can be present (only one of them)	At least one of: 1. Photophobia, phonophobia 2. Nausea, vomiting	At least one of ipsilateral: 1. Conjunctival injection, lacrimation 2. Nasal conjection or rhinorrhoea 3. Eyelid oedema 4. Forehead, facial sweating 5. Miosis, ptosis 6. Restlessness, agitation
Duration	30 min – 7 days, or may be continuous	4–72 h	15–180 min
Most frequent subtypes	Infrequent episodic tension-type headache Frequent episodic tension-type headache Chronic tension-type headache	Migraine without aura Migraine with aura	Cluster headache Other trigeminal autonomic cephalalgias

attacks can be triggered by alteration in stress level, diet, sleep pattern or hormonal status (Goadsby, 2006b). Before a migraine attack, about half of the patients suffer from premonitory symptoms such as feeling tired or weary, difficulty concentrating or food cravings (Silberstein, 2004). A minority of migraineurs (approximately 30%) experience aura that consists of reversible focal neurological symptoms – most frequently visual or sensory – which precede, accompany, or (rarely) follow an attack (Silberstein, 2004; Goadsby, 2007b). Based on surveys at least 50% of patients with migraine have never been diagnosed, or have received an inappropriate diagnosis, and nearly three-quarters of potential migraineurs were only self-medicated (Brandes, 2008).

Cluster headache attacks typically occur in series (cluster periods) lasting for weeks or months and interrupted by remission periods usually lasting months or years (Headache Classification Committee of the International Headache Society, 2004). Although it is a rare type of headache, it is important to include it in this review because nitrate therapy may induce or exacerbate cluster attacks (Ekbom *et al.*, 2004).

Characteristics of NO donor-induced headaches

As previously mentioned, the beneficial effects of nitrates relate to their NO-donating ability (Ignarro *et al.*, 2002) and it has been suggested that NO release is also responsible for nitrate-induced headaches (Iversen, 1995). Subsequently, nitrate models of vascular headache have been developed, initiating a more systematic approach to the study of nitrate-induced headaches (Magis *et al.*, 2007); a recent review (Olesen, 2008) summarizes the results of these studies in detail.

Healthy subjects

In healthy volunteers, glycerol trinitrate (GTN) infusion (max 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$ over 15–20 min) caused a reproducible and immediate headache that eased rapidly after termination of the infusion. This headache was mild or moderate with pulsating quality and aggravated by physical activity but without any accompanying symptoms (e.g. photo-and phonophobia, nausea and vomiting) (Iversen, 1995). In the first 30–60 min following sublingual administration of GTN (0.5–0.9 mg), approximately 30% of healthy subjects experienced a non-specific headache that did not fulfil the International Headache Society (IHS) criteria for migraine without aura, lasted only a few minutes and disappeared spontaneously (Juhasz *et al.*, 2003b; 2004; Sances *et al.*, 2004). A delayed, migraine-type headache developed in healthy subjects only if they had a predisposition for migraine (e.g. family history of migraine) (Juhasz *et al.*, 2003b; Afridi *et al.*, 2004; Sances *et al.*, 2004). However, extreme NO-donor exposure [5-isosorbide-mononitrate (5-ISMN) 30 mg three times daily] was able to provoke migraine even in healthy subjects without risk factors for migraine (Christiansen *et al.*, 2000b).

Tension-type headache patients

Only one study reported the effect of GTN infusion (max 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$ over 15–20 min) in episodic tension-type headache patients. Seven out of nine developed immediate headache with intensity and duration intermediate between those of migraineurs and controls (Olesen *et al.*, 1993). In chronic tension-type headache patients, GTN infusion induced immediate headache similarly to episodic tension-type headache patients (Ashina *et al.*, 2000). Furthermore, chronic tension-type headache patients experienced a delayed headache with similar characteristics to their usual headache. The intensity of this delayed pain reached its maximum about 8 h after the infusion and half of the patients had to use rescue medications (Ashina *et al.*, 2000).

Migraine patients

Migraine sufferers are the most systematically investigated population regarding NO donor-induced headaches and the

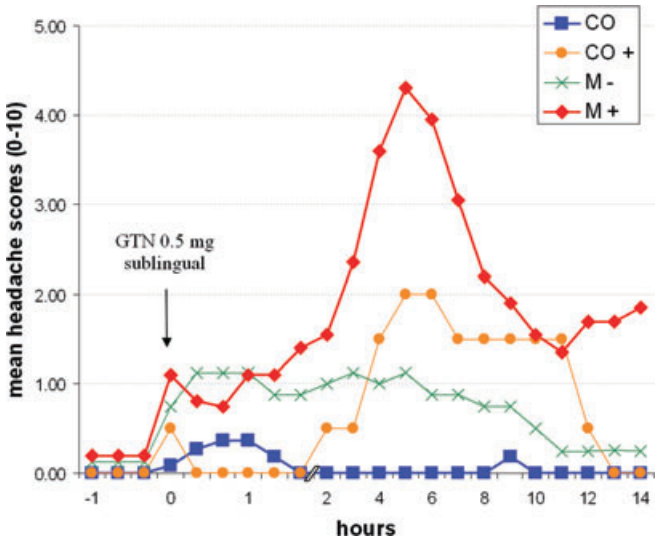

Figure 1 Headache intensity (0–10 verbal scale) after sublingual nitroglycerin (GTN, 0.5 mg) administration in controls (CO; $n = 11$), in controls with risk factor for migraine (CO+; $n = 2$), in migraineurs who did not develop delayed migraine type headache (M–; $n = 8$) and in migraineurs who developed typical delayed migraine without aura (M+; $n = 20$). (Combined data from Juhasz *et al.*, 2003b; 2005.)

main findings of these studies have been reviewed recently (Magis *et al.*, 2007). In this section we will focus on the characteristics of the induced headaches.

During GTN infusion (max 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$ over 15–20 min), migraineurs experienced stronger immediate headaches (some with additional migraine-characteristic symptoms), compared with controls or to tension-type headache patients. However, in most cases this immediate headache did not fulfil the criteria for migraine because accompanying symptoms were not present (Thomsen and Olesen, 1997). In contrast to the healthy controls, approximately 80% of the migraineurs reported delayed headaches 3–6 h after the infusion, which they labelled as a typical migraine attack. These headaches fulfilled the IHS criteria for migraine without aura, were moderate or severe, with similar accompanying symptoms to those reported during spontaneous migraine attacks (Thomsen and Olesen, 1997; Christiansen *et al.*, 2000a; Afridi *et al.*, 2004).

Sublingual GTN (0.5 mg) administration provoked similar headache patterns to the GTN infusion in migraineurs. Twice as much migraineurs (67%) than controls experienced an immediate headache that developed within the first hour (mean latency: 10.5 ± 2.8 min; mean duration 30.0 ± 7.7 min); but these headaches did not fulfil the IHS criteria for migraine without aura and eased spontaneously. However, a typical migraine attack without aura subsequently developed in 71% of migraine patients with a peak headache intensity about 5–7 h after the GTN intake, but not in the controls without risk factors for migraine (Figure 1) (Juhasz *et al.*, 2003b; 2004). Using sublingual GTN in a higher dose (0.9 mg) even more migraineurs developed an immediate headache (83% with frequent migraine-characteristic symptoms), and 78% developed a delayed migraine-type headache with a mean latency of 140 min (Sances *et al.*, 2004).

It is interesting to note that GTN provocation only rarely (0–14%) caused typical aura symptoms (Christiansen *et al.*, 1999; Bank, 2001; Sances *et al.*, 2004; Afridi *et al.*, 2005) and patients with familial hemiplegic migraine (FHM) did not develop a delayed migraine-type headache after GTN infusion (Hansen *et al.*, 2008a,b). FHM is a rare, severe, genetically heterogeneous autosomal dominant subtype of migraine with aura, characterized by at least some degree of weakness (hemiparesis) during the aura (Headache Classification Committee of the International Headache Society, 2004).

Cluster headache patients

Cluster attacks were induced by sublingual GTN only in the cluster headache patients whose disorder was in an active phase (i.e. in a cluster period, see paragraph about classification of headaches) (Fanciullacci *et al.*, 1997; Ekbom *et al.*, 2004). Cluster attacks started 30–45 min after GTN intake; pain became severe or very severe within 5–15 min and was indistinguishable from a spontaneous attack (Fanciullacci *et al.*, 1997).

Nitrate-induced headaches in cardiovascular patients

Much less is known about nitrate-induced headaches as side-effects in clinical practice. Describing the safety profile of a medicine is not usually the main goal of a clinical study; the primary end-point is to examine the pharmacokinetics of the proposed medicine (phase I) or assess its effectiveness (phases II and III). Since 1949 several clinical trials have been designed and conducted to test the efficacy of nitrates in cardiovascular disorders (Dewar *et al.*, 1959; Colditz *et al.*, 1988; Yusuf *et al.*, 1988; Scheidt, 1990; Held, 1992; Thadani and de Vane, 1992; Armstrong and Moe, 1993; Jugdutt, 1994; Abshagen, 1996; Parker, 1996; Thadani, 1997). Headache was reported as a common side-effect but without any deeper analysis (Thadani and Rodgers, 2006; Thadani and Ripley, 2007). Based on the estimation that the annual incidence rate of nitrate use (prescription of glyceryl trinitrate, isosorbide dinitrate and isosorbide mononitrate including sublingual, aerosol, transdermal and oral preparations for new patients) is about 1.5% in the European countries (Hemingway *et al.*, 2006) and that 10% of nitrate-treated patients report unbearable headaches (Thadani and Rodgers, 2006) then in Europe alone more than 700 000 patients every year suffer from serious nitrate-induced headaches. However, there is no indication of the characteristics of the headache or of the time–intensity relationship.

Some studies have investigated the occurrence of nitrate-induced headaches in various pathological conditions. For example, the probability of suffering a GTN-induced headache was much lower in chest-pain patients with obstructive coronary artery disease, than in chest-pain patients with normal coronaries or with minimal coronary artery disease (Hsi *et al.*, 2005); and using acute nitrate therapy the incidence of reported headache was low in acute myocardial infarction but high in unstable angina (Thadani and Ripley, 2007).

Migraine-like adverse effects after nitrate therapy have been reported only sparsely in the past; for example, Muller *et al.*

reported hemicrania (Mueller and Meienberg, 1983) and Bank reported migraine with aura in a patient suffering from angina pectoris (Bank, 2001). Cluster headaches were also observed during nitrate therapy (Ekbom *et al.*, 2004). In subjects with coexisting cluster headache and angina pectoris it has been shown that sustained nitrate therapy induced extra headache periods, but during active clusters angina pectoris became remitted (Ekbom *et al.*, 2004).

Potential mechanisms of NO-provoked headache types

Vasodilation

The main effects of nitrates are attributed to their vasodilatory effect by releasing NO (Moncada *et al.*, 1988). Although the relationship between NO and headaches has been the subject of considerable recent discussion and research, the exact mechanisms of the different types of NO-induced headaches are still not clear (van der Kuy and Lohman, 2003; Goadsby, 2007b; Olesen, 2008).

One hypothesis suggests that NO, as a powerful vasodilator, might be responsible for nitrate-induced migraine headaches through dilation of the large cranial arteries that have been reported during spontaneous migraine headaches (Iversen *et al.*, 1990). However, some recent data contradict any direct relation between NO-induced migraine attacks and NO-evoked vasodilation (Akerman *et al.*, 2002b; Goadsby, 2006b; Juhasz *et al.*, 2007); a human 3T magnetic resonance angiography study found that migraine attacks were not associated with vasodilation of cerebral or meningeal blood vessels (Schoonman *et al.*, 2008). Furthermore, sildenafil induces migraine without any change in middle cerebral artery diameter through activation of the cyclic guanosine monophosphate (cGMP) pathway, which is part of the NO-mediated signalling cascade (Kruuse *et al.*, 2003), while the potent vasodilator vasoactive intestinal polypeptide causes a weak immediate headache in healthy volunteers (Hansen *et al.*, 2006) but does not trigger a migraine attack in migraine patients (Rahmann *et al.*, 2008).

Other data provide further evidence for the dissociation of vasodilator and migraine-inducing effect of nitrates. The 5-HT_2 receptor agonist meta-chlorophenylpiperazine (m-CPP) caused migraine-type headaches with similar characteristics to those induced by nitrates in the same patient group at risk for NO donor-induced migraine. However, in contrast to nitrates, this compound causes vasoconstriction and sympathoadrenal activation rather than vasodilation (Bagdy *et al.*, 1988; Bagdy, 1998)

Taken together these observations suggest that although NO plays a role in some aspects of the pathophysiology of migraine it is unlikely simply to be a vascular effect. A more likely explanation of the mechanism of action of NO in migraine could be, for example, through the release of calcitonin gene-related peptide (CGRP) (Juhasz *et al.*, 2003b; 2005). This will be discussed in the next section.

Previous studies have suggested that immediate headaches may be related to vasodilation caused by direct activation of the NO-cGMP pathway (Figure 3) (Akerman *et al.*, 2002b; Juhasz *et al.*, 2003b). Through protein kinase G (PKG) the NO-cGMP pathway is able to control the function of various ion channels, including potassium channels that modify the smooth muscle contractility and vascular tone (Sobey, 2001). It is well documented that during NO-donor administration, meningeal and cerebral vasodilation occur both in animals (Dong *et al.*, 1998; Bergerot *et al.*, 2006) and in humans (Schoonman *et al.*, 2008) and human subjects also report immediate headaches paralleling the time-course of the vasodilation (Hansen *et al.*, 2007). It was observed recently that NO-donor GTN-induced dural and pial vasodilation involved the opening of calcium-activated potassium ($K_{Ca}1.1$ also known as BK or Slo1) channels (Gozalov *et al.*, 2007), which suggests that the $K_{Ca}1.1$ channels may play an important role in the NO-induced immediate headaches.

Calcitonin gene-related peptide (CGRP)

Elevated CGRP levels have been found in patients during spontaneous migraine (Goadsby *et al.*, 1990; Gallai *et al.*, 1995; Bellamy *et al.*, 2006b) and cluster headache attacks (Goadsby and Edvinsson, 1994; Fanciullacci *et al.*, 1995), which suggests the activation of the trigeminovascular system (Goadsby, 2007b). The proven efficacy of CGRP antagonists in migraine attacks supports the pivotal role of CGRP in migraine pain (Doods *et al.*, 2007; Edvinsson, 2008). Besides CGRP antagonists, animal studies indicate a potential usefulness of CGRP antibodies in the treatment of migraine as these antibodies showed a long inhibitory effect (Zeller *et al.*, 2008).

GTN-induced migraine is the most studied human migraine model and shows considerable similarities with spontaneous migraine attacks (Olesen, 2008). Indeed, previous studies have demonstrated that peripheral plasma CGRP concentration increased significantly also during the GTN-induced delayed migraine attack (Figure 2) (Juhasz *et al.*, 2003b; 2005), which provides additional support for the hypothesis that migraine attacks are a result of trigeminovascular activation (Akerman *et al.*, 2002b).

In contrast, the plasma CGRP concentration did not increase significantly during the NO-induced immediate headache (Ashina *et al.*, 2001; Juhasz *et al.*, 2003b). These data support the hypothesis that the initial headache may be related to a direct action of the NO-cGMP pathway via vasodilation, independently of the CGRP release (Ashina *et al.*, 2001; Akerman *et al.*, 2002b) as we discussed in the previous section. It seems that GTN triggers a cascade of events, initially leading to vasodilation, and subsequently to the release of CGRP which is involved in the causation of migraine in subjects who are at risk.

The mechanism of NO-induced delayed migraine is not clear. CGRP receptors can be found in the trigeminocervical complex (Storer *et al.*, 2004) and CGRP and neuronal NO synthase (nNOS) are frequently co-localized in neurons of the trigeminal ganglion (Hou *et al.*, 2001) and NO regulates the expression of the CGRP gene (Bellamy *et al.*, 2006a). Animal studies have demonstrated that NO donors caused biphasic activation of the trigeminal nucleus caudalis measured by c-fos expression (Tassorelli *et al.*, 2000; Akerman *et al.*, 2002b). The second phase reached its maximum after 4 h (Pardutz *et al.*, 2000; Tassorelli *et al.*, 2000) similar to the latency of

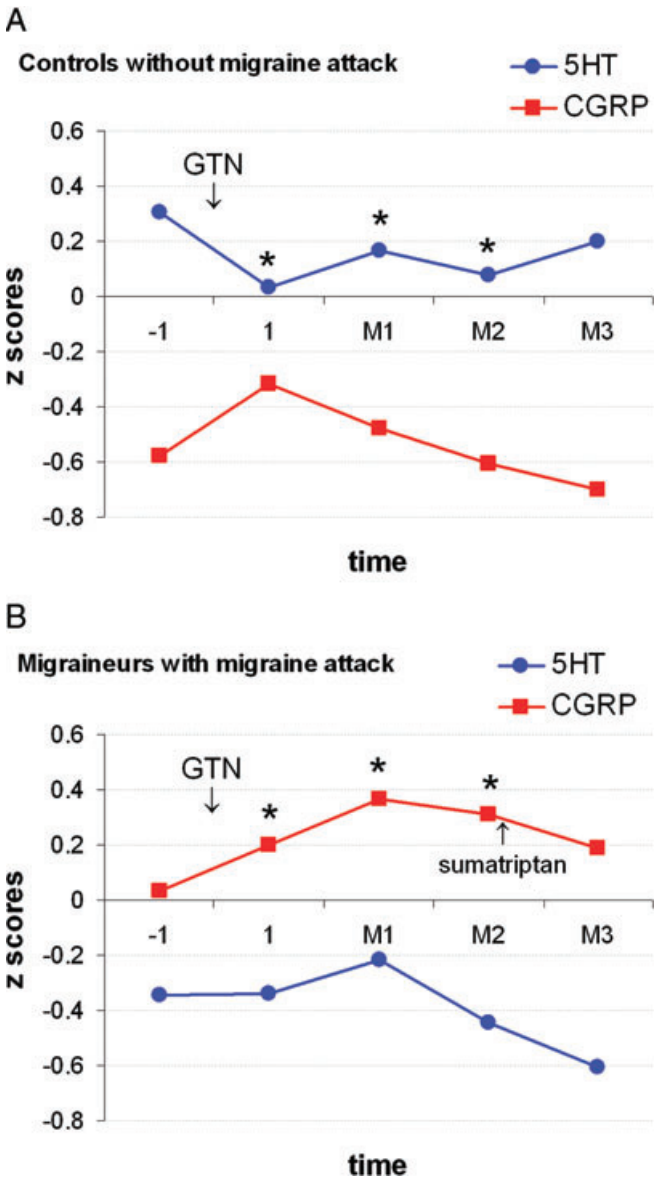

Figure 2 Effects of nitroglycerin (GTN, 0.5 mg sublingual) on plasma calcitonin gene-related peptide (CGRP) and platelet 5-HT concentrations in controls without delayed migraine attack (n = 10; A) and in migraineurs with delayed migraine attack (n = 19; B). We calculated z scores for plasma CGRP and platelet 5-HT concentrations [(subgroup mean – grand mean)/subgroup SD] to compare their changes during the GTN challenge. Baseline blood samples were collected at 7.00 a.m. (–1). A secondary blood sample was taken 1 h after sublingual application of GTN, at 9.00 a.m. (1). The next three blood samples were taken 60 min (M1), 120 min (M2) and 180 min (M3) after the beginning of the migraine attack. In controls, similar time schedules were used based on preliminary data (M1: 5 h, M2: 6 h and M3: 7 h after GTN respectively). Migraine patients took 20 mg sumatriptan nasal spray immediately after the M2 blood sampling. *Significant changes after sublingual GTN compared with baseline ($P < 0.05$). (Combined data from Juhasz *et al.*, 2003b; 2005.)

delayed headache attacks. At the same time (4 h after NO-donor administration) both neuronal (Pardutz *et al.*, 2000) and inducible (Reuter *et al.*, 2001) NO synthase (nNOS and iNOS) expressions were up-regulated. This cascade of events eventually leads to CGRP release from the trigeminal ganglion (Pardutz *et al.*, 2000; Tassorelli *et al.*, 2000; Akerman *et al.*, 2002b; Greco *et al.*, 2008b). The CGRP release seems to involve activation of mainly $Ca_v2.1$ (P/Q-type) and Ca_v3 (T-type) voltage-gated Ca^{2+} channels (Bellamy *et al.*, 2006a; Xiao *et al.*, 2008). On the other hand, blockers of Ca_v1, $Ca_v2.1$ and Ca_v3 channels could inhibit CGRP release and consequently the dural vasodilation indicating an important role of these channels in trigeminovascular nociception (Akerman *et al.*, 2003). In addition, both endogenous and exogenous CGRP-induced vasodilations have been attenuated by the $K_{IR}6$ (also known as K_{ATP}) channel inhibitor glibenclamide (Gozalov *et al.*, 2008), which suggests that $K_{IR}6$ channels, under certain circumstances, are involved in the CGRP-induced actions and might have a role in migraine pain (Figure 3).

Serotonin (5-HT)

Serotonin has been implicated in migraine pathophysiology for several years. In our previous studies, platelet serotonin concentration was significantly lower in migraineurs (Juhasz *et al.*, 2003a), and after GTN administration, migraineurs developed delayed migraine-type headaches much more frequently than did controls (Juhasz *et al.*, 2003b). Chronically low 5-HT levels in plasma and brain have been reported to increase the sensitivity of the trigeminovascular pathway (Hamel, 2007), and serotonin depletion to increase nNOS activity (Tagliaferro *et al.*, 2001). In animal studies, hyposerotoninergic conditions enhanced the vascular effects of GTN, especially 30–60 min after administration (Srikiatkhachorn *et al.*, 2000). In addition, up-regulation of 5-HT_2 receptors led to increased NO production, through activation of nNOS (Figure 3), and this process increased the chemically induced pain sensitivity in animals and might be responsible for chronic headaches in humans (Srikiatkhachorn *et al.*, 2002; Mehrotra *et al.*, 2008). These data support the observation that migraineurs are hypersensitive to NO donors.

In our GTN challenge study we could not demonstrate significant changes in platelet serotonin concentration in those who developed delayed migraine, but in those who remained migraine-free we observed an early decrease in platelet serotonin concentration, suggesting release (Figure 2) (Juhasz *et al.*, 2003b). Our results suggest that a significant release of serotonin in subjects who did not develop migraine attack may act on $5\text{-HT}_{1B/1D}$ receptors, as do triptans, and thus prevent trigeminovascular activation (Juhasz *et al.*, 2003b; 2005). Indeed, triptans were effective in NO donor-induced migraine attacks (Juhasz *et al.*, 2005), prevented NO-induced immediate headache in healthy controls (Iversen and Olesen, 1996) and also blocked NO-induced blood vessel dilatation and trigeminovascular activation in an animal model (Akerman *et al.*, 2002a).

Other neurotransmitters

In general, NO is a modulator of several neuronal functions in the brain and these effects are mainly mediated by cGMP (Prast and Philippu, 2001; Esplugues, 2002; Garthwaite, 2008). The potential mechanisms of NO-provoked headache types are summarized in Figure 3.

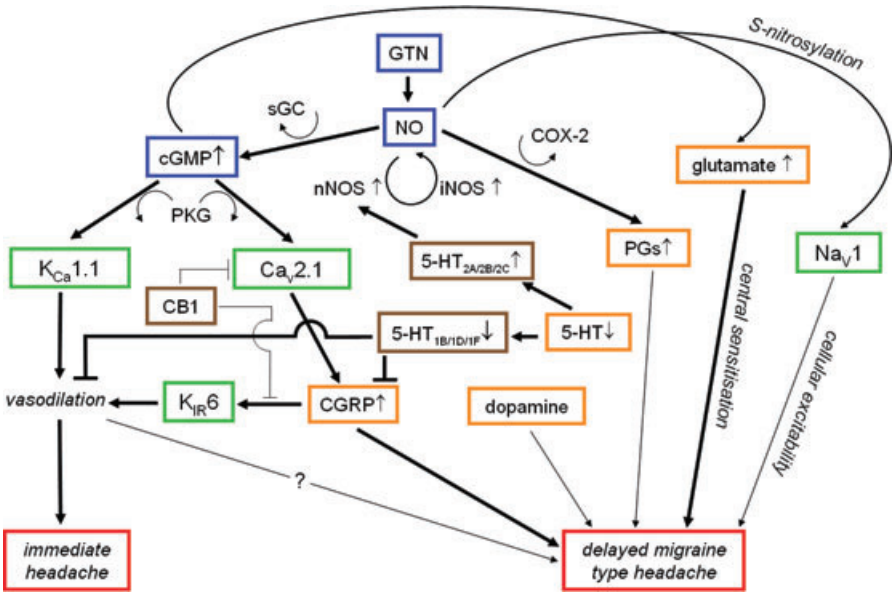

Figure 3 Nitrate derived NO actions relevant to nitroglycerin (GTN)-induced immediate and delayed (migraine-type) headaches. The NO donor GTN activates the NO-cGMP pathway via the soluble guanylate cyclase enzyme (sGC). Through protein kinase G the NO-cGMP pathway is able to control the function of various ion channels, including the calcium-activated potassium channels $K_{Ca}1.1$ (also known as BK or Slo1) that modify smooth muscle contractility and may play an important role in immediate headaches. The NO-cGMP pathway also increases calcitonin gene-related peptide (CGRP) release mainly by activation of $Ca_v2.1$ (P/Q-type) voltage-gated calcium channels. CGRP can cause vasodilation via $K_{IR}6$ channels (also known as K_{ATP}) and can cause delayed migraine-type headaches via mechanisms which are not fully understood yet. The CB1 receptor is able to inhibit both CGRP- and NO-induced dural vessel dilation. It is possible that this is partially due to the inhibition of $Ca_v2.1$ (P/Q-type) voltage-gated calcium channels but the mechanism is still unclear. The trigeminovascular activation, both vasodilation and CGRP release, can be blocked via the activation of 5-$HT_{1B/1D/1F}$ receptors. In chronic migraine patients up-regulation of 5-HT_2 receptors due to the low baseline 5-HT level may occur, and an increase in nNOS activity and elevated NO release due to an acute increase in 5-HT release could be expected. The NO-cGMP pathway also increases the release of glutamate that has major role in central sensitization and can lead to migraine attacks. Dopamine, prostaglandins and sodium channels also play a role in NO-induced migraine-type headaches but further studies are needed to elucidate the exact mechanism. Red box: different type of headaches, green boxes: ion channels, orange boxes: neurotransmitters, brown boxes: neurotransmitters and modulators, blue boxes: GTN-NO-cGMP pathway, arrows: activation lines: blockade of action, thick lines: evidence based pathways, thin lines: hypothesized pathways.

It has been demonstrated that NO donors increase the release of glutamate (via the NO-cGMP pathway), while NOS inhibitors decrease glutamate output (Prast and Philippu, 2001; Garthwaite, 2008). The elevated glutamate level might contribute to the central neuronal sensitization to incoming sensory signals, which is an important factor in migraine (Calabresi *et al.*, 2007). A previous study showed that cerebrospinal and plasma glutamate levels were increased in chronic migraine patients (Peres *et al.*, 2004). Antiepileptic drugs which are effective in migraine prevention target the glutamate-mediated excitatory and/or GABA-mediated inhibitory systems in conjunction with a modulation of voltage-gated sodium (Na_v1) and calcium (Ca_v) channels; indeed chronic administration of valproic acid prevented GTN-induced delayed migraine attack (Tvedskov *et al.*, 2004). However, further studies are needed to investigate the role of central sensitization and glutamate in NO-induced headaches and migraine.

Dopamine is also believed to play an important role in the pathophysiology of migraine although there are several conflicting results. Migraineurs are hypersensitive to dopamine agonists, while dopamine antagonists have beneficial effects in migraine treatment (Akerman and Goadsby, 2007). Dopamine is also implicated in premonitory symptoms, such as yawning and nausea, and may also contribute to hypotensive changes (Akerman and Goadsby, 2007). Currently there is little available information concerning dopamine and GTN-induced headaches although, in animals, intact central dopaminergic neurotransmission is essential for the GTN-induced activation of brain areas involved in migraine attacks, as well as for the hyperalgesic response to painful stimuli elicited by the GTN (Greco *et al.*, 2008a). It is important to note that dopamine neurotransmission interacts robustly with the 5-HT system. Serotonin receptors, such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C}, that have been implicated in migraine pathophysiology (Hamel, 2007), also modulate dopamine release (Alex and Pehek, 2007).

Finally, we recently showed that variations in the cannabinoid receptor 1 gene were significantly associated with migraine headaches (Juhasz *et al.*, 2009). Akerman *et al.* demonstrated that anandamide, an endogenous ligand to the cannabinoid CB_1 receptor, decreases CGRP and NO induced dural vasodilations by 30% and 40%, respectively, in animal models (Akerman *et al.*, 2004). The exact mechanism of this action is not fully understood. However, as the CB1 receptor is able to inhibit voltage-gated calcium channels and activate inwardly rectifying potassium channels it is possible that ion channels have a major role in its effect.

Cyclooxygenase-2 (COX-2) – prostaglandin pathway

A previous study reported that, in the jugular venous blood, the level of algogen prostaglandins (PGs), namely the PGE_2

Table 3 Ion channels involved in NO effects (modified from Ahern *et al.*, 2002)

Ion channel	*Site*	*Effect*	*References*
cGMP-modulated channels			
Na_v channel	Olfactory receptors	↑ (PKG)	Kawai and Miyachi (2001)
	Cardiomyocytes	↓ (PKG)	Ahmmed *et al.* (2001)
$K_{Ca}1.1$ channel	Pituitary nerve	↑ (PKG)	Klyachko *et al.* (2001)
	Pituitary cell line	↑ (PKG)	White (1999)
	Smooth muscle	↑ (PKG)	Pfeifer *et al.* (1998)
	Dermal fibroblast	↑	Roh *et al.* (2007)
	Endothelial cell	↑	Dong *et al.* (2008)
$K_v1.5$ channel	Cardiomyocytes	↓	Nunez *et al.* (2006)
$K_v4.3$ channel	Cardiomyocytes	↓	Gomez *et al.* (2008)
$K_{IR}6$ channel	Cardiomyocytes	↑ (PKG)	Han *et al.* (2001)
Ca_v1 channel	Cardiomyocytes	Multiple actions	Fischmeister and Mery (1996)
	Cardiomyocytes	↓	Bai *et al.* (2004)
	Hippocampal neuron	↓ (PKG)	Doerner and Alger (1988)
$Ca_v2.2$ channel	Retinal ganglion cell	↓ (PKG)	Hirooka *et al.* (2000)
	Dorsal root ganglion	↓ (PKG)	Yoshimura *et al.* (2001)
Ca_v3 channel	Olfactory receptor	↑ (PKG)	Kawai and Miyachi (2001)
S-nitrosylated channels			
Na_v channel	Nodose ganglia	↓	Li *et al.* (1998)
	Posterior pituitary	Persistence	Ahern *et al.* (2000)
	Cardiomyocytes	Persistence	Ahern *et al.* (2000)
	Hippocampus	Persistence	Hammarstrom and Gage (1999)
	Spinal cord neuron	↓	Ashki *et al.* (2008)
$K_{Ca}1.1$ channel	Brain	↑	Shin *et al.* (1997)
	Posterior pituitary	↑	Ahern *et al.* (1999)
	Hippocampal neuron	↑	Tjong *et al.* (2007; 2008)
	Smooth muscle	↑	Bolotina *et al.* (1994); Lang and Watson (1998)
$K_v1.5$ channel	Cardiomyocytes	↓	Nunez *et al.* (2006)
$K_v2.1$ channel	Cardiomyocytes	↑	Gomez *et al.* (2009)
$K_v4.3$ channel	Hippocampus	↓	Liu *et al.* (2007)
Ca_v1 channel	Cardiomyocytes	↓	Hu *et al.* (1997)
$Ca_v1.2$ channel	Smooth muscle	↓	Kang *et al.* (2007)

and the stable product of prostacyclin ($PGF_{1\alpha}$), significantly increased during the late phase (2–4 h after the onset) of spontaneous migraine attacks (Sarchielli *et al.*, 2000). In animal models of migraine, GTN activated the COX-2 – PGE_2 pathway in the brainstem 4 h after GTN administration (Tassorelli *et al.*, 2007). However, it has been demonstrated that GTN-induced vasodilation *in vivo* is independent of the PG system (Ahlner *et al.*, 1991) thus it is unlikely that the COX-2 – PGE_2 pathway has a major role in immediate GTN-induced headaches but its role in delayed migraine-type headaches warrants further studies.

NO-induced ion channel modifications

NO modifies the function of ion channels responsible for excitability, predominantly in two ways (Ahern *et al.*, 2002) – indirectly and directly. According to the classical view, all the actions of NO are mediated by cGMP; the three principal targets of which are PKG, cyclic nucleotide gated channels and cyclic nucleotide phosphodiesterase. Of these, PKG provides the broadest means for controlling ion-channel functions, as these proteins contain PKG phosphorylation sites that are strongly conserved. This classical view was overturned following findings that NO could modify proteins through direct chemical reactions. One such modification, termed S-nitrosylation, occurs at the thiol side-chains of cysteine residues through a complex chemical mechanism without the assistance of enzymes. However, S-nitrosylation requires higher concentrations of NO than the activation of the cGMP pathway. S-nitrosylation of various ion channels can be mediated not only directly by NO, but also by NO metabolites, peroxynitrite and other reactive oxidant species (e.g. NO_2, OH, H_2O_2) (Kang *et al.*, 2007; Liu *et al.*, 2007; Ashki *et al.*, 2008; Dyachenko *et al.*, 2009). Nevertheless it was supposed that the nitrosative and oxidative actions of peroxynitrite may play a role both in regulation of normal cellular functions and in its well-known cytotoxic effects (Ferdinandy, 2006). Table 3 summarizes NO actions on various ion channels, focusing mainly on those that are thought to play a role in migraine pain.

Sodium channels

The primary route by which NO modulates Na_v1 channels appears to be directly via S-nitrosylation although Na_v1 channels of sensory neurons differentially respond to NO (Hammarstrom and Gage, 1999; Ahern *et al.*, 2002). In one class of sensory neurons (nodose ganglion) S-nitrosylation inhibits both tetrodotoxin (TTX)-sensitive and -insensitive Na_v1 channels (Li *et al.*, 1998), while in dorsal root ganglia neither TTX-sensitive nor -resistant action potentials were affected by NO (Yoshimura *et al.*, 2001).

A different form of Na_v1 channel modulation has been described for the 'persistent' Na^+ current: many excitable tissues have a component of Na^+ current that is resistant to inactivation. This 'persistent' Na^+ current is believed to play an important role in the integration of synaptic inputs, the generation of rhythmic oscillations, and the pathological

changes in electrical excitability associated with many disease states including cardiac arrhythmias, ischaemic stroke, epilepsy and probably migraine (Segal and Douglas, 1997). NO donors have been shown to increase persistent Na^+ current in posterior pituitary nerve terminals (Ahern *et al.*, 2000) and hippocampal neurons (Hammarstrom and Gage, 1999). This action of NO on Na_v1 channels is thought to be a direct chemical reaction with a protein thiol (S-nitrosylation) as described above (Hammarstrom and Gage, 1999; Ahern *et al.*, 2000; Evans and Bielefeldt, 2000). In neurohypophysial nerve terminals and in ventricular myocytes NO reduced Na^+ current inactivation thus inducing persistent Na^+ currents. This effect was independent of cGMP, and was blocked by the alkylating agent N-ethylmaleimide thus, apparently NO acts directly on the channel or on a closely associated protein. Persistent Na^+ current could also be induced by endogenous NO, generated enzymatically by nNOS (activated via Ca^{2+} loading). Both rises in cellular NO and modulation of Na^+ channels were blocked by NOS inhibitors. These findings suggest that NO is a potential physiological regulator of Na_v1 channel persistence and this is likely to have important consequences for the regulation of cellular excitability (Ahern *et al.*, 2000).

The potential role of Na_v1 channels in the pathophysiology of migraine is supported by the fact that among the well-known prophylactic drugs amytriptyline, valproate and topiramate also affect Na_v1 channel function (Meldrum and Rogawski, 2007), and also by the association between Na_v1 channel mutations and FHM (see section 'Migraine as a channelopathy').

Calcium channels

cGMP differentially modulates a variety of Ca_v channels depending on tissue, species and age. cGMP depresses Ca^{2+} current in hippocampal neurons (Doerner and Alger, 1988), inhibits the activation of $Ca_v2.2$ (N-type Ca^{2+}) channels in dorsal root ganglion neurons (Yoshimura *et al.*, 2001), but has no effect on $Ca_v2.2$ and Ca_v1 (L-type) channels in pituitary nerve terminals (Klyachko *et al.*, 2001). NO can also inhibit cardiac Ca_v1 channels directly, via S-nitrosylation (Hu *et al.*, 1997). High voltage-activated (HVA) Ca^{2+} channels (Ca_v1, $Ca_v2.1$, $Ca_v2.2$ and Ca_v3) play a prominent role in trigeminovascular nociception as they are localized to trigeminal presynaptic nerve terminal in the dura and the trigeminal nucleus caudalis. It is likely that mainly $Ca_v2.1$ channels are involved with initiation of migraine attacks and/or aura symptoms as well as with nociceptive transmission because they participate in controlling the release of CGRP (Xiao *et al.*, 2008). Indeed, mutations in $Ca_v2.1$ channels are among the established genetic risk factors for FHM (see section 'Migraine as a channelopathy'). Several Ca^{2+} channel blockers (dihydropyridines, flunarizine and anti-epileptics with Ca^{2+} channel inhibitory activity) are used as prophylactic drugs against migraine (Evers, 2008; Meldrum and Rogawski, 2007). Recently, it has become apparent that the molecular targets for gabapentin and pregabalin are α_2-δ proteins of HVA Ca^{2+} channels (Davies *et al.*, 2007); this interaction may explain the anti-epileptic and antinociceptive actions of these drugs (Davies *et al.*, 2007).

Ca^{2+}-activated K^+ ($K_{Ca}1.1$) channels

Large-conductance, calcium-activated potassium ($K_{Ca}1.1$) channels are expressed in both excitable and non-excitable cells and are involved in many cellular functions, such as action potential repolarization, neuronal excitability, transmitter release and hormone secretion. $K_{Ca}1.1$ channels are special among K^+ channels, being sensitive to both intracellular Ca^{2+} concentrations and voltage. These features make $K_{Ca}1.1$ channels ideal negative feedback regulators in many cell types: by hyperpolarizing the membrane they decrease voltage-dependent Ca^{2+} entry. Located in dendrites, axons and synaptic terminals, $K_{Ca}1.1$ channels thus play an important role in controlling the excitability of neurons (Ghatta *et al.*, 2006; Nardi and Olesen, 2008). Both PKG and S-nitrosylation enhance the activity of $K_{Ca}1.1$ channels. In smooth muscle the cGMP-dependent pathway predominates (Pfeifer *et al.*, 1998) but S-nitrosylation also seems to be involved (Bolotina *et al.*, 1994; Lang and Watson, 1998), and both pathways are important in nerve terminals (Ahern *et al.*, 2002).

As we mentioned previously, animal studies suggest that $K_{Ca}1.1$ channels are involved in the NO-donor GTN-induced dural and pial vasodilation or acetylcholine induced rabbit middle cerebral artery dilation and thus may play an important role in the NO-induced immediate headaches (Dong *et al.*, 1998; Bergerot *et al.*, 2006; Gozalov *et al.*, 2007).

Other potassium channels

The NO-cGMP cascade regulates several other potassium channels. S-nitrosylation enhances the activity of K_v channel in arterial smooth muscle (Yuan *et al.*, 1996). PKG modulates the neuronal voltage-gated potassium channels $K_v3.1$ and $K_v3.2$ (Moreno *et al.*, 2001). In vestibular hair cells cGMP inhibits the delayed rectifier K^+ current and shifts its activation curve to more positive direction (Behrend *et al.*, 1997). In hippocampal CA1 neurons peroxynitrite donor caused an inhibition of transient outward potassium current (I_{to}, K_v 4.3 channel) and delayed rectifier potassium current (I_K) (Liu *et al.*, 2007). PKG enhances the activity of cardiac $K_{IR}6$ channels (Han *et al.*, 2001). In cardiac cells NO modifies K^+ currents in a complex fashion. NO activates the slow component of the delayed rectifier current (I_{Ks}) (Bai *et al.*, 2004) and the inwardly rectifying K^+ current (I_{K1}, $K_{IR}2.1$ channel) (Gomez *et al.*, 2009), but inhibits the fast component (I_{Kr}) (Taglialatela *et al.*, 1999), the $hK_v1.5$ channel which generates the ultrarapid delayed rectifier current (I_{Kur}) (Nunez *et al.*, 2006) and the transient outward current (I_{to}) ($K_v4.3$ channel) (Gomez *et al.*, 2008).

There is growing evidence that K_v7 (KCNQ) potassium channels, especially the neuronal $K_v7.2$ and $K_v7.3$, play a role in neurological diseases (Miceli *et al.*, 2008). Neuronal K_v7 gene defects have been implicated in two rare forms of genetically determined human channelopathies, namely benign familial neonatal seizures and non-syndromic autosomal-dominant hearing loss. Compounds acting as direct activators of neuronal K_v7 channels have been approved recently for clinical use as analgesics and anticonvulsants (Gribkoff, 2008; Miceli *et al.*, 2008). As neuronal hyperexcitability is a marker of other neurologic diseases, the role of this type of potassium channel has been suggested in migraine also, although direct evidence is lacking.

Nitrate tolerance and headaches

The main limitation of nitrate therapy, especially when continuous application is required, is nitrate tolerance. Nitrate tolerance is characterized by the decrease of vasodilator effect of organic nitrates that can reach complete loss of vasodilatation within 24–48 h (Mayer and Beretta, 2008). This is a complex, still not fully understood process that has been related to molecular changes in intrinsic vascular processing, such as desensitization of soluble guanylyl cyclase, oxidative stress, uncoupling of endothelial NO synthase reduced GTN activation because of vascular thiol depletion and inactivation of mitochondrial aldehyde dehydrogenase (ALDH-2) (see detailed reviews from Mayer and Beretta, 2008 and Daiber *et al*., 2008). Neurohormonal responses, such as sympathoadrenal axis activation and/or renin–angiotensin system activation, also contribute to the decreased nitrate efficacy (Daiber *et al*., 2008; Mayer and Beretta, 2008).

Regarding GTN-induced headaches attenuation can be seen after 5–7 days of the initiation of therapy (Ahlner *et al*., 1991). However, this timeframe does not coincide with the vascular nitrate tolerance mentioned above. Christiansen *et al*. showed that in healthy volunteers daily 3 × 30 mg 5-ISMN provoked the most frequent and intense headaches in the first 3 days, followed by gradual decrease in headache symptoms and tolerance had developed by the sixth day (Christiansen *et al*., 2000b; 2008). They proposed that extracerebral arteries, which showed only partial nitrate tolerance after 24 h, might contribute to GTN-induced headaches besides other mechanisms. On the other hand, there is no available data about those patients whose headache was initially unbearable. Indeed headache side-effects are the major cause of discontinuation of nitrate therapy (Ahlner *et al*., 1991) and detailed data on these patients' headaches are not reported so far. As nitrates exert diverse effects in different tissues – for example myocardial anti-ischaemic effect has been preserved even after vascular nitrate tolerance (Csont and Ferdinandy, 2005) – it can be hypothesized that tolerance to GTN-induced severe headaches are limited or absent in these patients.

Genetic and inherited vulnerability to NO-induced headaches

To the best of our knowledge the genetic risk factors for NO-induced delayed migraine or unbearable headache have not been investigated yet. However, healthy subjects with a family history of migraine are more sensitive to NO-provoked headaches (Juhasz *et al*., 2003b; 2004; Afridi *et al*., 2004; Sances *et al*., 2004), which supports the hypothesis that inherited vulnerability factors play a significant role. Therefore in this section we will summarize the relevant results from genetic studies about migraine, but for detailed reviews see the work of van den Maagdenberg *et al*. (van den Maagdenberg *et al*., 2007) and Wessman *et al*. (Wessman *et al*., 2007).

Migraine as a channelopathy

Migraine is a complex genetic neurovascular disorder (Goadsby, 2007b). Many chromosomal regions are reported to be potentially involved, but mutations in the three genes for FHM – CACNA1A, ATP1A2 and SCNA1A – form the only established molecular knowledge of migraine (van den Maagdenberg *et al*., 2007). From a clinical point of view, FHM and migraine may be part of the same spectrum and may share some pathogenetic mechanisms. Therefore, FHM seems a valid model to study genetic factors of migraine in general.

FHM1 (CACNA1A gene)

This gene encodes the pore-forming α_{1A} subunit of $Ca_v2.1$ calcium channels (Ophoff *et al*., 1998) which modulate release of neurotransmitters at peripheral and particularly central excitatory synapses. Many CACNA1A mutations have been analysed with electrophysiological techniques in neuronal and non-neuronal cell models (Pietrobon, 2005; Jeng *et al*., 2006; Pietrobon, 2007). Because of the different experimental circumstances, varying and conflicting results have been obtained (Pietrobon, 2007). While the consistent change found with FHM1 mutations was an enhanced single channel Ca^{2+} influx with an increased channel open probability producing a gain-of-function of $Ca_v2.1$ channels (Hans *et al*., 1999; Tottene *et al*., 2002; 2005), other data obtained from transfected cells indicated the opposite effect – a loss-of-function (Cao *et al*., 2004; Jeng *et al*., 2006). Theoretically, the observed gain-of-function of single channels should lead to an easier opening of channels in neurons, resulting in increased $Ca_v2.1$-dependent neurotransmitter release from cortical neurons.

FHM2 (ATP1A2 gene)

This gene encodes the α_2 subunit of sodium–potassium pump ATPase (De fusco *et al*., 2003). Glial and neuronal Na^+/K^+ ATPase modulate the re-uptake of K^+ and glutamate from the synaptic cleft into neurons and astrocytes. Molecular studies of a few FHM2 mutations show different functional changes from a complete loss of function to a reduced function in the Na^+/K^+ ATPase activity to variable degrees. However, the common consequences of these mutations are reduced reuptake of K^+ and glutamate from the synaptic cleft (see review by Pietrobon, 2007). This decreased clearance of K^+ and glutamate by astrocytes during cortical neuronal activity could depolarize neurons resulting in an impaired recovery from neuronal excitation.

FHM3 (SCNA1 gene)

This gene encodes the α_1 subunit of neuronal voltage-gated sodium (Nav1.1) channels that play an important role in the generation and propagation of action potentials. Only a few mutations (Q1489K, T1174S, L1649Q) have so far been identified, confirming the relationship between SCNA1 and FHM3 (Dichgans *et al*., 2005; Gargus and Tournay, 2007; Vanmolkot *et al*., 2008). However, mutation scanning of a large number of other FHM families suggests that the SCNA1 gene is a rare cause of FHM. Mutation Q1489K, located in the cytoplasmic linker between domains IIIS6 and IVS1 which is critical for fast inactivation, has been studied for its functional effects (Dichgans *et al*., 2005). This mutation causes a two to four

times faster recovery from fast inactivation. These findings suggest a gain-of-function mechanism in FHM3 with predicted enhanced neuronal excitability and release of neurotransmitters.

Common consequence of FHM mutations

The common consequence of FHM1, FHM2 and FHM3 mutations seems to lead to increased levels of glutamate and K^+ in the synaptic cleft causing an increased propensity for cortical spreading depression (CSD) (Ferrari and Goadsby, 2006). There is accumulating evidence that NO production is markedly augmented during CSD (Olesen, 2008). In this way, although mutations in FHM genes (CACNA1A in FHM-1 and ATP1A2 in FHM-2) are not associated with hypersensitivity to NO in GTN-induced headache models (Hansen *et al.*, 2008a,b), both CSD and GTN provocation result in higher NO levels in the central nervous system and may act on a common pathological pathway. In conclusion, even though the FHM genes are probably not directly involved in common migraines, the study of the cellular mechanisms of enhanced susceptibility to CSD and enhanced cortical excitability in FHM knock-in animal models may provide unique insights into possible mechanisms of common migraines (Pietrobon, 2007) and so also help to understand NO-induced headaches.

Nitric oxide synthase and other genes

Hypersensitivity of migraineurs to NO in the GTN-induced headache model directed research interest towards NOS genes. However, there is no confirmation that they play a major role in the vulnerability to these headaches (van den Maagdenberg *et al.*, 2007; Wessman *et al.*, 2007; Montagna, 2008; Olesen, 2008). Furthermore, there are no convincing replicated results that genetic variations in the serotonergic, dopaminergic, or other plausible pathways/systems are associated with common forms of migraine – with or without aura (van den Maagdenberg *et al.*, 2007; Wessman *et al.*, 2007; Montagna, 2008). One possible explanation for this failure is that headache diagnoses based on the IHS criteria (1988; 2004) might not represent biological pathways influenced by specific genetic variations (Anttila *et al.*, 2006; Russell, 2007; van den Maagdenberg *et al.*, 2007; Wessman *et al.*, 2007). However, the episodic nature of migraine suggests an ionopathic disturbance (Goadsby, 2007a) that can be primary (as in FHM) or secondary – namely dysfunction in the ion channels' controlling networks.

Treatment of NO donor-induced migraine

Based on experimental headache provocation studies, GTN-induced headaches respond to the same drugs that are used to treat primary headache disorders (Fanciullacci *et al.*, 1997; Ashina *et al.*, 2000; Tvedskov *et al.*, 2004; Juhasz *et al.*, 2005; Magis *et al.*, 2007). Thus, guidelines for treating primary headache patients can be adapted, after taking into account individual circumstances such as general medical condition and age (Evers *et al.*, 2006; Goadsby, 2006a; Martelletti *et al.*, 2008). In general, triptans should be avoided in patients with a history of coronary vascular pathology or multiple risk factors for cardiovascular disease, although the evidence suggests that they are generally safe and well-tolerated (Dodick *et al.*, 2004).

An accurate headache diagnosis and the recognition of the possible role of nitrates depend on taking a careful medical history. Unfortunately, headaches at emergency circumstances are frequently under-diagnosed and/or under-treated, both in Europe and in the United States (Gupta *et al.*, 2007; Martelletti *et al.*, 2008). After exclusion of other secondary headaches, the first choice of treatment is oral or venous non-steroidal anti-inflammatory drugs. Analgesics with evidence of efficacy on the acute migraine treatment and their recommended doses can be found in the European Federation of Neurological Societies guideline, e.g. acetylsalicylic acid 1000 mg, ibuprofen 200–800 mg, naproxen 500–1000 mg, diclofenac 50–100 mg, paracetamol 1000 mg (Evers *et al.*, 2006). Depending on the symptoms anti-emetic drugs might be necessary, e.g. metoclopramide: 10–20 mg oral, 10 mg i.v., i.m. or s.c., or 20 mg suppository; domperidon 20–30 mg oral (Ashina *et al.*, 2000; Evers *et al.*, 2006; Gupta *et al.*, 2007; Martelletti *et al.*, 2008). It is hoped that in future, new specific drugs without cardiovascular side-effects, such as CGRP antagonists, might be available for relevant at-risk patients (Doods *et al.*, 2007).

Previous studies demonstrated that in most cases tolerance developed to nitrate-induced headaches; this tolerance was independent of the type of headache (whether it fulfilled the IHS criteria for migraine without aura or not) (Christiansen *et al.*, 2000b; Thadani and Rodgers, 2006). However, a revised treatment plan was necessary for about 10% of patients whose nitrate-induced headache was unbearable (Thadani and Rodgers, 2006). Migraine and cluster headache patients are more susceptible to NO-induced severe headaches, so it is possible that patients with unacceptable headaches may suffer from migraine, or in some cases from cluster headaches, although there is no published evidence for this hypothesis to the best of our knowledge. Thus further studies are needed to investigate which subgroup of cardiovascular patients are not likely to tolerate nitrate therapy due to severe headache side-effects. Future research could also study the possible diagnostic and prognostic value of nitrate-induced headaches.

Conclusions

Nitrate compounds are frequently used as therapeutic drugs, despite evidence that they often trigger serious migraine attack in migraine patients (although not in healthy persons devoid of primary headache in their medical history). However, migraine is neither listed as an adverse effect of these compounds, nor noted in the summaries of product characteristics. Headache is a known side-effect of nitrates, but it is not generally known that NO donors may cause two markedly different types of headaches; (i) immediate headaches, which can occur in anyone (not just patients at risk of migraine); although these are uncomfortable, they are not serious and disappear spontaneously, and (ii) migraine attacks, which occur in migraineurs (or those at risk), usually start several hours after initial drug administration, are

serious, debilitating, and of long duration needing special treatment – usually anti-attack drugs, such as triptans. However, nitrates are commonly used to treat angina pectoris, and triptans are contraindicated for this condition. Recent studies regarding the mechanism of action and side-effects of nitrates provide evidence that the two headaches are caused by different mechanisms: immediate headaches are associated with vasodilation caused by NO release, while migraines are triggered by other actions such as CGRP release, cGMP-modulated or S-nitrosylation-mediated changes in ion channel functions.

In conclusion, these data suggest that correction of summaries of nitrate product characteristics is desirable. In addition, there is an urgent need to develop new types of anti-migraine drugs, effective in migraine attacks that could be used in patients at risk for angina pectoris.

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Conflict of Interest

The authors declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. Dr Pál Riba occasionally has acted as an expert for the Hungarian drug regulatory agency (National Institute of Pharmacy, Hungary).

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