Migraine headache is not associated with cerebral or meningeal vasodilatation—a 3T magnetic resonance angiography study

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Migraine headache is widely believed to be associated with cerebral or meningeal vasodilatation. Human evidence for this hypothesis is lacking. 3 Tesla magnetic resonance angiography (3T MRA) allows for repetitive, non-invasive, sensitive assessment of intracranial vasodilatation and blood flow. Nitroglycerine (NTG) can faithfully induce migraine attacks facilitating pathophysiological studies in migraine. Migraineurs ($n = 32$) randomly received NTG (IV $0.5\,\mu g/kg/min$ for 20 min; $n = 27$) or placebo ($n = 5$; for blinding reasons). Using 3T MRA, we measured: (i) blood flow in the basilar (BA) and internal carotid arteries (ICA) and (ii) diameters of the middle meningeal, external carotid, ICA, middle cerebral, BA and posterior cerebral arteries at three timepoints: (a) at baseline, outside an attack; (b) during infusion of NTG or placebo and (c) during a provoked attack or, if no attack had occurred, at 6 h after infusion. Migraine headache was provoked in 20/27 (74%) migraineurs who received NTG, but in none of the five patients who received placebo. The headache occurred between 1.5 h and 5.5 h after infusion and was unilateral in 18/20 (90%) responders. During NTG (but not placebo) infusion, there was a transient 6.7–30.3% vasodilatation ($P < 0.01$) of all blood vessels. During migraine, blood vessel diameters were no different from baseline, nor between headache and non-headache sides. There were no changes in BA and ICA blood flow during either NTG infusion or migraine. In contrast to widespread belief, migraine attacks are not associated with vasodilatation of cerebral or meningeal blood vessels. Future anti-migraine drugs may not require vasoconstrictor action.

Introduction

Migraine is a neurovascular disorder typically characterized by attacks of severe, throbbing, unilateral headache, associated autonomic symptoms and, in one third of patients, focal neurological aura symptoms (Goadsby *et al.*, 2002). Since the seminal work by Wolff and colleagues (Wolff, 1948), showing that stimulation of cerebral and meningeal arteries caused headache, there is a widespread belief that vasodilatation of intracranial blood vessels is the underlying mechanism for migraine headache (Ferrari and Saxena, 1993). This hypothesis was further fed by a number of other observations. Balloon dilatation of the middle cerebral artery (MCA) may cause migraine-like headache

(Nichols *et al.*, 1990). Vasoactive substances such as the nitric oxide donor nitroglycerine (NTG) (Thomsen *et al.*, 1994) and calcitonin gene related peptide (CGRP) (Lassen *et al.*, 2002) can trigger migraine in susceptible subjects. In fact, the recent development of novel CGRP antagonists for treating migraine attacks was at least partly based on the hypothesis that prevention or reversal of vasodilation would block migraine headache (Olesen *et al.*, 2004; Doods *et al.*, 2007). Animal and *in situ* pharmacological experiments (Goadsby *et al.*, 2002; Tfelt-Hansen *et al.*, 2000) and human *in vivo* studies using transcranial Doppler (Iversen *et al.*, 1990; Friberg *et al.*, 1991; Thomsen *et al.*, 1995) have shown that acute anti-migraine agents (ergots and triptans)

constrict cerebral and meningeal blood vessels (Edvinsson et al., 2005). In fact, the triptan class was specifically designed to selectively constrict intracranial blood vessels (Ferrari and Saxena, 1993).

The role of vasodilatation in migraine has been vividly debated in the past [for review see: (Humphrey and Goadsby, 1994)] and more recently (Goadsby et al., 2002; Parsons and Strijbos, 2003). Some researchers view vasodilation of meningeal or cerebral blood vessels as a primary trigger for migraine headaches, and consider vasoconstriction necessary for acute anti-migraine efficacy (Villalon et al., 2003). Others feel that vasodilation is a secondary phenomenon, due to activation of the trigeminovascular system and release of vasoactive neuropeptides. Vasodilation would primarily be involved in sustaining and worsening of the headache during migraine attacks (Waeber and Moskowitz, 2005). A third line of thinking holds that vasodilation is irrelevant or, at best, 'an innocent bystander' in the pathogenesis of migraine headache. Consequently, vasoconstriction may not be needed to treat migraine headaches (Hoskin et al., 1996a, b; Goadsby, 2005). This would be an enormous advantage as the currently available most effective anti-migraine agents, triptans and ergots, all possess (sometimes strong and sustained) vasoconstrictor activity (Ferrari et al., 2001). They may cause myocardial and cerebral ischaemia in patients with (risk factors for) vascular disease (Dodick et al., 2004). Novel anti-migraine agents, which are devoid of vasoconstrictor activity, would be safer and could thus also be used by the many migraineurs with vascular disease.

Remarkably, the three opposing views on the role of vasodilation in migraine are all primarily based on extrapolations of observations in experimental animal models, with very little evidence from human studies. This is primarily due to lack, until recently, of sensitive non-invasive imaging techniques to directly and reliably assess intracranial blood flow and blood vessel diameters in humans. Previous studies have used invasive methods such as carotid angiography (Masuzawa et al., 1983), or could only indirectly estimate diameter changes of cerebral blood vessels using transcranial Doppler (Friberg et al., 1991; Markus, 2000). Meningeal blood vessels proved too small to be investigated quantitatively. With the advent of 3 Tesla magnetic resonance imaging (3T MRA) a sensitive and non-invasive imaging technique has become available to reliably measure intracranial blood flow and diameter changes of cerebral and meningeal blood vessels (Krabbe-Hartkamp et al., 1998) as small as the middle meningeal artery (MMA) (Schoonman et al., 2006).

Infusion of NTG can reliably and faithfully provoke migraine headaches in migraineurs (Thomsen, 1997; Sances et al., 2004; Afridi et al., 2005b). The response to NTG infusion is typically biphasic: an initial, brief and mild bilateral headache during the infusion in nearly all migraine and non-migraine study subjects (Afridi et al., 2005b), followed by a typical migraine, 4–5h later, in 60–80%

of migraine, but not in non-migraine study subjects (Thomsen et al., 1994; Sances et al., 2004). The symptomatology of provoked attacks is no different from that of spontaneous attacks of migraine without aura (Thomsen et al., 1994), including premonitory symptoms (Afridi et al., 2004), response to anti-migraine drugs (Iversen and Olesen, 1996), and increase of CGRP, a marker for activation of the trigeminovascular system (Juhasz et al., 2003). This provocation model has greatly facilitated the logistics of studying pathophysiological changes during migraine attacks.

In the present study, we used 3T MRA to intraindividually compare: (i) blood flow in the basilar (BA) and internal carotid arteries (ICA) and (ii) the diameters of the external carotid arteries (ECA), ICA, MCA, BA, posterior cerebral arteries (PCA) and MMA between three conditions: (a) at baseline, outside an attack; (b) during infusion of NTG or placebo (to assess the immediate vascular effects of NTG) and (c) during NTG-provoked migraine attacks or, if no attack had occurred, at 6h post-infusion (to assess whether migraine attacks are associated with vasodilatation). We will demonstrate that there is no detectable vasodilation of cerebral or meningeal blood vessels during NTG-provoked migraine attacks, suggesting that vasoconstriction may not be required to treat migraine headaches.

Methods
Subjects

In total 32 migraine patients ($n = 5$ with aura; $n = 27$ without aura) were recruited from the neurology outpatient clinic of Leiden University Medical Centre. Inclusion criteria were: (i) age between 18 years and 55 years; (ii) diagnosis of migraine according to the diagnostic criteria of the International Headache Society (Headache Classification Committee of the International Headache Society, 2004); (iii) an average attack frequency between 1 and 8 attacks/ 2 months in the 6 months prior to the study and (iv) moderate or severe headache during spontaneous migraine attacks. Exclusion criteria included: (i) >10 days of headache per month; (ii) inability to differentiate between migraine and other forms of headache; (iii) contra-indications for the use of triptans; (iv) current use of vasoactive drugs and (v) MRI-specific contra-indications (such as claustrophobia). The study was approved by the local medical ethics committee and the subjects gave informed consent prior to the start of the study.

Experimental procedure and NTG provocation

All subjects arrived at the hospital between 8 a.m. and 10 a.m. on the day of the study. No medication, coffee, tea or alcohol was allowed in the 12 h prior to the start of the experiment. From 1 hour before the experiments until the very end of the experiments, study subjects were not allowed to smoke. Patients had to be free of migraine for at least the 3 days prior to the study day and they could not have any form of headache at the beginning of the experiment.

Migraine patients ($n = 32$) were scanned: (i) at baseline, outside an attack; (ii) during randomly allocated and double-blind infusion of NTG (0.5 µg/kg/min over 20 min; $n = 27$) or placebo

($n = 5$) and (iii) during an ensuing migraine attack or, if no migraine had occurred, at 6 h after infusion. The duration of the scan sessions was ~25 min. The study subjects remained in the scanner between the baseline and the NTG or placebo infusion scanning sessions, which began 10 min after onset of the infusion. Heart rate and blood pressure were monitored during the experiments. Two days after the experiment, subjects were contacted by telephone to check whether a migraine attack had occurred beyond the 6-hour time window (Ferrari and Saxena, 1993).

Placebo administration was included in the protocol to minimize patient and observer's bias for diagnosing whether or not NTG infusion had provoked a migraine headache [as this diagnosis is based on subjective assessment of symptoms (Headache Classification Committee of the International Headache Society, 2004)]. We choose for an unequal and incomplete allocation to receiving NTG or placebo mainly for two reasons. First, NTG administration was only used as a well-established tool to provoke migraine attacks. Our study objective was primarily to assess intra-individual changes from baseline, rather than comparing the effect of NTG with that of placebo. Secondly, we wanted to minimize the number of patients who would contribute only very little to the study results (placebo was only given for masking reasons) to reduce unnecessary burden to patients, investigators and MRI scanning time (the study protocol was very time consuming).

MRA

The MR investigations were performed on a 3.0-Tesla whole-body system (Philips Medical Systems, The Netherlands). The MRA protocol consisted of two parts, one to assess blood vessel diameter changes and one to assess blood flow changes.

The 'blood vessel diameter protocol' consisted of a thick 2D phase contrast sagittal localizer survey through the circle of Willis, followed by a 3D time-of-flight MRA sequence to visualize the BA and ECA, ICA, PCA and MCA on both sides. This scan had the following imaging parameters: repetition time/echo time: 22 ms/3.5 ms; flip angle 15°; field of view: 220 × 220 mm; number of excitations: 1; slice orientation: transverse; slice thickness: 0.65 mm; number of slices: 200; scan percentage 100%, matrix reconstruction size: 512 × 512 resulting in a nominal voxel size (*x*, *y*, *z*) of 0.43 × 0.43 × 0.65 mm; total acquisition time: 4 min 30 s. Based on the reconstruction of this 3D-time-of-flight, a second 3D-time-of-flight with a higher spatial resolution was performed to visualize the extra- and intra-cranial parts of the MMA on both sides. This scan had the following imaging parameters: repetition time/echo time: 15 ms/2.1 ms; flip angle 15°; field of view: 200 × 200 mm; number of excitations: 1; slice orientation: transverse; slice thickness: 0.25 mm; number of slices: 130; scan percentage 100%, matrix reconstruction size: 512 × 512 resulting in a nominal voxel size (*x*, *y*, *z*) of 0.39 × 0.39 × 0.25 mm; total acquisition time: 8 min 31 s.

For the 'blood flow protocol', a 2D phase contrast section was positioned on the basis of two thick slab localizer MRA scans in the coronal and sagittal plane at the level of the skull base, perpendicular on the ICA and BA, to measure the flow volume. The MRA flow volume measurements in the present study are derived from previously developed and optimized protocols (Spilt *et al.*, 2002*a*, *b*; Bakker *et al.*, 1995, 1996). Acquisition parameters: repetition time/echo time: 16 ms/8.5 ms; flip angle 10°; field of
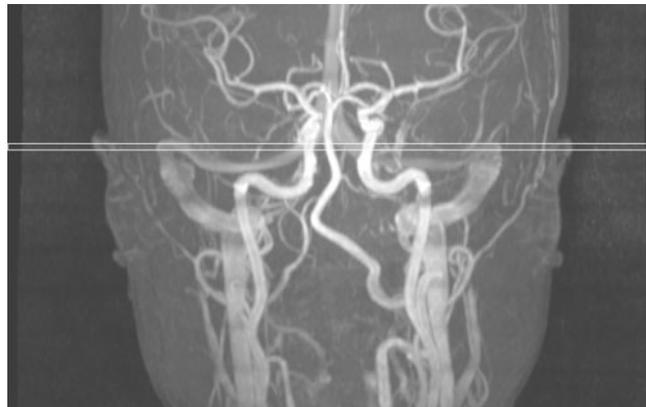

Fig. I MRA, coronal maximum intensity projection. Horizontal line indicates the positioning of the 2D phase contrast section through the ICA and the BA.

view: 150 × 150 mm; number of excitations: 20; slice orientation: transverse; slice thickness: 5.0 mm; number of slices: 1; scan percentage 100%; phase contrast velocity encoding: 140 cm/s; matrix reconstruction size: 256 × 256 resulting in a nominal voxel size (*x*, *y*, *z*) of 0.59 × 0.59 × 50 mm; total acquisition time: 56 s. Figure 1 illustrates the positioning of the 2D phase contrast section through the ICA and BA. On an independent workstation, quantitative flow values were calculated in each vessel by integrating across manually drawn regions of interest that enclosed the vessel lumen closely.

Image post-processing: diameter calculations

All MRA images were transferred to a remote workstation for quantitative analysis using the Quantitative-MRA (QMRA) software package developed at our institution. A full description of the contour detection methods used and the validation have been described previously (de Koning *et al.*, 2003). The software provides automated contour detection and quantification of the luminal boundaries in selected vessel segments in 3D MRA datasets. The only user interaction required is the definition of the vessel segment of interest by placing a proximal and distal point in the 3D dataset. Subsequently, the software detects a 3D path line following the centre of the vessel lumen and cross-sectional multiplanar recontructions are generated perpendicular to the centreline at 0.5 mm intervals. In each of these multiplanar recontructions, a contour around the vessel lumen is detected automatically. From these contours, based on the assumption of circular vessel cross-sections, the average diameter of the selected vessel segment is derived. Blood vessel segments were selected as follows: (i) the MMA was measured in an extra-cranial segment (from the origin at the maxillary artery to the end, 5–6 mm distally; Fig. 2); (ii) the ECA from the origin at the superficial temporal artery to the end, 10 mm proximally; (iii) the ICA from just proximally of the syphon to the end, 15 mm distally; (iv) the MCA, onset after A1 segment and end 8 mm distally; (v) the BA, from the origin at the PCA to the end 12 mm proximally; (vi) the PCA, beginning at the origin at BA and end 8 mm distally. Location of measured vessel segments were kept constant within subjects.

Fig. 2 MRA of the MMA region and position of the measured segment: (**A**) maxillary artery, (**B**) MMA.

Statistical analysis

We first tested the left-to-right differences in diameters for bilateral blood vessels (MMA, ICA, ECA, MCA and PCA) using paired *t*-tests. Since the differences were not statistically significant, we only present the mean diameters for the right and left blood vessels throughout the manuscript. The effect of NTG and migraine attack on blood vessel diameters and blood flow were tested using a linear mixed model. Patients with a migraine attack ($n = 20$) were compared with patients without an attack after NTG ($n = 7$). Data from patients receiving placebo were not used for statistical testing. $P < 0.05$ was considered statistically significant.

Results
Clinical effects of infusion of NTG or placebo

In total 32 migraine patients were randomly infused with either NTG ($N = 27$) or placebo ($N = 5$). Demographic characteristics of the study population are summarized in Table 1. No attack occurred after placebo (0/5). In contrast, infusion of NTG provoked a migraine attack (all without aura) in 20/27 (74%) migraine patients after a median time of 3.75 h (range: 1.5–5.5 h). In 18/20 attacks the headache was unilateral (left: $n = 9$; right $n = 9$). The clinical characteristics of the patients who developed a migraine attack in response to NTG and the clinical features of the provoked attacks are summarized in Supplementary Table s1.

Side-to-side differences for blood vessel diameters

There were no ($P > 0.05$) right-to-left differences for the diameters of the four bilateral blood vessels (MMA, ICA,

Table I Demographic characteristics of study participants

Intervention Attack	Migraine ($n = 32$)		
	NTG (27)		Placebo ($n = 5$)
	Yes ($n = 20$)	No ($n = 7$)	No
Age in years (SD)	45.5 (8.5)	34 (8.9)	44.8 (13.3)
Ratio female to males	15 : 5	7 : 0	3 : 2
Ratio MO to MA	17 : 3	6 : 1	4 : 1
Attack frequency; mean (SD)	2.6 (1.0)	2.1 (0.38)	2.4 (1.1)

MO = migraine without aura; MA = migraine with aura.

ECA, MCA, PCA) in any of the three conditions (data not shown), except for the MCA during session three ($P = 0.024$). This difference was considered not significant after correction for multiple testing. Similarly, in the 18 patients with a unilateral headache, there were no significant ($P > 0.05$) differences between the diameters on the headache and the non-headache side (Supplementary Table s4). Therefore, the mean diameters of the right and left blood vessels are presented throughout the article.

Diameter and blood flow changes during infusion of NTG or placebo

During NTG infusion there was a significant vasodilatation of all blood vessels compared with baseline (Fig. 3A–F and Supplementary Table s2; $P < 0.01$ for all blood vessels). The diameter increase was greatest in the extra-cerebral blood vessels (MMA and ECA), ranging from 16.4% to 30.3%, as compared with 6.7–20.7% diameter increase in the intra-cranial blood vessels (ICA, MCA, BA and PCA). During infusion of placebo, there were no changes in diameter for any of the blood vessels. There were no changes in ICA or BA blood flow during infusion of NTG or placebo (Fig. 4A, B and Supplementary Table s3).

Diameter and blood flow changes during migraine attacks

Compared with baseline, there were no significant ($P > 0.05$) diameter changes during attacks for any of the blood vessels (Table 2 and Fig. 3A–F). This was also true when controlling for the headache side in the 18 patients with an unilateral headache; the changes on the headache side were no different compared with those on the non-headache side (Supplementary Table s4). Similarly, there were no significant ($P > 0.05$) differences when comparing the mean diameter changes (baseline versus attack) in the 20 patients who developed a migraine attack after NTG with the changes (baseline versus 6 h post-infusion) in the seven patients who did not develop an attack and were measured 6 h after infusion. The attack versus no-attack change-differences were for the MMA = 0.06 mm (95% CI: −0.8 to 0.21), for the ECA = 0.05 mm (95% CI: −0.14 to 0.24),
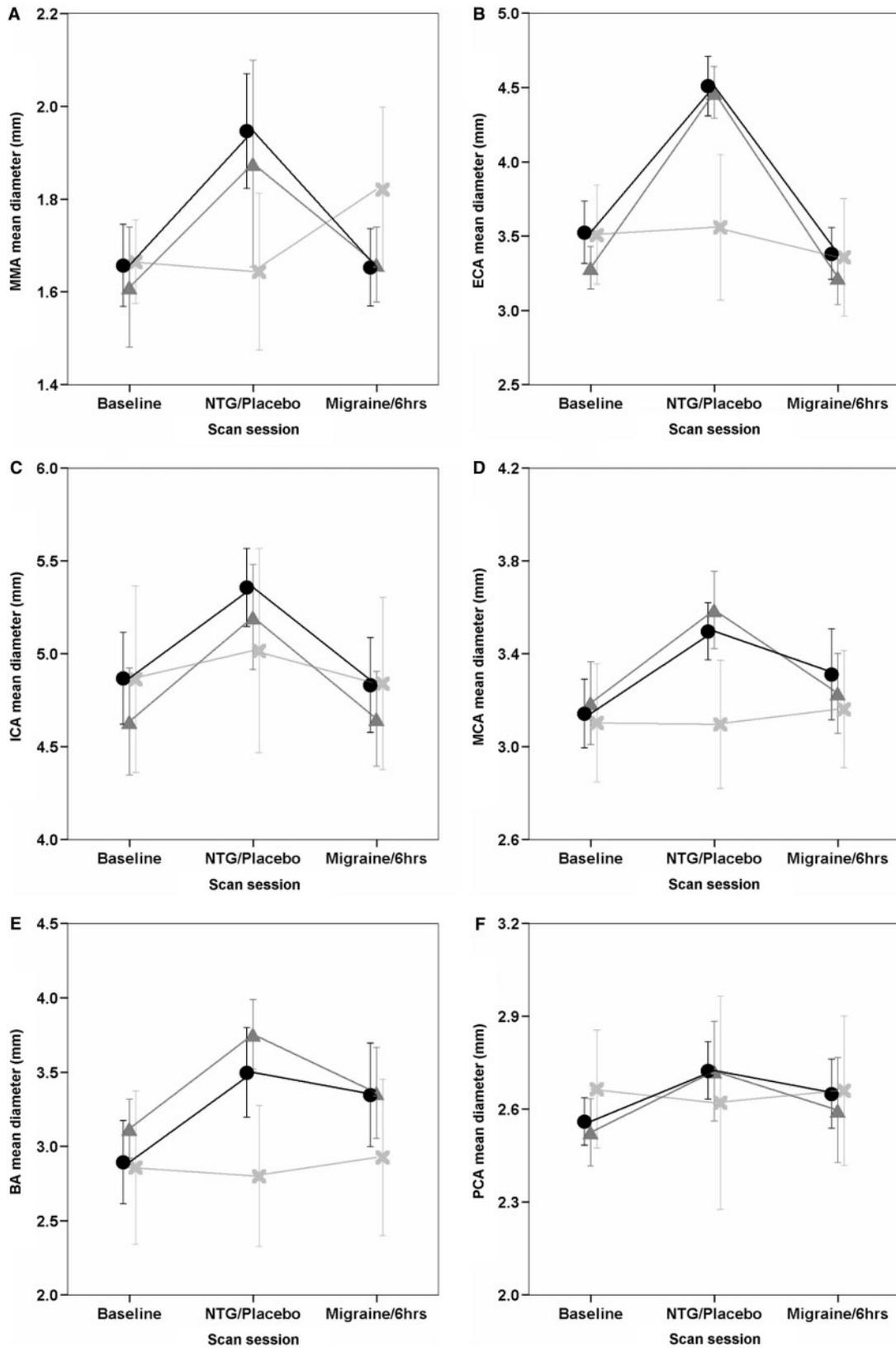
Fig. 3 (**A**–**F**) Mean blood vessel diameter changes (mean of left and right in bilateral vessels) in six selected intracranial blood vessels at baseline, during infusion of NTG or placebo, and during an NTG-provoked migraine or, if no attack had occurred, at 6 h after infusion. [filled circle = migraine patients (NTG) with a provoked attack, filled triangle = migraine patients (NTG) without an attack, cross = migraine patients (placebo) without an attack].

Fig. 4 (**A** and **B**) Mean blood flow in ICA (mean of left and right) and BA at baseline, during infusion of NTG or placebo, and during an NTG-provoked migraine or, if no attack had occurred, at 6 h after infusion. [filled circle = migraine patients (NTG) with a provoked attack, filled triangle = migraine patients (NTG) without an attack, cross = migraine patients (placebo) without an attack].
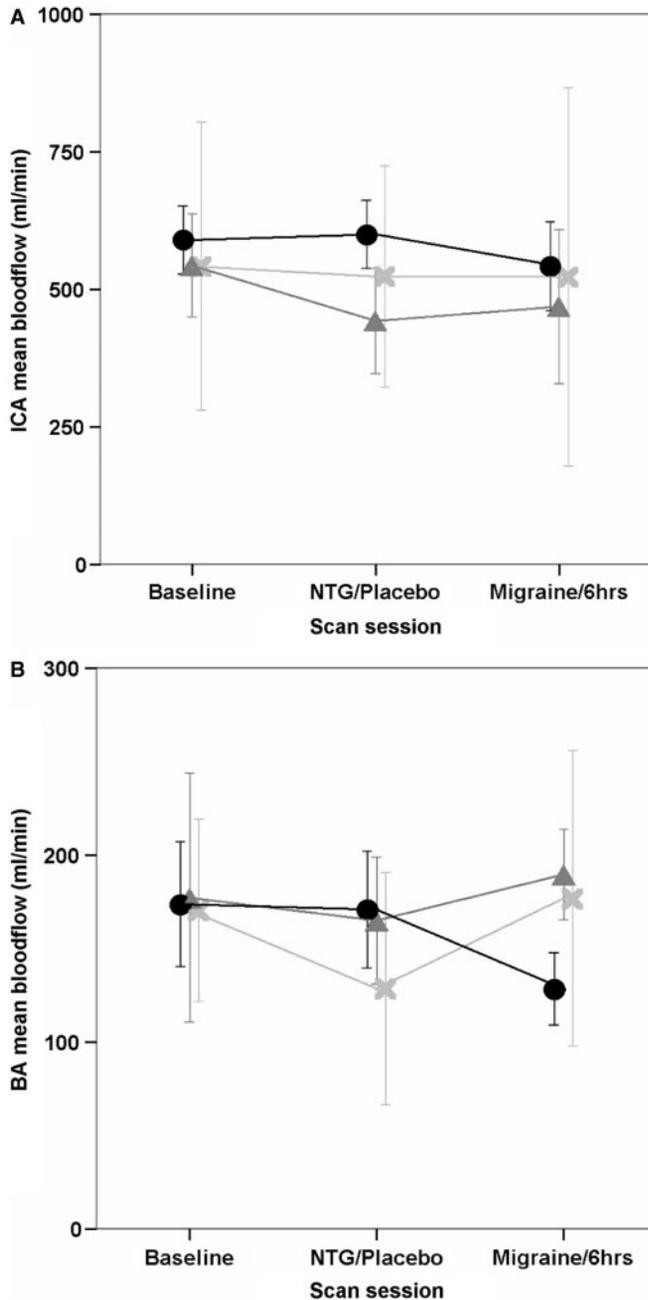
for the ICA = 0.06 mm (95% CI: −0.19 to 0.31), for the MCA = −0.13 (95% CI: −0.41 to 0.14), for the BA = −0.24 (95% CI: −0.59 to 0.11) and for the PCA = −0.02 (95% CI: −0.22 to 0.18). There were also no significant ($P > 0.05$) changes in total-, BA- or ICA-blood flow during a migraine attack when compared with baseline, nor were there significant ($P > 0.05$) differences in the changes observed during attacks when compared with the changes in the

patients who did not develop an attack and were measured 6 h after infusion (Table 3) (Supplementary Table s4).

Discussion

We used a well-established NTG provocation model to induce faithfully migraine attacks and a highly sensitive, non-invasive 3T MRA technique to visualize and measure even small intra-individual diameter changes of cerebral and meningeal blood vessels. Contrary to longstanding and widespread belief, we failed to detect any evidence for a clinically relevant vasodilatation of major cerebral or meningeal blood vessels during migraine attacks. This finding has important implications for the understanding of the pathophysiology of the migraine headache and the development of future anti-migraine agents. Novel anti-migraine treatments may not require vasoconstrictor activity as predicted earlier (Goadsby *et al.*, 1990).

In our provocation experiments, we infused NTG over a 20 min period and observed a vessel-dependent 7–30% vasodilatation at 10 min after beginning of the infusion. The vasodilatatory effect is believed to be due to a direct local effect of nitric oxide on vascular smooth muscle cells (Andresen *et al.*, 2006) or through the release of vasoactive peptides such as CGRP (Strecker *et al.*, 2002; Wei *et al.*, 1992). Our findings on the early vascular effect of NTG are in accordance with those of (Hansen *et al.*, 2007). Using 1.5T MRA they found a peak vasodilatation at 10–15 min after beginning of the NTG infusion and a normalization of the vascular diameters back to baseline at 45 min after stopping of the infusion. For logistic reasons, we did not scan at 45 min after the infusion to confirm normalization of the blood vessel diameter. However, in view of the well known short duration of action of NTG (Abrams, 1985) and the observed time course of the early vascular responses by (Hansen *et al.*, 2007), we feel confident that blood vessel diameters had returned to baseline by 1 h after the second (infusion) scan. Therefore, it seems justified to compare measurements during attacks with those obtained at baseline, before infusion.

The most important finding of the present study is that migraine headache was not associated with a clinically relevant vasodilatation of major cerebral or meningeal blood vessels, not even when controlled for headache side. We feel confident that this was not due to too low a sensitivity of the detection method. The very fact that we were able to detect an early transient vasodilatation in response to NTG of as low as 7% shows that the method we used is sufficiently sensitive to measure even small diameter changes. The clinical relevance of smaller changes is doubtful as during NTG infusion, we observed an up to 30% increase in blood vessel diameter without associated migraine headache. Our results are also in agreement with at least some older transcranial Doppler studies failing to show blood velocity changes indicative for vasodilatation during migraine attacks (Caekebeke *et al.*, 1992;

Table 2 Mean blood vessel diameters (mean of right and left for bilateral blood vessels) of six selected intracranial blood vessel at baseline and during an NTG-provoked migraine attack or, if no attack had occurred, at 6 h after infusion in 32 migraine patients

Blood vessel	Inter-vention	Migraine attack	N	(A) Baseline mm (SD)	(B) During migraine or at 6 h mm (SD)	Change (B versus A) mm (percentage from A)
MMA	NTG	Yes	20	1.66 (0.19)	1.65 (0.17)	−0.01 (−0.6)
	NTG	No	7	1.61 (0.12)	1.66 (0.08)	0.05 (3.1)
	Placebo	No	5	1.67 (0.73)	1.82 (0.14)	0.16 (9.6)
ECA	NTG	Yes	20	3.53 (0.42)	3.38 (0.36)	−0.12 (−3.4)
	NTG	No	7	3.29 (0.16)	3.22 (0.19)	−0.07 (−2.1)
	Placebo	No	5	3.51 (0.27)	3.36 (0.32)	−0.15 (−4.3)
ICA	NTG	Yes	20	4.87 (0.53)	4.83 (0.53)	−0.04 (−0.8)
	NTG	No	7	4.64 (0.31)	4.65 (0.28)	0.01 (0.2)
	Placebo	No	5	4.86 (0.41)	4.84 (0.37)	−0.02 (−0.4)
MCA	NTG	Yes	20	3.14 (0.32)	3.31 (0.41)	0.17 (5.4)
	NTG	No	7	3.19 (0.19)	3.23 (0.19)	0.04 (1.3)
	Placebo	No	5	3.10 (0.20)	3.16 (0.20)	0.06 (1.9)
BA	NTG	Yes	20	2.89 (0.60)	3.35 (0.72)	0.48 (16.6)
	NTG	No	7	3.12 (0.21)	3.36 (0.33)	0.24 (7.7)
	Placebo	No	5	2.86 (0.42)	2.93 (0.42)	0.07 (2.5)
PCA	NTG	Yes	20	2.56 (0.16)	2.65 (0.23)	0.09 (3.5)
	NTG	No	7	2.52 (0.12)	2.60 (0.18)	0.07 (2.8)
	Placebo	No	5	2.67 (0.15)	2.66 (0.19)	0.01 (0.4)

There were no significant changes in diameter during the migraine attack.

Table 3 Blood flow in the BA and ICA (mean of left and right) in migraine patients at baseline and during a migraine attack or, if no attack had occurred, at 6 h after infusion of NTG or placebo

Blood vessel	Inter-vention	Migraine attack	N	(A) Blood flow baseline ml/min (SD)	(B) Blood flow during migraine or at 6 h ml/min (SD)	Difference (B versus A) ml/min
BA	NTG	Yes	20	173.7 (69.4)	128.5 (40.1)	−46.2
	NTG	No	7	177.2 (71.9)	189.7 (26.3)	12.5
	Placebo	No	5	170.5 (39.4)	176.9 (63.6)	6.4
ICA	NTG	Yes	20	589.6 (128.5)	542.0 (166.8)	−57.7
	NTG	No	7	542.9 (101.2)	468.6 (151.2)	−74.3
	Placebo	No	5	542.0 (211.1)	522.8 (276.7)	−19.2
Total cerebral blood flow	NTG	Yes	20	763.3 (124.1)	670.5 (166.6)	−92.8
	NTG	No	7	720.1 (97.7)	658.3 (153.4)	−61.8
	Placebo	No	5	712.6 (202.4)	699.7 (253.9)	−12.8

Difference between patients with an attack compared with patients without an attack after NTG were not significant.

Zwetsloot *et al.*, 1993; Limmroth *et al.*, 1996; Gori *et al.*, 2005) Finally, BA and ICA blood flow did also not change during migraine attacks. Cerebral blood flow is dependent on cardiac output, arterial calibre and vasomotor tone in small resistance vessels (Guyton, 2006). As blood pressure (as a measure for cardiac output; data not shown) and the BA and ICA diameters had not changed, it seems likely that there were also no changes in the intracranial resistance microvasculature during migraine attacks. In conclusion, our data seem to refute an important role of cerebral or meningeal vasodilatation in causing migraine headache. This would certainly be in accordance with observations that

non-vascular mechanisms, such as exposure to sildenafil, (Kruuse *et al.*, 2003) are capable of inducing migraine attacks.

Potential limitations of our study include that we did not measure just before or at the onset of the migraine headache. We could thus have missed a brief transient vasodilatation at the very beginning of the migraine headache. Although unlikely, we cannot exclude this possibility. Another important question is whether and to what extent NTG-provoked migraine attacks are similar to spontaneous attacks. There are strong clinical and pathophysiological arguments in favour of this notion. The clinical symptoms and features, including the occurrence of

premonitory symptoms several hours before the headache (Afridi *et al.*, 2004) and the response to anti-migraine drugs (Iversen and Olesen, 1996), are strikingly similar between spontaneous and NTG-induced attacks. Likewise, in both there is an increase of CGRP in jugular venous blood (Goadsby *et al.*, 1990; Juhasz *et al.*, 2003) and activation of the dorsal rostral brainstem on positron emission tomography (Weiller *et al.*, 1995; Bahra *et al.*, 2001). The fact that NTG provokes migraine aura's only rarely, even in patients with migraine with aura (Christiansen *et al.*, 1999; Afridi *et al.*, 2005a), seems to point at a trigger site of action beyond the aura triggering mechanism. We thus feel confident that our findings in NTG-provoked attacks can be extrapolated to spontaneous migraine headaches.

In this study, we did not observe significant changes in blood vessel diameter or blood flow during the headache phase of provoked migraine attacks. However, there were some (non-significant) changes in the posterior circulation that need to be discussed. First, the diameter of the BA did not return to baseline levels, unlike the other blood vessels. This was, however, true for both patients who had developed a delayed migraine headache and for those who had not. Secondly, the blood flow in the BA was decreased (although not significantly) from 174 ml/min at baseline to 129 ml/min in patients who had developed a migraine headache after GTN, whilst there was no such change in patients who had not developed a migraine headache. Whether these findings are clinically relevant, needs to be explored. A tentative correlation, for instance, could be made with previous findings of in previous studies our group has shown our group demonstrating increased prevalence of pontine hyperintensities and cerebellar infarcts in migraineurs from the general population (Kruit *et al.*, 2004, 2006).

We conclude that, contrary to a longstanding and widespread belief, cerebral and meningeal diameter changes in migraine attacks, if at all happening, appear not to be of primary importance to the pathophysiology of the migraine headache.

Supplementary material

Supplementary material is available at *Brain* online.

Acknowledgements

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