Migraine and anti-migraine drugs

Citation for published version (APA):

de Hoon, J. N. J. M. (2000). *Migraine and anti-migraine drugs: focus on cardiovascular aspects*. [Doctoral Thesis, Maastricht University]. Universiteit Maastricht. <https://doi.org/10.26481/dis.20000928jh>

Document status and date:

Published: 01/01/2000

DOI:

[10.26481/dis.20000928jh](https://doi.org/10.26481/dis.20000928jh)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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- The final published version features the final layout of the paper including the volume, issue and page numbers.

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MIGRAINE AND ANTI-MIGRAINE DRUGS

Focus on Cardiovascular Aspects

Cover illustration: Indirect immunohistochemistry highlighting the presence of calcitonin gene-related peptide containing perivascular sensor-motor nerves in a whole mount preparation of a mesenteric resistance artery of the rat. Courtesy of Gregorio Fazzi and Jo De Mey, Vascular Pharmacology Unit, Dept. of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht (CARIM), Universiteit Maastricht, The Netherlands.

Migraine and anti-migraine drugs: focus on
cardiovascular aspects / Jan Nelly Jozef Maria de Hoon
ISBN 90-9014076-X
NUGI 741/746

The financial support of the following companies and organisations
is gratefully acknowledged:

Belgium: AstraZeneca (main sponsor for publication), Boehringer Ingelheim, Organon, Pfizer, Servier R&D Benelux. The Netherlands: Anglo-Dutch Migraine Association, GlaxoWellcome, Merck Sharp & Dohme, Pie Medical Benelux.
France: Institut de Recherche Pierre Fabre.

MIGRAINE AND ANTI-MIGRAINE DRUGS

Focus on Cardiovascular Aspects

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht
op gezag van de Rector Magnificus,
Prof. dr. A.C. Nieuwenhuijzen Kruseman,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op donderdag 28 september 2000 om 10.00 uur

door

JAN NELLY JOZEF MARIA DE HOON

Geboren te Turnhout op 8 mei 1964

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*Aan Ann, ons kindje
en mijn ouders*

*Het is met het leven
als met een proefschrift,
het komt meer op de inhoud
dan op de lengte aan.*

(naar E. Laurillard,
ter nagedachtenis aan nonkel Joe, 1928-1985)

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Part I

Introduction

*“... there was given to me a thorn in the flesh,
the messenger of Satan to buffet me ...”*

(Saint Paul, 4-64)

Chapter 1

General Introduction: Migraine and Anti-Migraine Drugs

1.1 Epidemiology of migraine

As migraine is a highly prevalent syndrome affecting up to 10% of the general population it comprises a major public health problem [1]. Among females the incidence of migraine peaks between 12 and 17 years of age, among males between 5 and 11 [2]. Migraine affects about 18% of women and 6% of men. It is 2 to 3 times more frequent in females compared to males and has the highest prevalence in persons aged 25 to 55 years [3, 4].

1.2 Clinical features and diagnostic criteria

One of the first major treatises on the subject of migraine was written by Edward Liveing in 1873 [5]. In his book *“On megrim, sick-headache and some allied disorders”* he compared migraine with epilepsy and referred to it as *“the pathology of nerve-storms”*. By carefully observing his patients Liveing meticulously described the clinical features of migraine as it was reported by his patients:

“ ... Every three or four weeks I am liable to an attack, coming on for the most part in consequence of some unhealthy influence, such as long fasting, a fatiguing evening entertainment, and so forth. I thereupon awake the next morning with a general feeling of disorder, and a slight pain in the region of the right temple, ... reaching its greatest intensity at mid-day; towards evening it usually passes off. While at rest the pain is bearable, but it is increased by motion to a high degree of violence. ... It responds to every beat of the temporal artery. ... At the height of the attack there is nausea, ... it has only once occasioned vomiting.”

Table 1.1 Classification and diagnostic criteria for migraine according to the Headache Classification Committee of the International Headache Society (1988) [6].

Migraine without aura:

- A. At least 5 attacks fulfilling B-D.
- B. Headache attacks, lasting 4-72 hours (untreated or unsuccessfully treated).
- C. Headache has at least two of the following characteristics:
 - 1. Unilateral localisation
 - 2. Pulsating quality
 - 3. Moderate or severe intensity (inhibits or prohibits daily activities)
 - 4. Aggravation by walking stairs or similar routine physical activity
- D. During headache at least one of the following:
 - 1. Nausea and/or vomiting
 - 2. Photophobia and phonophobia
- E. At least one of the following:
 - 1. History, physical- and neurological examinations do not suggest association with head trauma, vascular or non-vascular intracranial disorders, use of or withdrawal from noxious substances, non-cephalic infections, metabolic disorders or disorder of cranial or facial structures.
 - 2. History and/or physical- and/or neurological examinations do suggest such disorder, but it is ruled out by appropriate investigations.
 - 3. Such disorder is present, but migraine attacks do not occur for the first time in close temporal relation with the disorder.

Migraine with aura:

- A. At least 2 attacks fulfilling B.
 - B. At least 3 of the following 4 characteristics:
 - 1. One or more fully reversible aura symptoms indicating focal cerebral cortical - and/or brain stem dysfunction.
 - 2. At least one aura symptom develops gradually over more than 4 minutes or, 2 or more symptoms occur in succession.
 - 3. No aura symptom lasts more than 60 minutes. If more than one aura symptom is present, accepted duration is proportionally increased.
 - 4. Headache follows aura with a free interval of less than 60 minutes. (It may also begin before or simultaneously with the aura).
 - C. (See above under E. for migraine without aura).
-

To improve the diagnosis and scientific study of migraine, the Headache Classification Committee of the International Headache Society formulated diagnostic criteria in 1988 (Table 1.1) [6]. According to these criteria, migraine is characterised by attacks of moderate to severe headache accompanied by autonomic and neurological symptoms. Typical migraine headache lasts 4 to 72 hours and presents as unilateral, pulsating pain worsening upon physical activity. In case of migraine *without aura* (previously referred to as *common migraine*) headache is associated with nausea and vomiting as well as photophobia and phonophobia. However, in up to one-third of patients migraine headache is preceded by aura symptoms (previously referred to as *classical migraine*) which last up to one hour. Aura symptoms mainly consist of visual symptoms such as scintillating scotomas and visual field defects, but may also include sensory and motor symptoms such as paresthesia and paresis [7].

1.3 Pathophysiology of migraine: a historical overview

About hundred years ago, Freud's theories on the pathogenesis of migraine focused on neurophysiological hypotheses [8]. He stated "*that migraine represents a toxic effect produced by sexual stimulating substances when this cannot find sufficient discharge*". Since that time the pathophysiology of migraine has been much debated with the pendulum of interest swinging between a primary vascular and a primary neuronal theory (Table 1.2) [9]. However, recent findings indicate that elements of both theories can be reconciled in a unifying hypothesis which regards migraine as a neurovascular disorder.

The primary vascular theory

The concept of migraine being due to an inappropriate vasodilatation and increased arterial pulsations of cranial blood vessels dominated the thinking of migraine pathophysiology for several centuries. It was first recognised by the Swiss physician Wepfer [10] in the 17th century and confirmed by Graham and Wolff in 1938 [11].

Table 1.2 Migraine pathophysiology: a historical overview.

55 - 56	II Corinthians 12: 7	Was St Paul's thorn in the flesh ... migraine?
1727	J. Wepfer	Arterial distension and perivascular oedema?
1873	E. Liveing	Megrim: <i>The pathology of "nerve storms"</i> .
1895	S. Freud	Neurophysiological hypotheses.
1937	J. Graham & H. Wolff	Increased arterial pulsations causing pain.
1944	A. Leão	Cortical spreading depression causing aura.
1969	H. Heyck	Shunting through arteriovenous anastomoses.
1979	M. Moskowitz	Activation of the trigeminovascular system.
1988	P. Goadsby	Release of calcitonin gene-related peptide.
1998	M. Ferrari	Cerebral calcium channelopathy?

In their pioneer work Graham and Wolff observed an increase in the amplitude of the superficial temporal artery pulsations during the headache phase of migraine, while after the administration of ergotamine the amplitude was reduced and pain was often relieved [11]. From these data they reasoned that migraine headache is caused by stretching of branches of the external carotid artery and that it is alleviated by vasoconstriction. In 1969, during the headache phase Heyck observed a decreased arteriovenous oxygen difference in the extracranial circulation which normalised after the administration of dihydroergotamine [12]. Thus he postulated that the increased blood flow in the extracranial circulation might result from the shunting of blood through arteriovenous anastomoses which are predominantly present in the skin of the scalp and in the dura mater [13, 14]. This would also explain the paleness frequently observed during a migraine attack and is in line with recent research demonstrating that many acute anti-migraine drugs constrict arteriovenous anastomoses by 5-HT_{1B}-receptor activation in the porcine carotid circulation [15, 16].

Only recently adequate tools became available to investigate the vascular changes during migraine headache in more detail. Based on indirect measurements Drummond and co-workers [17] concluded that only in a minority of patients

dilatation of the superficial temporal artery contributes to migraine headache. In contrast, using ultrasound Iversen et al. [18] showed that the diameter of the temporal artery is significantly larger on the symptomatic than on the non-symptomatic side during an attack. Although conflicting results were obtained, it is likely that in a substantial number of migraine patients headache is associated with *extracranial* arterial vasodilatation.

Intracranial changes in arterial diameter cannot be measured directly [19, 20]. Only relative changes can be estimated by the combination of regional cerebral blood flow (rCBF) measurements, which refer to tissue perfusion controlled by resistance vessels, and transcranial Doppler sonography (TCD), which measures blood flow velocity in large arteries. If rCBF is unaffected it is assumed that a decrease or increase in arterial blood flow velocity reflects vasodilatation or vasoconstriction, respectively. Friberg et al. [21] reported that both in patients *with* and *without* aura, migraine headache is associated with a decreased blood flow velocity in the middle cerebral artery while rCBF was unaffected, indicating intracranial arterial vasodilatation. In addition, sumatriptan increased blood flow velocity, suggesting reversal of middle cerebral artery vasodilatation. Thus taken together, both intra- and extracranial arterial vasodilatations have been reported during an acute migraine attack. These observations support the primary vascular theory which states that vasodilatation causes headache by the activation of perivascular sensory nerves [22].

The transient neurological symptoms preceding headache during migraine *with* aura, have been proposed to result from cerebral hypoperfusion secondary to intracranial vasoconstriction [20]. In the early phase of migraine *with* aura, Olesen et al. [23] reported focal posterior cerebral hypoperfusion which was proportional to the severity of aura symptoms. Using novel functional magnetic resonance imaging techniques (fMRI) a decrease in cerebral blood flow in the occipital cortex was recently confirmed in migraine with aura [24, 25]. If due to hypoperfusion, transient ischaemia would affect neuronal function these data would favour a primary vascular event.

Although attractive, several lines of evidence cast doubt on the vascular theory. Firstly, in migraine patients *without* aura at no point in time focal changes in cerebral

blood flow are observed and there is no consistent relationship between arterial diameter, cerebral blood flow and headache [26, 27]. Secondly, some cranial vasodilators (e.g. nitrates and histamine) [28, 29] are capable of inducing headache, while others are not (e.g. papaverine) [30] suggesting that vasodilatation *per se* does not cause headache. Although nitric oxide (NO) donors (e.g. nitroglycerine and nitroprusside) can induce headache which resembles migraine [31, 32], this does not prove arterial vasodilatation to be the primary event. On the contrary, recent animal studies by Wei and co-workers demonstrated that vasodilatation by NO-donors results from the activation of perivascular sensory nerves and the subsequent release of calcitonin gene-related peptide (CGRP) [33]. If the same is true for man, this might explain the delayed onset of headache after the administration of nitrates. Thirdly, vasodilatation cannot only be the cause of perivascular nerve stimulation, but it can also be caused by it [22].

The primary neuronal theory

Clinical practice shows that up to 24 hours before the actual attack, migraine patients report prodromes including craving for foods, mood changes, yawning and fatigue which all indicate hypothalamic dysfunction [34]. In case of migraine *with* aura, both visual disturbances and sensor-motor symptoms are clearly of a neuronal origin. According to the neuronal theory the aura might be caused by “cortical spreading depression” (CSD) which was first reported by Leão in 1944 [35]. He described the phenomenon of CSD as a slow-moving wave of depressed cortical activity which can be induced in animals by a variety of stimuli. The speed at which the neuronal depression wave moves across the cortex (2-3 mm/min) corresponds well with the spreading of cerebral hypoperfusion which moves from occipital to frontal regions during the aura phase [36]. In line with these observations, the primary neuronal theory states that depressed neuronal activity causes the aura symptoms while arteriolar vasoconstriction, resulting in reduced cerebral perfusion, is merely an epiphenomenon due to the reduced metabolic demand. However, proof of the existence of CSD in human is not yet available [37].

Figure 1.1 Pathophysiological mechanism of migraine (Reproduced with permission from Moskowitz MA, *Trends Pharmacol Sci* 1992; 13: 307-311).

The unifying hypothesis: the trigeminovascular system

The current view is that migraine is a neurovascular disorder of which the trigeminovascular system is the anatomical substrate [38]. The trigeminovascular system comprises of pain sensitive intracranial structures (i.e. meninges and blood vessels) and the major afferent pathway for transmitting pain from these structures constituted by the first division of the trigeminal nerve and its central projections into the trigeminocervical complex (i.e. the trigeminal nucleus caudalis and the C₁-C₂ superficial dorsal horns). From these brainstem regions nociceptive information is transmitted to the thalamus and cortical areas to produce pain while projections into the nucleus tractus solitarius mediate autonomic symptoms including nausea and vomiting [39].

In 1979, Moskowitz hypothesised that activation of the trigeminovascular system is essential for the generation of migraine headache (Fig. 1.1) [40]. Both in animal and in human it has been shown that the activation of this system, by stimulation of the trigeminal ganglion, results in the central transmission of pain and the retrograde release of neurotransmitters [41, 42]. These neurotransmitters including CGRP, substance P (SP) and neurokinin A (NKA) are vasodilator neuropeptides contained within unmyelinated perivascular sensory nerves [38]. The effects of SP and NKA, which also promote plasma protein extravasation, are mediated by interaction with NK₁ and NK₂ receptors, respectively [43]. Activation of these receptors is coupled to the stimulation of phospholipase C and involves the endothelial release of NO. CGRP exerts its biological effects by interaction with CGRP-receptors (CGRP₁ and CGRP₂) which, based on *in vitro* data, involves both endothelium-dependent and endothelium-independent mechanisms [44]: (i) activation of vascular smooth muscle cell adenylate cyclase [45-47]; (ii) the endothelial release of NO [48, 49] and (iii) the activation of adenosine-5'-triphosphate-sensitive potassium channels (K⁺_{ATP}-channels) [50-52]. However, *in vivo* human research on the vasodilator mechanism of action of CGRP is still lacking (see Chapter 5).

In animal experiments, neuropeptide release promotes neurogenic inflammation which is characterised by vasodilatation, plasma protein extravasation, platelet aggregation and mast cell degranulation [22, 41]. Although neurogenic inflammation is suggested to be a defence mechanism to noxious stimuli, the substances released by it might sensitise sensory nerves and induce sustained headache. Recently, Goadsby and co-workers were the first to demonstrate that during migraine headache the trigeminovascular system is indeed turned on and CGRP is released into the cranial circulation [53]. However, plasma protein extravasation has not yet been shown to constitute an important component in the pathophysiology of migraine [54].

Taken together, according to the neurovascular theory, activation of the trigeminal pathway is the primary event in migraine headache and results in the secondary perivascular release of vasodilating neuropeptides (e.g. CGRP and SP) initiating neurogenic inflammation. Alternatively, the centrally projecting C-fibres release neurotransmitters (e.g. CGRP, SP and glutamate) which activate second order

neurones within the trigeminal nucleus and result in the central transmission of pain [22]. In this cascade, recent evidence indicates an important role for glutamate and N-methyl-D-aspartate (NMDA) receptor activation for the transmission of nociceptive information within the trigeminocervical complex [55-58].

Although the trigeminovascular theory provides a sound framework for linking the neuronal and vascular changes observed in migraine headache, the coupling mechanism between cerebral events (i.e. aura symptoms) and the activation of the trigeminovascular system remains uncertain. One of the potential links is NO. During CSD, glutamate is released and NMDA receptor-operated calcium channels are activated [59]. The resulting influx of calcium is responsible for the propagation of spreading depression and the calcium-dependent activation of NO-synthase [60]. Interestingly, NO has been shown to activate perivascular sensory nerves and plays a regulatory role in the release of neuropeptides [33, 61].

The trigger initiating migraine

To date, none of the previously discussed hypotheses has been able to identify the trigger initiating the vascular and/or neuronal changes associated with migraine headache. As an isolated migraine attack can occur in any subject, it is not the individual attack but the repeated occurrence of it which is considered abnormal and defines migraine as a disease [1]. Therefore, it is suggested that migraine patients are either susceptible subjects with a low “migraine threshold” or are exposed to frequent and/or strong migraine-inducing triggers.

Based on epidemiological data, many environmental factors have been reported to precipitate migraine [62]. Among these stress, weather changes, hormonal fluctuations, exposure to bright light, hunger and fatigue are most frequently mentioned. In addition, about 30% of patients report food-induced migraine and commonly refer to alcohol (red wine in particular), chocolate and certain cheeses (containing tyramine or phenylethylamine) as precipitants for headache [63]. In view of the ease and safety with which these triggers can be manipulated, their role in the management of migraine should not be forgotten.

According to Olesen and co-workers [64, 65] NO (or endothelium derived relaxing factor, EDRF) is a likely candidate for the triggering molecule initiating migraine headache. It was demonstrated that migraine patients suffer a more intense migraine-like headache and display an increased vasodilatation of the middle cerebral artery after the administration of NO-donors (i.e. nitrates) compared to healthy subjects [66]. Based on these data it was concluded that migraine patients display an arterial supersensitivity to NO. This view is supported by the observation that other substances including prostacyclin, methacholine and adenosine which are known to induce the endothelial release of NO, can also induce migraine headache [67-69]. Although NO is likely to be involved in the pathophysiology of migraine, it remains unknown whether it exerts its effects predominantly at the level of the blood vessel wall, at the level of perivascular nerves or both. However, the latency period between the exposure to organic nitrates and the onset of migraine-like headache suggests that NO initiates a slow pathological process which eventually leads to the attack [65]. This pathological process could well be the activation of the trigeminovascular system (see Chapter 4).

With regard to the “migraine threshold”, biochemical [70], neurophysiological [71] and magnetic resonance spectroscopic [72] data suggest an increased cortical excitability and increased responses during the interictal period. In this context, there is increasing evidence that genetic factors might set the scene for migraine [73]. For instance, in familial hemiplegic migraine, a rare autosomal dominant type of migraine with aura which is associated with hemiparesis, mutations have been reported in the *CACNA1A* gene encoding the α_{1A} -subunit of a brain specific voltage-gated P/Q-type calcium channel [74]. Mutations in the *CACNA1A* gene alter calcium channel function and are likely to be involved in typical migraine both with and without aura [75, 76]. As mutant calcium channels might be implicated in CSD and abnormal neurotransmitter release, Ferrari states that migraine can be regarded as a “cerebral calcium channelopathy” [1].

Finally, studies using positron emission tomography (PET) have identified a brainstem region which is activated during migraine headache and remains activated after pain relief by sumatriptan [77]. These data are suggestive for the existence of a

central “migraine generator” triggering the attack and persisting after successful treatment which alleviates symptoms without affecting the “migraine generator” itself.

1.4 Anti-migraine drugs

The physiological mechanisms by which anti-migraine drugs relieve migraine headache are still not fully elucidated. Thinking of migraine in terms of an activation of the trigeminovascular system, three distinct pharmacological sites of action can be identified: (i) the cranial blood vessels (both meningeal and cerebral), (ii) the peripheral trigeminal sensory nerves innervating these vessels, and (iii) the central projections of the trigeminal nerve and the trigeminocervical complex [78]. The desired pharmacological effects at each of these levels comprise of (i) vasoconstriction, (ii) inhibition of perivascular nerve activation and/or neurogenic inflammation and (iii) attenuation of the excitability of trigeminal neurones, respectively. In the present overview the pharmacology of drugs used in the acute treatment of migraine is discussed. Attention is focused on both anti-migraine activity and cardiovascular effects.

Non-specific drugs

Using a stepped-care approach, migraine patients should first be treated with non-specific drugs including simple analgesics (e.g. paracetamol and acetylsalicylic acid) and non-steroidal anti-inflammatory drugs (NSAIDs, e.g. ibuprofen and naproxen) [79]. Because gastric stasis often accompanies migraine attacks, anti-emetics are frequently associated to increase drug absorption and enhance the effectiveness of the treatment [80]. Compared to specific anti-migraine drugs, several non-specific drug regimens (e.g. the combination acetylsalicylic acid and metoclopramide) have been shown to be equally effective [81]. The mode of action of NSAIDs probably relates to their ability to block neurogenic inflammation by inhibiting plasma protein extravasation [82].

Ergot alkaloids

The ergot alkaloids were the first specific compounds reported to be successful in the acute treatment of migraine [83]. Ergotamine was isolated in 1918 from the fungus *Claviceps purpurea* and reported effective as an anti-migraine drug in 1926. However, because of its unfavourable side effect profile the dihydrogenated derivative dihydroergotamine (DHE, Fig. 1.2) was developed and became available in 1945 [84]. Both ergotamine and DHE are non-selective pharmacological compounds interacting with serotonergic (5-HT₁ and 5-HT₂-receptors), adrenergic (α_1 and α_2 -receptors) and dopaminergic receptors (D₂-receptors) [84, 85]. DHE differs from ergotamine in that it is less toxic, less emetic and claimed to display a decreased peripheral vasoconstrictor activity [84, 86].

Despite their wide acceptance as anti-migraine drugs, ergot alkaloids have a very low oral and rectal bioavailability (5% or less) and limited placebo-controlled data are available on their clinical efficacy [87]. The anti-migraine activity of the ergot alkaloids results from cranial vasoconstriction and inhibition of plasma protein extravasation [88, 89]. The low incidence of headache recurrence observed in clinical practice has been attributed to their long lasting vasoconstrictor effects [90].

Because of sustained systemic and coronary vasoconstriction ergot alkaloids are contraindicated in patients with cardiovascular disease [91-93]. The vasopressor effects of the ergot alkaloids are the combined result of actions on (i) pre-synaptic α_2 and D₂-receptors, inhibiting noradrenaline efflux, (ii) post-synaptic α_1 , 5-HT_{1B} and 5-HT_{2A} receptors, mediating vasoconstriction, and (iii) central blood pressure control mechanisms [94]. Concerning arterial effects, *in vitro* data show that ergotamine and DHE induce a sustained contraction of human arteries [93, 95, 96]. For ergotamine, long lasting arterial contractions and a discrepancy between plasma concentrations and arterioconstrictor activity have also been reported in human [91]. Both in animal [97] and in human [98-100] the same discrepancy has been demonstrated for the venoconstrictor activity of DHE. In contrast, data on the arterial and arteriolar effects of DHE in human are scarce. Although based on indirect measurements, Andersen et al. [101] concluded that DHE lacks peripheral arterial effects, direct assessments of the arterial effects of DHE have never been performed in human (see Chapter 7).

Name	Structure
F 11356	
Sumatriptan	
Zolmitriptan	
Rizatriptan	
Naratriptan	
Avitriptan	
Eletriptan	
Frovatriptan	
Almotriptan	
Dihydroergotamine	
Alniditan	

Figure 2.1 Chemical structures of anti-migraine drugs.

Sumatriptan

Prior to the development of sumatriptan, attention focused on the role of the disturbance in 5-hydroxytryptamine (5-HT, serotonin) metabolism in migraine patients [102]. Compared to healthy control subjects, between attacks 5-HT plasma concentrations are lower in migraine patients [103]. During migraine headache, platelet 5-HT content falls while plasma 5-HT concentration and urinary 5-hydroxyindole-3-acetic acid (5-HIAA) secretion, a metabolite of 5-HT, are increased [104]. The ictal rise in plasma 5-HT concentration probably reflects a self-defence mechanism. This is supported by the observations that reserpine, which lowers plasma 5-HT concentrations, can induce migraine in susceptible subjects while the intravenous administration of 5-HT can abort the attack [105]. In view of these data, it was hypothesised that compounds might be designed which retain the efficacy of 5-HT in the treatment of migraine headache but are devoid of its unacceptable side effects (e.g. platelet aggregation, bronchoconstriction and generalised vasoconstriction).

As a result of this research in the early 1990s sumatriptan (GR 43175) was the first selective 5-HT_{1B/1D}-receptor agonist to become available as an anti-migraine drug and still is one of the most effective and generally well tolerated treatments [106, 107]. Disadvantages include its poor oral bioavailability, hydrophilicity limiting central nervous system penetration and short plasma half-life responsible for frequent headache recurrence in patients who initially respond well (Table 1.3) [108-111]. However, the major limitation for the use of sumatriptan are chest symptoms which are reported by up to 15% of patients [112-114]. Although coronary vasoconstriction is an unlikely cause for these chest symptoms in most patients, sumatriptan has occasionally been associated with both myocardial infarction and sudden death [115, 116]. In addition to coronary vasoconstriction, the systemic vasoconstrictor effects of sumatriptan have also clearly been demonstrated in human and rendered the drug contraindicated in patients with cardiovascular disease [93, 117-119]. Therefore, recent research aimed at the development of “sumatriptan-like” drugs which should be at least equally effective but display increased cardiovascular safety and an improved pharmacokinetic profile.

Table 1.3 Pharmacokinetic and efficacy data of sumatriptan and second-generation triptans after oral intake.

<i>Triptan</i> (recommended dose)	<i>Bioavailability</i> (%)	<i>t_{max}</i> (h)	<i>t_{1/2,z}</i> (h)	<i>Lipophilicity^a</i> (Log <i>D_{pH7.4}</i>)	<i>Clinical efficacy^b</i> (%)
Almotriptan (6.25-12.5 mg) ¹²⁵	70	2.5	3.5	NAV	57 ^d
Eletriptan (40-80 mg) ¹²⁶	50	1-1.3	4	+0.5	65-77
Frovatriptan (2.5 mg) ¹²⁷	22-30	2.3-3	26	NAV	38-48 ^d
Naratriptan (2.5 mg) ¹²⁰	63, 74 ^c	2-3	6	-0.2	45-51
Rizatriptan (10 mg) ^{121,122}	45	0.9	2.0	-0.7	67-80
Sumatriptan (50-100 mg) ^{110, 111}	14	1.5	2.0	-1.5	51-61
Zolmitriptan (2.5-5 mg) ^{123, 124}	40	2.5	2.5, 3.0 ^c	-1.0	59-70

t_{max}: time to reach maximum plasma concentration; *t_{1/2,z}*: terminal half-life; NAV: not available. ^aLipophilicity refers to Log*D_{pH7.4}* values defined as the logarithm of the distribution coefficients in a two-phase system of octanol/buffer at pH 7.4; the more positive, the more lipophilic and the more negative, the more hydrophilic. ^bClinical efficacy refers to pain relief at 2 h, i.e. the proportion of patients whose headache severity improved from moderate or severe at baseline to mild or no pain on a four-point scale within 2 hours of treatment. ^cValues refer to men and women respectively. ^dOnly very limited data available.

Table 1.4 Receptor binding properties of triptans at human recombinant 5-HT₁ receptors (pK_i values)^{78, 132-134}.

Receptor	Almotriptan	Eletriptan	Frovatriptan	Naratriptan	Rizatriptan	Sumatriptan	Zolmitriptan
5-HT _{1A}	6.3	7.35	7.3	7.12	6.37	5.96	6.64
5-HT _{1B}	8.0	7.82	8.23	9.32	8.14	8.5	9.08
5-HT _{1D}	8.0	8.82	8.52	9.16	8.63	8.68	9.66
5-HT _{1E}	NAV	7.25	<6.0	7.69	6.77	5.79	7.73
5-HT _{1F}	NAV	7.72	7.20	8.4	6.86	7.78	7.54

NAV: not available.

Second generation triptans: the "triptan war"

Together with sumatriptan, the majority of the more recently developed 5-HT_{1B/1D}-receptor agonists are tryptamine derivatives which are collectively referred to as "triptans" (Fig. 1.2) [78]. Naratriptan (GR 85548) [120], rizatriptan (MK 462) [121, 122] and zolmitriptan (311C90) [123, 124] were recently marketed while almotriptan (LAS 31416) [125], eletriptan (UK 116044) [126] and frovatriptan (VML 251/SB 209509) [127] will shortly become available. Although these triptans have a higher bioavailability, longer plasma half-life and increased lipophilicity compared to sumatriptan (Table 1.3), their clinical efficacy increased only marginally and headache recurrence has not decreased substantially [79, 111, 128]. F 11356 [129] and IS-159 [130] are in clinical development whereas, despite their effectiveness, avitriptan (BMS 180048) [131] and alniditan (R 091274, a non-indole derivative) [132] will not be commercialised.

Triptans: pharmacology and mode of action

Based on their pharmacological profile the triptans as a class can be regarded as selective 5-HT₁-receptor agonists (Table 1.4) [78, 133-135]. They bind to human 5-HT_{1B} (previously known as 5-HT_{1DB}) [136] and 5-HT_{1D} (previously known as 5-HT_{1DW}) [136] receptors with high affinity, and to a lesser extent to 5-HT_{1F} receptors. The triptans have virtually no affinity to the other classes of 5-HT receptors (5-HT₂₋₇). Notable exceptions are zolmitriptan and F 11356 which display affinity to the 5-HT₇ (pK_i 7.02) and 5-HT_{2A} (pK_i 6.57) receptor, respectively. A higher affinity to the 5-HT_{2B} receptor is noted for zolmitriptan (pK_i 7.19) and rizatriptan (pK_i 6.59).

The distribution of 5-HT_{1B} and 5-HT_{1D} receptors and their functional involvement in the anti-migraine activity of triptans have been clarified by pharmacological, immunocytochemical and molecular techniques [137-140]. It was demonstrated that within the trigeminovascular system 5HT_{1B} receptors are only present on meningeal smooth muscle cells while 5-HT_{1D} receptors are expressed on trigeminal sensory neurones and their peripheral and central projections. It is now generally accepted that the anti-migraine activity of both sumatriptan and second-generation triptans is the combined result of cranial vasoconstriction by 5-HT_{1B}-receptor activation and

inhibition of neuropeptide release by presynaptic 5-HT_{1D}-receptor activation [141-143]. In addition, there is convincing evidence that increased lipophilicity permits second-generation triptans to penetrate into the brain and suppress neuronal activity within the trigeminal nuclei by 5-HT_{1D}-receptor stimulation [144-146]. As a consequence, central pain transmission is interrupted and autonomic symptoms are reduced.

Although sumatriptan has also been suggested to interfere with central pain transmission, because of its hydrophilic nature it is not expected to penetrate into the brain. However, it cannot be excluded that during the process of neurogenic inflammation the blood-brain barrier is disrupted and access is gained into the central nervous system [147, 148].

Triptans: focus on cardiovascular effects

Most second-generation triptans are selective 5-HT_{1B/1D}-receptor agonists characterised by an *in vitro* pharmacology comparable to that of sumatriptan in terms of receptor selectivity, potency and efficacy (Table 1.4) [78]. Thus, from a pharmacological point of view, there is little reason to expect that second-generation triptans will display a profoundly different cardiovascular profile.

Which are the receptors involved in the vascular effects of the triptans? The vascular effects of 5-HT and its derivatives are determined by interactions with 5-HT receptors both on vascular smooth muscle cells, including 5-HT_{1B}, 5-HT_{2A} and 5-HT₇ receptors, and on the endothelium, mainly 5-HT_{1B} and 5-HT_{2B} receptors [149-151]. The 5-HT₁ receptor subtypes are coupled with inhibition of adenylate cyclase, 5-HT₂ receptor subtypes with stimulation of phospholipase C and the 5-HT₇ receptor with enhancement of adenylate cyclase activity. Endothelial 5-HT receptors mediate the release of vasodilator substances, including NO/EDRF, resulting in vasodilatation [152]. Interactions with 5-HT_{1B} and 5-HT_{2A} receptors on smooth muscle cells mediate vasoconstriction whereas 5-HT₇ receptors probably induce vasodilatation in the absence of endothelium [149, 153].

Thus, 5-HT and 5-HT analogues interact in a complex way with different 5-HT receptors which display opposite functional effects in the regulation of vasomotor

tone. If the endothelium plays a pivotal role in providing a counteractive mechanism for the vasoconstrictor effects of triptans, this constitutes a sound rational why patients with endothelial dysfunction are at increased risk for unwanted coronary vasoconstriction after the intake of 5-HT-receptor agonists [154, 155].

In contrast with cerebral blood vessels in which 5-HT_{1B} receptors predominate, there is a low expression of 5-HT_{1B} receptors in peripheral and coronary arteries [141]. In these vessels 5-HT_{2A} receptors play a more important role in 5-HT-induced vasoconstriction [156, 157]. This differential anatomical distribution of 5-HT_{1B} receptors provides a pharmacological basis for the craniovascular selectivity of 5-HT_{1B/1D}-receptor agonists which display a low affinity for 5-HT_{2A} receptors. However, as the clinical efficacy of the currently available triptans is at least in part related to their ability to constrict cranial vessels, they are all likely to cause peripheral and coronary vasoconstriction as well by 5-HT_{1B} receptor activation. Thus, although second-generation triptans are often claimed to display an increased cranioselectivity it is not entirely clear how to gain this increased selectivity.

In vitro data indicate that many second generation triptans contract both human coronary and cranial arteries with a potency and maximal effect comparable with that of sumatriptan [93, 141, 158, 159]. However, as the clinical relevance of these *in vitro* data remains speculative, *in vivo* human research is needed to better define the cardiovascular profile of these drugs by comparing their vascular effects at clinically relevant plasma concentrations. Thus, until proven otherwise, peripheral and coronary vasoconstriction can be regarded a class effect of the triptans (see Chapter 6).

Beyond the triptans

To date, the clinical use of all acute anti-migraine drugs is limited by their potential to induce systemic and coronary vasoconstriction by 5-HT_{1B} receptor activation [93]. Therefore, current research is aimed at the development of effective drugs devoid of 5-HT_{1B} receptor affinity which could, if proven clinically effective, represent a neuronal site of action and display an increased cardiovascular safety [138].

The development of selective 5-HT_{1F}-receptor agonists (referred to as "SSOFRA") and/or 5-HT_{1D}-receptor agonists is one possible approach. Based on

studies with the selective 5-HT_{1F}-receptor agonist LY 334370, these receptors most likely inhibit neurotransmitter release within the trigeminal nucleus caudalis [160, 161]. Presynaptic inhibition of neurotransmitter release probably blocks the activation of second-order neurones and the central transmission of nociceptive stimuli. Both *in vitro* data and preliminary human data indicate that LY 334370 lacks vasoconstrictor properties while it was proven to be clinically effective at high dose [162, 163]. However, as alniditan and rizatriptan are clinically effective triptans with a low affinity for 5-HT_{1F}-receptors, the role of 5-HT_{1F}-receptor activation in the treatment of migraine remains obscure [78, 164]. In contrast with selective 5-HT_{1F}-receptor agonists, the selective 5-HT_{1D}-receptor agonist CP-122,288 was ineffective in the treatment of migraine [165], questioning the potential of this approach.

A second approach might be the development of inhibitors of plasma protein extravasation. However, many compounds which potently inhibit plasma protein extravasation in animal experiments do not abort migraine attacks in clinical practice. These compounds include CP-122,288 [165], bosentan (endothelin-receptor antagonist) [166] and the NK₁-receptor antagonists RPR 100893 [79] and lanepitant (LY 303870) [167]. Surprisingly, avitriptan [131] and BMS-181885 [168], which are effective drugs, do not reduce dural plasma protein extravasation. These observations indicate that inhibition of plasma protein extravasation in animal models may not be related to clinical efficacy [78].

As indicated, NK₁-receptor antagonists interfering with neurogenic inflammation, did not prove to be clinically effective. In contrast with SP, CGRP and NO are considered much more important in initiating neurogenic vasodilatation [22]. Consequently, the development of both CGRP-receptor antagonists and NO-synthase inhibitors became a major field of research which recently resulted in the first potent, selective non-peptidergic CGRP antagonist (BIBN4096BS) [169]. BIBN4096BS completely inhibits neurogenic vasodilatation in animal experiments and is currently being evaluated for its clinical efficacy as an anti-migraine drug.

Alternatively, NO-synthase inhibitors are being developed as well. As previously discussed, NO might be involved at several stages of migraine pathophysiology: (i) as a triggering molecule initiating migraine [170], (ii) as a

coupling mechanism between CSD and activation of the trigeminovascular system [60], (iii) as a regulator of neuropeptide release [33] and (iv) as EDRF mediating the vasodilating effects of CGRP and SP [43]. Although selective NO-synthase inhibitors are not yet available for clinical use, preliminary results obtained in migraine patients with L-N^G-methylarginine (546C88) suggest that NO-synthase inhibition may represent a promising therapeutic principle in the treatment of migraine [171].

Finally, the role of NMDA-receptor mediated activation of the trigeminocervical complex by glutamate is gaining increasing interest as a potential target for future anti-migraine drugs. Obviously, in addition to selective 5-HT_{1F}-receptor agonists, selective NMDA-receptor antagonists might offer an alternative approach for interrupting the central transmission of pain [59].

1.5 Migraine and cardiovascular disease

During a migraine attack vascular changes are not limited to the cranial circulation. Iversen et al. [18] reported that a migraine attack is characterised by a generalised peripheral vasoconstriction and concluded that migraine headache is accompanied by abnormal reactions of the arterial system. In addition, the association of migraine with angina pectoris due to coronary artery vasospasm (i.e. Prinzmetal's angina) [172-174] and the increased frequency of Raynaud's syndrome in migraine patients [175], suggest that migraine might be part of a generalised vasospastic disorder [172]. Furthermore, migraine patients have repeatedly been reported to be at increased risk for the development of ischaemic stroke [176-178], supporting the notion of migraine being associated with vascular abnormalities. As vascular endothelial mechanisms and autonomic transmission are intimately linked, it has even been suggested that migraine might be an "endotheliopathy" [179]. Indeed, many vasoactive substances including adenosine-5'-triphosphate (ATP), SP, CGRP, 5-HT and acetylcholine (ACh) have a role in the autonomic nervous system and each of them interacts with endothelial receptors mediating the release of NO.

Taken together, it cannot be excluded that migraine is accompanied by vascular changes which extend beyond the cranial circulation and are also present during the interictal period. However, neither the structural nor the functional vascular properties and endothelial function have ever been investigated *in vivo* in migraine patients (see Chapters 3 and 4).

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*"The ability to convert ideas to things
is the secret of outward succes".*

(Henry Ward Beecher)

Outline and Aims of the Present Thesis

Migraine is a neurovascular disorder in which the trigeminovascular system plays a pivotal role. Consequently, research has focused on the central nervous system, cranial blood vessels and the connection between both provided by the trigeminal nerve. Although structural and functional extracranial vascular research has hardly ever been performed in migraine patients, peripheral vascular disorders are probably associated with it, vascular changes are at least part of the headache phase, and acute anti-migraine drugs affect the cardiovascular system as a whole. Therefore, the objective of the present thesis was to study migraine and its treatment by performing cardiovascular research on migraine pathophysiology and acute anti-migraine drugs. The specific aims were the following:

1. To make an inventory of the cardiovascular properties of migraine patients during the interictal period compared to those of matched control subjects (*Chapter 3*).
2. To challenge the concept of nitric oxide (NO) supersensitivity and to assess the vascular reactivity to calcitonin gene-related peptide (CGRP) in migraine patients (*Chapter 4*).
3. To investigate the vasodilator mechanism of action of CGRP at clinically relevant plasma concentrations *in vivo* in human (*Chapter 5*).
4. To compare the effects of selective 5-HT_{1B/1D}-receptor agonists on large artery wall properties, resistance vessels and endothelial function in migraine patients (*Chapter 6*).
5. To investigate the time-course of the peripheral vascular effects of dihydroergotamine and its pharmacokinetics after a single subcutaneous administration to healthy subjects (*Chapter 7*).

All research was performed in migraine patients during the interictal period or in healthy volunteers. The most important techniques for haemodynamic assessments used in the present thesis (*Chapter 2*) comprise of non-invasive ultrasound measurements of cardiovascular parameters and venous occlusion plethysmography applied to the “perfused forearm model”. In the last part (*Chapters 8 and 9*) the research results are discussed in terms of their implications for migraine pathophysiology and for the future development of anti-migraine drugs.

Chapter 2

Methods for Haemodynamic Assessments Used in the Present Thesis

All haemodynamic assessments reported in this thesis, were performed in a quiet and temperature controlled room. The subjects had been lying in the supine position for at least 15 and 30 minutes before assessments were started in the non-invasive and invasive study protocols, respectively.

2.1. Blood pressure, pulse pressure and heart rate

Oscillometric measurements

Using a semi-automated oscillometric device (Dinamap® 950, Critikon Inc., Florida) resting heart rate (beats per min, bpm), brachial artery systolic blood pressure (SBP, mmHg) and diastolic blood pressure (DBP, mmHg) were measured at the left upper arm [1]. Depending on the study, every 1 to 3 minutes recordings were made in order to obtain at least 3 measurements within the available time frame; the mean of these recordings was taken as the subject's reading. Brachial artery pulse pressure was calculated as SBP-DBP.

Applanation tonometry

Using applanation tonometry, pressure waveforms were recorded at the common carotid, brachial and femoral artery (*Chapters 3, 6 and 7*). The applanation tonometer (Micro-tip® transducer, Millar Instruments Inc., Texas) is a pencil-like probe incorporating a small pressure sensitive transducer which is placed in contact with the skin and permits continuous and accurate recording of pressure waveforms of

superficial arteries [2]. Although, applanation tonometry has been proposed for local pulse pressure measurement, the accuracy of absolute pulse pressure values obtained with this technique remains a matter of debate. Therefore, an alternative approach was developed for local non-invasive pulse pressure assessment at peripheral arteries in human by calibrating the original pressure waveforms (see section 2.2) [3].

Invasive blood pressure measurements

Invasive blood pressure measurements were performed during those studies in which the “perfused forearm model” was used (see section 2.3 and *Chapters 4 and 5*).

2.2 Large artery wall properties: a novel approach

Arterial diameter and distension

In the studies presented in this thesis the echo-tracking system developed by Hoeks and collaborators [4] was used to measure arterial diameter and distension. The system consists of an ultrasound device (Scanner 350, Pie Medical, The Netherlands) equipped with a 7.5 MHz linear-array transducer connected to a data acquisition and processing unit (Wall Track System[®], Pie Medical, The Netherlands). For a detailed description of the principles of the vessel wall movement detector system we refer to the paper by Hoeks et al. [4]. In brief, the system makes use of Doppler signal processing to assess non-invasively (transcutaneous) the displacement of arterial walls during the cardiac cycle. The resulting distension waveform, i.e. the diameter as a function of time, allows for the off-line calculation of the change in arterial diameter during the cardiac cycle (distension) relative to its initial end diastolic diameter for each heart beat. The system has previously been validated and has shown acceptable reproducibility for the measurement of large artery wall properties of the common carotid, brachial and common femoral artery [5]. In addition, because of its putative importance in the pathophysiology of migraine (see *Chapter 1*), the variability of temporal artery diameter measurements was assessed during the preparation of the protocols presented in this thesis. The intra- and inter-session coefficients of variation (expressed as percentage) averaged 5.6 ± 2.1 and $5.0 \pm 3.1\%$, respectively.

All data on arterial measurements presented in this thesis are the mean of at least 3 recordings of diameter and distension; at the temporal artery diameter was recorded at least 5 times. As each recording lasted approximately 5 seconds (about 5 heart beats), data refer to the mean of about 15 heart beats (25 for the temporal artery). If within one recording of approximately 5 heart beats diameter or distension was unstable (i.e. standard deviation >10% of the mean), the recording was discarded and repeated.

At the common carotid artery, ipsilateral vessel wall movements and pressure waveforms were recorded consecutively as a function of time. At the brachial and femoral artery recordings were performed simultaneously. Therefore, after obtaining a satisfactory image of the artery under investigation for vessel wall tracking, the ultrasound transducer was fixed with a robotic arm (without touching the skin) while pressure waveforms were recorded at the contralateral side. In order to minimise motion artifacts, temporal artery diameter measurements were also obtained by fixing the ultrasound transducer.

Vessel wall properties: the “classical” approach

Distensibility, a measure of elasticity, and cross-sectional compliance, a measure of buffering capacity, are parameters which refer to local mechanical vessel wall properties [6]. Using end diastolic diameter (D, mm) and distension (ΔD , μm) the end diastolic cross-sectional area (CSA, equation 2.1) and the change in cross-sectional area during the cardiac cycle (ΔCSA , equation 2.2) were calculated:

$$\text{CSA} = \pi(D/2)^2 \quad \text{mm}^2 \quad (2.1)$$

$$\Delta\text{CSA} = \pi((D+\Delta D)/2)^2 - (D/2)^2 \quad \text{mm}^2 \quad (2.2)$$

Subsequently, the distensibility coefficient (DC, equation 2.3) and cross-sectional compliance (CC, equation 2.4) were calculated by making use of the local pulse pressure (ΔP) [7]:

$$\text{DC} = (\Delta\text{CSA}/\text{CSA})/\Delta P \quad \text{kPa}^{-1} \quad (2.3)$$

$$\text{CC} = \Delta\text{CSA}/\Delta P \quad \text{mm}^2/\text{kPa} \quad (2.4)$$

In man, pulse pressure as measured at the level of the brachial artery (i.e. $\Delta P_{\text{BA}} = \text{SBP} - \text{DBP}$), has often been used to calculate vessel wall properties of different peripheral

arteries. This approach will be further referred to as the “classical” approach. However, as pulse pressure increases centrifugally because of a significant increase in SBP [8], this results in an overestimation of pulse pressure for more central arteries (e.g. carotid artery) and an underestimation for more peripheral arteries (e.g. radial artery). Therefore, an algorithm was developed which permits to *calculate* local pulse pressure by making use of pressure waveforms recorded with applanation tonometry and calibrating them with DBP and MAP [3].

Vessel wall properties: a “novel” approach

In order to assess the pulse pressures of the common carotid (ΔP_{CCA}), brachial (ΔP_{BA}) and femoral artery (ΔP_{FA}) the pressure waveforms recorded with the applanation tonometer were calibrated [3]. By definition, ΔP_{BA} as measured with applanation tonometry equals pulse pressure as measured with the oscillometric device at the upper arm. Thus at the brachial artery the nadir and peak of the pressure waveform can be equalled to DBP and SBP, respectively. Subsequently, by integrating the area under the pressure-time curve of the brachial artery and dividing it by the duration of the cardiac cycle, MAP was calculated. As MAP and DBP hardly change throughout the arterial tree [9], the nadir and mean (assessed by integration) of the pressure waveforms of the common carotid and femoral artery were equalled to DBP and MAP, respectively. Finally, the corresponding local SBP, ΔP_{CCA} and ΔP_{FA} were calculated.

Table 2.1 summarises the data (obtained by one investigator) on reproducibility and variability of *absolute* values of pulse pressures as measured with applanation tonometry (ΔP_{tono}) compared to *calculated* pulse pressures (ΔP_{cal}) according to the approach outlined in the previous paragraph. The data were obtained in a group of ten young, healthy volunteers (8 males, 2 females). Analysis was performed by calculating repeatability coefficients (RC, according to Bland-Altman [10]) and coefficients of variation (CV, expressed as percentage) for two sets of 3 recordings each. Compared to ΔP_{tono} , these data show that ΔP_{cal} is more reproducible. In addition, as illustrated by figure 2.1, in our hands calculated pulse pressures as obtained by the method outlined above, result in values which are in agreement with the known centrifugal increase in pulse pressure.

Table 2.1 Repeatability and variability of measured compared to calculated pulse pressure of peripheral arteries in human (n=10 for each artery).

	Common carotid artery	Radial artery	Brachial artery	Femoral artery
<i>Repeatability coefficient (mmHg)</i>				
ΔP_{tono}	6.2	4.3	14.6	7.9
ΔP_{cal}	4.7	4.9	5.3	3.3
<i>Coefficient of variation* (%)</i>				
ΔP_{tono}	4.8 ± 1.0	4.5 ± 0.6	8.5 ± 1.9	4.9 ± 1.2
ΔP_{cal}	2.5 ± 0.4	2.1 ± 0.5	5.3 ± 2.2	2.1 ± 0.3

* Coefficient of variation expressed as percentage (CV, %) and presented as mean $\pm$ SEM.

ΔP_{tono} : pulse pressure measured with applanation tonometry; ΔP_{cal} : calculated pulse pressure.

Based on these observations we decided to use this “novel” approach for calculating vessel wall properties in the studies presented in this thesis (*Chapters 3, 6 and 7*).

Figure 2.1 Calculated pulse pressures (mean) of the carotid, brachial and femoral artery in a group of 50 migraine patients by using the “novel” approach outlined in detail in the text (for the complete data, see *Chapter 3*).

Diameter-time recordings (ultrasound) and pressure-time recordings (applanation tonometry) were simultaneously analysed off-line using a custom made Matlab® script developed by the biophysics department of the university. Because of different phase-characteristics between the echo-tracking algorithm and the pressure amplifier, a phase shift between both signals was prevented by multiplying the signals coming out of both systems by each others phase-characteristics during the analysis. In addition, on merging the separately recorded diameter and pressure signals of the common carotid artery, both signals were aligned at the start of their up-slope (i.e. the beginning of systole, Fig. 2.2). By doing so, vessel wall properties can be calculated both over the operating pressure (i.e. local pulse pressure) and over a pre-defined fixed pressure interval (e.g. 85-95 mmHg). The latter are referred to as vessel wall

properties obtained under isobaric conditions: isobaric distensibility coefficient (DC_{iso}) and isobaric cross-sectional compliance (CC_{iso}) [6]. This approach is particularly useful to investigate changes in vessel wall properties after the administration of drugs influencing blood pressure. In such case, changes in vessel wall properties resulting from drug-induced changes in the pressure-diameter relation can be differentiated from changes resulting from a change in blood pressure (cfr. *Chapters 6 and 7*).

Figure 2.2 Example of aligned diameter-time (echo-tracking, fat line) and pressure-time recording (applanation tonometry, thin line) at the common carotid artery.

Using the “novel” approach as presented in the previous paragraphs, the variability of large artery wall properties was assessed in a group of young, healthy volunteers (8 males, 2 females) and compared to that of a previous study [5] using the “classical” approach (Table 2.2). These data show that by (i) making use of a robotic arm to measure vessel wall movements, (ii) calculating local pulse pressures and (iii) simultaneously analysing diameter-time and pressure-time recordings, in our hands

Table 2.2 Variability (CV, %) of large artery wall properties comparing the “novel” approach with the “classical” approach* (n=10).

	Diameter	Distension	Distensibility	Compliance
<i>Intra-session variability</i>				
Common carotid artery	1.2 ± 0.2 (4.5 ± 1.1)	2.6 ± 0.6 (7.9 ± 1.6)	3.9 ± 0.8 (8.3 ± 1.3)	3.9 ± 1.0 (9.1 ± 2.6)
Brachial artery	1.7 ± 0.4 (2.5 ± 0.5)	7.4 ± 1.3 (13.4 ± 2.8)	10.1 ± 1.1 (16.1 ± 2.5)	6.6 ± 1.2 (15.6 ± 2.6)
Femoral artery	1.3 ± 0.3 (2.7 ± 0.6)	6.8 ± 1.9 (12.4 ± 2.2)	7.2 ± 2.2 (13.4 ± 2.2)	6.9 ± 1.9 (12.5 ± 2.1)
<i>Inter-session variability</i>				
Common carotid artery	0.8 ± 0.2 (2.1 ± 0.5)	6.7 ± 1.3 (9.2 ± 0.9)	8.0 ± 1.6 (7.1 ± 2.2)	7.1 ± 1.2 (8.5 ± 1.5)
Brachial artery	1.6 ± 0.4 (3.3 ± 0.6)	8.5 ± 2.3 (12.5 ± 2.8)	11.9 ± 3.9 (14.2 ± 2.5)	11.9 ± 3.6 (12.6 ± 2.8)
Femoral artery	2.2 ± 0.6 (3.0 ± 1.1)	6.4 ± 1.5 (12.8 ± 3.0)	8.9 ± 1.6 (15.2 ± 3.7)	7.6 ± 1.6 (14.2 ± 2.5)

* Coefficient of variation expressed as percentage (CV, %) and presented as mean ± SEM.
“Novel” approach: refers to the simultaneous analysis of pressure-time and diameter-time recordings using calculated local pulse pressure.
“Classical” approach (data between brackets): refers to vessel wall properties calculated with brachial artery pulse pressure for all arteries.

the variability of the data was at least as good as previously reported whereas large artery wall properties might be calculated more accurately.

In summary, the outlined approach offers the following potentials: (i) a reproducible calculation of local pulse pressures, (ii) a more accurate calculation of large artery wall properties and (iii) the possibility to calculate isobaric distensibility and cross-sectional compliance.

2.3 Blood flow and vascular resistance

Ultrasound

Doppler ultrasound provides a non-invasive and reproducible technique to measure blood flow in conduit arteries [11]. Centre stream velocity profiles in the common carotid, temporal and brachial artery were measured using (7.5 MHz) pulsed-Doppler at a 70° angle to the vessel and a sampling volume in the centre of the artery. For each cardiac cycle the mean flow velocity was calculated electronically by multiplying the area under the velocity profile curve (corrected for angle) by the heart rate. Of each artery at least 8 velocity profiles were recorded; the mean of these recordings was taken as the subject's reading. Blood flow (ml/min) was calculated by multiplying the mean flow velocity by the end diastolic cross-sectional area (CSA) as measured with the echo-tracking system. Vascular resistance of the vascular bed distal of the artery under investigation was calculated as MAP divided by local blood flow and expressed as $\text{kPa.l}^{-1}.\text{s}$.

Venous occlusion plethysmography

Venous occlusion plethysmography offers an alternative technique to study limb blood flow [12]. Whereas early studies mainly involved measurements of the lower limb, the forearm is currently most frequently used in pharmacological studies [13]. The underlying principle of the technique is straightforward: the rate of swelling of a limb during obstruction of the venous return is proportional to the rate of arterial inflow. Using mercury-in-rubber strain gauge devices, changes in limb circumference are detected as changes in electrical resistance of the gauge. As changes in limb

circumference are measured, a measure of blood flow per unit volume of tissue (e.g. ml/min/100 ml of tissue) is obtained. Absolute blood flow can be calculated by measuring limb volume, for instance by water displacement, and multiplying it with blood flow per unit volume of tissue. For more detailed information on venous occlusion plethysmography we refer to recent excellent reviews by Webb [13] and Benjamin [14].

In the present thesis, venous occlusion plethysmography was used to measure forearm arterial blood flow (FBF). Total FBF consists of blood flow through skeletal muscle (50 to 70% of total FBF under resting conditions) and skin perfusion. Blood flow through skin varies markedly with temperature and has a different physiology and pharmacology compared with skeletal muscle perfusion. Therefore, hand perfusion, which predominantly refers to skin perfusion, is usually excluded during FBF measurements [15]. In practice, we measured FBF with ECG-triggered venous occlusion plethysmography using mercury-in-Silastic strain gauges. Venous outflow was obstructed by intermittently inflating upper arm cuffs to a pressure of 45 mmHg. Hand perfusion was excluded by wrist cuffs inflated to 240 mmHg one minute before FBF measurements were started.

Venous occlusion plethysmography is used in different settings [13, 14]. First, it is used to assess changes in limb perfusion after the *systemic* administration of drugs (e.g. dihydroergotamine, *Chapter 7*). This approach takes into account drug-induced systemic haemodynamic effects which make the direct vascular effects of drugs more difficult to interpret. Alternatively, venous occlusion plethysmography is used to investigate the direct vascular effects of vasoactive drugs during *local* intra-arterial infusion (e.g. into the brachial artery). Obviously, the major advantage of this technique, referred to as the “perfused forearm model”, is that local high dosages of vasoactive substances can be administered without causing any systemic side effects. In addition, by simultaneously measuring FBF both in the infused and non-infused arm, the latter provides an internal control which takes into account short-term changes in FBF affecting both arms and being unrelated to the drug under investigation. For a detailed description of the experimental set-up of the “perfused forearm model” we refer to the methods sections of *Chapters 4* and *5*.

Figure 2.3 Calcitonin gene-related peptide (CGRP) induced changes in forearm blood flow (mean $\pm$ SEM) simultaneously measured in the infused (■, solid line) and non-infused (□, dashed line) arm in healthy subjects (n=40). (For the complete data, see *Chapter 5*).

Provided that perfusion pressure (arterial blood pressure) is constant, changes in FBF reflect changes in smooth muscle tone of resistance vessels and measurements can be expressed as FBF per unit of forearm volume (Fig. 2.3). However, small alterations in perfusion pressure or sympathetic arousal which influence FBF have to be taken into account [14, 16]. Therefore, FBF measurements are either expressed in terms of calculated forearm vascular resistance (FVR) defined as perfusion pressure divided by FBF and expressed as arbitrary units (AU) or, if FBF is being measured simultaneously in both arms, results are expressed as the ratio in FBF in the infused over the control arm (FBF-ratio).

2.4 In vivo assessment of endothelial function

After Furchgott and Zawadzki [17] demonstrated the obligatory role of the endothelium in modulating vascular tone, numerous studies have evaluated

endothelial function using invasive [18-21] and non-invasive [22-27] techniques in both normal and disease states. Healthy endothelium releases endothelium-derived relaxing factor (EDRF or nitric oxide, NO) which is a potent vasodilator and is generated through the action of endothelial nitric oxide synthase (eNOS or NOSIII) on its substrate L-arginine [28]. Endothelial NOS can be activated both by chemical stimuli (e.g. acetylcholine, histamine, serotonin, bradykinin and substance P) via receptor-mediated mechanisms and by the physical stimulus of shear stress via mechanoreceptors. However, many cardiovascular diseases have been shown to be associated with a decreased endothelium-dependent vasodilatation which is referred to as endothelial “dysfunction” and is regarded as an early stage of atherosclerosis. Consequently, techniques investigating endothelial function, in particular non-invasive approaches, are gaining increasing interest.

Non-invasive approach

Flow-mediated vasodilatation of the brachial artery is currently a frequently used non-invasive technique to assess endothelial function in human [26, 29, 30]. Flow-mediated vasodilatation of the brachial artery depends on the endothelial release of NO [31], resulting from an increase in shear stress, and correlates well with coronary endothelial function [32].

In the present thesis (*Chapters 4 and 6*) flow-mediated vasodilatation of the brachial artery was measured with the echo-tracking system (see section 2.2) using a protocol comparable with the one described by Celermajer and collaborators [27]. In practice, the brachial artery was scanned over a longitudinal section about 5 cm proximal of the elbow. When a satisfactory picture was obtained, both the position of the arm and the transducer remained fixed throughout the experiment. At baseline, 5 diameter measurements (each consisting of 4 to 5 cardiac cycles) and 8 velocity profiles of the arterial blood flow were recorded. These measurements were followed by inflation of a blood pressure cuff placed around the forearm to a pressure of 240 mmHg for 5 minutes. Thereafter, the cuff was released and the maximum velocity profile during the first 15 seconds was recorded, followed by a diameter measurement after 45 seconds of deflation. Finally, depending on the study protocol (*Chapters 4*

and 6), from 1 until 5 minutes after cuff deflation brachial artery diameter was measured at regular time intervals.

In the analysis the diameter change caused by flow-mediated vasodilatation was expressed as the percentage change relative to the mean baseline diameter. Basal blood flow was calculated by multiplying the basal mean flow velocity by the cross-sectional area of the artery at rest. Maximal blood flow (reactive hyperaemia) was calculated by multiplying the mean flow velocity upon reperfusion by the cross-sectional area of the artery 45 seconds after deflation of the cuff and was expressed as percentage change relative to the basal blood flow. From cuff deflation ($t=0$) until 5 minutes after reperfusion the area under the diameter-time curve ($AUC_{0 \rightarrow 5}$) was calculated.

Invasive approach

By using the “perfused forearm model”, both basal and stimulated endothelial NO production can be assessed *in vivo* in man [13]. Basal NO production can be evaluated by using the competitive NOS inhibitor L-N^G-monomethyl-arginine (L-NMMA); stimulated NO production can be investigated by the local infusion of substances inducing forearm vasodilatation by eNOS activation (*Chapters 4 and 5*).

In both the invasive and the non-invasive approach, endothelium-independent vasodilatation induced by an exogenous NO-donor (e.g. nitroprusside or organic nitrates) should be included as a control experiment to exclude that differences in endothelium-dependent vasodilatation are due to structural changes limiting the vasodilator capacity of the vascular bed under investigation.

2.5 Cardiac output and systemic vascular resistance

Cardiac output was measured using echocardiography (Vingmed CFM 750, Sonotron, Norway). Measurements were performed in the left lateral position. Aortic diameter was measured at least 3 times using pulsed ultrasound (2.5 MHz) from a standard two-dimensional long-axis parasternal view at the site of the aortic annulus; the mean of these readings was used in the subsequent calculations. Aortic blood velocity profiles

(at least 8) were obtained with continuous ultrasound using an apical window. Stroke volume was calculated from aortic cross-sectional area multiplied by the mean blood velocity across the aortic valve during systole. Cardiac output was calculated electronically by multiplying stroke volume and heart rate, as determined from the duration of the cardiac cycle [33]. Systemic vascular resistance (SVR, $\text{kPa}\cdot\text{l}^{-1}\cdot\text{s}$) was defined as MAP divided by cardiac output.

2.6 Intima-media thickness

Using an established ultrasound method [34] posterior wall intima-media thickness (IMT) of the right common carotid artery (1-2 cm proximal of the bulb) and brachial artery (at the level of the elbow) was assessed. The raw radio frequency (RF) signals from M-mode were used to measure local IMT. For this purpose sample volumes were placed at the blood-intima and media-adventitia interface of the amplitude signal of the end-diastolic RF-line displayed on the computer monitor. IMT was measured with an accuracy of two decimals (in mm) whereas the RF-line is discretised in steps of 35 μm . At each artery at least 5 measurements were performed; the mean was taken as the subject's reading.

2.7 Minimal forearm vascular resistance

Minimal forearm vascular resistance (minimal FVR, defined as $\text{MAP}/\text{maximal FBF}$) was calculated as a functional correlate of the morphological status of the forearm resistance vessels and expressed as arbitrary units (AU) [35, 36]. Maximal (post-ischaemic) FBF of the arm was measured using venous occlusion plethysmography (Periflow, Janssen Scientific Instruments, Belgium). After 3 minutes of basal FBF measurements, arterial occlusion of the left upper arm was obtained for 13 minutes by inflating a blood pressure cuff to a pressure of 240 mmHg. During the last minute of ischaemia dynamic exercise (10 to 20 hand contractions) was added. After release of the cuff FBF was measured for 2 minutes. Both the maximum FBF and the time to

reach maximum were used in the analysis. During FBF measurements, both at baseline and during reactive hyperaemia, 3 oscillometric MAP measurements were obtained (with 1-minute intervals) at the right upper arm to calculate minimal FVR.

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Part II

The Pathophysiology of Migraine: Focus on Vascular Aspects

“The art of life is the avoiding of pain.”

(Thomas Jefferson, 1743-1826)

Chapter 3

Migraine Patients Display Interictal Changes in Large Artery Wall Properties

Abstract - Background: Migraine has been suggested to be part of a generalised vasospastic disorder. Therefore it was hypothesised that migraine patients may display functional and/or structural vascular changes which extend beyond the cranial circulation and are present outside the headache phase. **Methods:** During the interictal period, in 50 migraine patients (with or without aura) and 50 matched control subjects vascular measurements were performed at the common carotid, temporal, brachial and common femoral artery. Using ultrasound and applanation tonometry arterial diameter, distension, intima-media thickness, blood flow velocity and pressure waveforms were recorded. Using echocardiography, cardiac output was measured. Subsequently, large artery wall properties and systemic vascular resistance were calculated. **Results:** Vascular parameters of the common carotid arteries, cardiac output and systemic vascular resistance did not differ between both groups. Right temporal artery diameter was larger ($P=0.03$) in migraine patients (mean difference $101\mu\text{m}$ with 95% confidence interval (95%CI) of $9/194\mu\text{m}$) compared to control subjects. At the brachial artery migraine patients displayed a smaller ($P=0.02$) distension (difference $-24\mu\text{m}$ and 95%CI $-45/-4\mu\text{m}$) and isobaric compliance (difference $-0.025\text{ mm}^2/\text{kPa}$ and 95%CI $-0.047/-0.003\text{ mm}^2/\text{kPa}$) whereas intima-media thickness tended ($P=0.07$) to be increased (391 ± 8 versus $411\pm 9\mu\text{m}$) compared to control subjects. At the right femoral artery distensibility tended to be smaller ($P=0.10$) in migraine patients.

Based on: J.N.J.M. de Hoon⁽¹⁾, J.M. Willigers⁽²⁾, J. Troost⁽³⁾, H.A.J. Struijker-Boudier⁽¹⁾, L.M.A.B. Van Bortel⁽¹⁾. Migraine patients display interictal changes in large artery wall properties. (submitted).

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Conclusions: During the interictal period migraine patients display both cranial and peripheral vascular changes, supporting the hypothesis that migraine might be part of a more generalised vascular disorder.

3.1. Introduction

The original concept of migraine being due to a vasodilatation of cranial blood vessels as postulated by Graham and Wolff [1] has shifted towards a more complex theory regarding migraine as a neurovascular disorder of the trigeminovascular system [2]. Although this theory highlights the activation of pain-producing perivascular sensory nerves, the concept of vascular changes taking place during a migraine attack is still generally accepted [3, 4]. These vascular changes comprise of cranial vasoconstriction and cerebral hypoperfusion during the aura phase followed by vasodilatation and hyperperfusion during the headache phase.

Studies on the pathophysiology of migraine have focused on the dilatation and increased pulsations of the external carotid artery and its branches, including the temporal artery [5, 6]. However, during a migraine attack vascular changes are not limited to the cranial circulation. Iversen et al. [5] reported that a migraine attack is characterised by a generalised peripheral vasoconstriction and concluded that migraine is accompanied by abnormal reactions of the arterial system. Moreover, the association of migraine with angina pectoris due to coronary artery vasospasm (i.e. Prinzmetal's angina) [7-9] and the increased frequency of Raynaud's syndrome in migraine patients [10], suggest that migraine might be part of a generalised vasospastic disorder [7]. In addition, migraine patients have repeatedly been reported to be at increased risk for the development of ischaemic stroke [11-13]. As each of these conditions might be the result of a common vascular abnormality, it cannot be excluded that migraine is accompanied by vascular changes which extend beyond the cranial circulation and are present outside the headache phase.

Based on these observations, the present study was designed to compare the cardiovascular properties of migraine patients with those of a matched control group of non-migraineurs. To this end, arterial structure and function of cranial (common

carotid and superficial temporal artery) and peripheral (brachial and common femoral artery) blood vessels were assessed and cardiac measurements were performed during the interictal period.

3.2 Subjects and methods

Subjects and subject selection

The cardiovascular parameters of 50 migraine patients (as defined by the International Headache Society migraine criteria [14]) were compared to those of 50 healthy control subjects (Table 3.1). Both populations were matched for gender, age, body mass index, blood pressure, cholesterol and smoking habits. Migraine patients were recruited from the neurology outpatient clinic and control subjects from among hospital and laboratory staff. Based on medical history, physical examination and routine laboratory tests, all participants were in good health. Major exclusion criteria were: history of cerebrovascular or cardiovascular disease, arterial hypertension (blood pressure >160/95 mmHg), diabetes mellitus, hyperlipidaemia (total cholesterol >6.5 mmol/l), pregnancy or lactation. The regular use of vasoactive drugs was not allowed. After approval by the ethics committee of the academic hospital of Maastricht this research was carried out in accordance with the Declaration of Helsinki (1989). All subjects gave written informed consent to participate.

Study protocol

After screening, eligible subjects were invited to return to the hospital for cardiovascular assessments. Before examination subjects were asked not to use any drug for at least 3 days and to abstain from caffeine or alcohol-containing beverages and from smoking for at least 12 hours. All measurements were performed by one investigator in a quiet, temperature controlled room with the subject lying comfortably in the supine position. Measurements started after 15 minutes of supine rest and lasted approximately 1.5 hour. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were recorded at the left upper arm using a semi-automated

oscillometric device (Dinamap® 950, Critikon Inc., Florida). Blood pressures and heart rate were measured every 3 minutes for at least 15 minutes. The mean of these readings was used as the subject's haemodynamic parameters.

Table 3.1 Subject characteristics at screening*.

<i>Characteristic</i>	<i>Control subjects (n=50)</i>	<i>Migraine patients (n=50)</i>
Sex (male/female)	12 / 38	12 / 38
Smoker/non-smoker	14 / 36	14 / 36
Age (years)	38.4 ± 9.9	38.3 ± 10.3
Body mass index (kg/m ²)	24.0 ± 3.3	24.2 ± 2.5
Systolic blood pressure (mmHg)	118 ± 12	114 ± 11
Diastolic blood pressure (mmHg)	70 ± 9	69 ± 9
Heart rate (bpm)	65 ± 7	63 ± 8
Total cholesterol (mmol/l)	5.1 ± 0.8	5.2 ± 1.0

*Data presented as number or mean±SD.

Vascular measurements were consecutively performed at the common carotid artery (bilateral), superficial temporal artery (bilateral) and at the right brachial and common femoral artery using an echo-tracking system (Wall Track System® combined with Scanner 350, Pie Medical, The Netherlands) and an applanation tonometer (Micro-tip® transducer, Millar Instruments Inc., Texas). Cardiac measurements were performed using echocardiography (Vingmed CFM 750, Sonotron, Norway).

Measurements

At the common carotid, brachial and femoral artery both pressure waveforms and vessel wall movements (i.e. arterial end diastolic diameter, D and diameter change, ΔD or distension) were recorded non-invasively using applanation tonometry [15]

and an echo-tracking system [16], respectively. At the temporal artery only D was measured. At the common carotid artery vessel wall movements and pressure waveforms were recorded ipsilateral and consecutively. At the brachial and femoral artery vessel wall movements (right) and pressure waveforms (left) were recorded contralateral and simultaneously. In the analysis, mean arterial pressure (MAP), local pulse pressures of the common carotid (ΔP_{CCA}), brachial (ΔP_{BA}) and femoral artery (ΔP_{FA}) and large artery wall properties were calculated [17]. Therefore, diameter-time recordings (ultrasound) and pressure-time recordings (applanation tonometry) were simultaneously analysed off-line using a custom made Matlab[®] script developed by the biophysics department of the university as previously reported [17]. By constructing diameter-pressure curves, distensibility coefficients (DC) and cross-sectional compliance (CC) were calculated over the operating pressure.

Using ultrasound, in the common carotid and temporal arteries centre stream velocity was measured in order to calculate local blood flow and vascular resistance. At the right common carotid artery and brachial artery posterior wall intima-media thickness was assessed using an established ultrasound method [18]. Finally, using echocardiography cardiac output and systemic vascular resistance were determined.

For a detailed description on all the measurements which were performed and the subsequent data analysis, we refer to *Chapter 2*.

Statistical analysis

It was calculated that with a significance level of 0.05 and a power of 80%, 40 subjects per group were required to detect between group differences of 20% in distensibility of the femoral artery, which is the artery with the lowest reproducibility. Comparison of clinical characteristics and cardiovascular parameters between both groups was done with the unpaired Student's *t*-test. Except for subject characteristics, which are presented as mean $\pm$ SD, data are presented as mean $\pm$ SEM. $P < 0.05$ was considered statistically significant.

Table 3.2 Common carotid artery: comparison of vascular parameters of migraine patients (n=50) versus control subjects (n=50)*.

	<u>Left common carotid artery</u>		<u>Right common carotid artery</u>	
	<i>Control subjects</i>	<i>Migraine patients</i>	<i>Control subjects</i>	<i>Migraine patients</i>
Diameter (mm)	6.73 ± 0.06	6.78 ± 0.08	6.74 ± 0.07	6.76 ± 79
Distension (µm) ^a	504 ± 18	522 ± 21	550 ± 20	545 ± 23
Distensibility (10 ⁻³ /kPa) ^a	26.7 ± 1.4	27.7 ± 1.2	29.9 ± 1.4	29.8 ± 1.3
Compliance (mm ² /kPa) ^a	1.00 ± 0.05	1.06 ± 0.04	1.14 ± 0.05	1.15 ± 0.05
Intima-media thickness (µm)	ND	ND	632 ± 13	614 ± 13
Flow (ml/min)	1,166 ± 28	1,144 ± 24	1,197 ± 27	1,140 ± 27
Resistance (kPa.l ⁻¹ .s)	620 ± 17	626 ± 12	602 ± 15	635 ± 17

*Data presented as mean±SEM. ^a P<0.05 by paired Student's *t*-test for comparison of left versus right common carotid artery within each group. No between group differences were observed. ND = not determined.

3.3 Results

Subject characteristics

Clinical characteristics of migraine patients (aged 19 to 58 year) and control subjects (aged 21 to 59 year) did not differ (Table 3.1). Migraine patients had been suffering from migraine for on average 15 ± 1 year. Eleven patients had a history of recurrent migraine with aura (classic migraine), 39 without aura (common migraine). Migraine attacks recurred with a frequency of 2.6 ± 0.4 per month and lasted on average 1.4 ± 0.1 days. Headache was one-sided in 43 patients: either left-sided ($n=15$), right-sided ($n=16$) or alternating ($n=12$). Nausea or vomiting ($n=39$) and photophobia or phonophobia ($n=46$) were frequently associated with it. As an abortive drug triptans ($n=21$), analgesics ($n=20$) and anti-inflammatory drugs ($n=15$) were most frequently used. Only 3 patients mentioned the use of ergot alkaloids. Oral contraceptives or hormonal replacement therapy were used by a comparable number of migraine patients ($n=13$) and control subjects ($n=14$).

Haemodynamic data

Mean arterial pressure did not differ between control subjects (88 ± 1 mmHg) and migraine patients (88 ± 1 mmHg). Pulse pressures of the common carotid, brachial and femoral artery of control subjects (41 ± 1 , 44 ± 1 and 47 ± 1 mmHg, respectively) were comparable with those of migraine patients (39 ± 1 , 43 ± 1 and 46 ± 1 mmHg, respectively).

Vascular measurements

At the common carotid artery (Table 3.2 and Fig. 3.1) vascular parameters did not differ between migraine patients and control subjects. In both groups distension, distensibility and compliance were smaller at the left common carotid artery compared to the right one. In a subgroup of 31 migraine patients who consistently reported unilateral migraine on the same side of the head, the vascular parameters of the “diseased” and “healthy” side were compared. At the level of the common carotid artery this subgroup analysis revealed no differences.

Table 3.3 Temporal artery: comparison of vascular parameters of migraine patients (n=50) versus control subjects (n=50)*.

	<u>Left temporal artery</u>		<u>Right temporal artery</u>	
	<i>Control subjects</i>	<i>Migraine patients</i>	<i>Control subjects</i>	<i>Migraine patients</i>
Diameter (mm)	1.37 ± 0.03	1.45 ± 0.04 ^b	1.34 ± 0.03	1.45 ± 0.04 ^a
Flow (ml/min)	31 ± 2	38 ± 3 ^b	29 ± 2	35 ± 3 ^b
Resistance (kPa.l ⁻¹ .s)	26,968 ± 1,712	23,429 ± 1,662	28,028 ± 1,426	25,953 ± 1,782

*Data presented as mean±SEM. ^aP<0.05 and ^bP≤0.1 by unpaired Student's *t*-test for comparison between groups.

Table 3.4 Right brachial and femoral artery: comparison of vascular parameters of migraine patients versus control subjects (n=50 in each group)*.

	<u>Right brachial artery</u>		<u>Right common femoral artery</u>	
	<i>Control subjects</i>	<i>Migraine patients</i>	<i>Control subjects</i>	<i>Migraine patients</i>
Diameter (mm)	4.17 ± 0.07	4.14 ± 0.09	8.68 ± 0.18	8.73 ± 0.18
Distension (µm)	119 ± 7	95 ± 8 ^a	261 ± 19	230 ± 14
Distensibility (10 ⁻³ /kPa)	9.6 ± 0.6	8.2 ± 0.6 ^b	10.3 ± 0.9	8.6 ± 0.5 ^b
Compliance (mm ² /kPa)	0.13 ± 0.01	0.11 ± 0.01 ^a	0.61 ± 0.05	0.53 ± 0.04
Intima-media thickness (µm)	391 ± 8	411 ± 9 ^b	ND	ND

*Data presented as mean±SEM. ND = not determined.

^aP<0.05 and ^b0.05≤P≤0.1 by unpaired Student's *t*-test for comparison between groups.

Figure 3.1 Comparison of the vascular parameters of the right common carotid, brachial and common femoral artery of migraine patients with those of control subjects. Differences are expressed as percentage difference versus control subjects. * $P < 0.05$ and # $P \leq 0.1$ by unpaired Student's *t*-test versus control subjects.

Temporal artery diameter (Table 3.3 and Fig. 3.2) was larger ($P = 0.03$, right) or tended to be larger ($P = 0.1$, left) in migraine patients compared to control subjects. Likewise, temporal artery blood flow tended to be larger ($P \leq 0.1$) in migraine patients while local vascular resistance did not differ significantly. A subgroup analysis comparing the temporal artery parameters of the “diseased” and “healthy” side of the head of migraine patients showed no differences.

At the brachial artery (Table 3.4 and Fig. 3.1) distension and compliance were smaller ($P = 0.02$) in migraine patients compared to control subjects. In addition, distensibility tended to be smaller ($P = 0.10$) and intima media thickness tended to be larger (5.3% versus control subjects, $P = 0.07$) in migraine patients. At the femoral artery (Table 3.4 and Fig. 3.1) no differences were demonstrated between both groups.

Figure 3.2 Comparison of the vascular parameters of the left and right temporal artery of migraine patients with those of control subjects. Differences are expressed as percentage difference versus control subjects. * $P < 0.05$, # $P \leq 0.1$ and NS (no statistically significant difference) by unpaired Student's *t*-test versus control subjects.

Cardiac measurements

Cardiac output and systemic vascular resistance of control subjects (4.8 ± 0.1 l/min and 149 ± 4 kPa.l⁻¹.s, respectively) and non-migraineurs (4.9 ± 0.1 l/min and 148 ± 4 kPa.l⁻¹.s, respectively) were comparable.

3.4 Discussion

The present study aimed at investigating whether migraine patients display different cardiovascular properties compared to matched control subjects during the interictal period. The most striking functional and structural vascular differences between both populations were observed at the medium sized brachial artery displaying an increased

arterial stiffness and a tendency towards an increased intima-media thickness in migraine patients. In addition, migraineurs showed a larger temporal artery diameter and tended to have an increased temporal artery blood flow compared to control subjects. However, neither at the left nor at the right common carotid artery any differences were observed between both groups. Likewise, cardiac output and systemic vascular resistance did not differ.

Vascular research has frequently been performed in migraine patients and mainly aimed at investigating changes in flow and diameter [5, 6, 19]. However, most studies only report on vascular changes observed during a migraine attack and on those vascular territories involved in the pathogenesis of migraine headache. In particular, research focused on the external carotid artery and its branches, including the temporal artery [5], which largely perfuse meningeal blood vessels and are considered the pain-producing structures in migraine [2, 20, 21]. Iversen et al. [5] have previously reported that in migraine patients the temporal artery diameters of both sides do not differ during the interictal period while migraine headache is associated with an increased arterial diameter at the affected side. Accordingly, in the present study the temporal artery diameters of both sides did not differ; neither in migraine patients (between attacks) nor in control subjects. However, even between attacks, temporal artery diameter was larger in migraine patients compared to control subjects. In addition, the trend towards an increased temporal artery blood flow in migraine patients points out to differences at the level of resistance vessels as well. Taken together, these data indicate that irrespective of migraine headache, patients display changes both of conduit arteries and resistance vessels in the vascular territory which is affected during an attack. A subgroup analysis of those patients who consistently reported unilateral headache did not reveal any interictal differences between the temporal artery of the affected and unaffected sides, arguing against localised vascular changes.

At the common carotid and brachial artery both functional (vessel wall properties) and structural (intima-media thickness) vascular parameters were investigated. The functional parameters distensibility (a measure of vascular elasticity) and cross-sectional compliance (a measure of buffering capacity) refer to mechanical

vessel wall properties and decrease with increasing arterial stiffness [22]. Distensibility and cross-sectional compliance of migraine patients and control subjects were compared at operating pressures which did not differ between both groups. The present study is the first to demonstrate that the distension and buffering capacity of the brachial artery are decreased in migraine patients. Thus, in addition to changes at the temporal artery, migraine patients are also characterised by changes at the peripheral brachial artery. However, in agreement with Barenbrock and co-workers [23], at the common carotid artery no vascular changes were observed. The different behaviour of the common carotid artery compared to the brachial artery is probably related to differences in vessel wall structure. In general, the viscoelastic properties of the vessel wall depend on a passive component due to elastic and collagenous connective tissue and on an active component due to smooth muscle activity [24, 25]. The common carotid artery is an elastic artery which has a high proportion of elastic fibres and a small quantity of vascular smooth muscle cells compared to the muscular brachial artery [24]. Thus, changes in vascular smooth muscle tone are likely to result in smaller changes of arterial stiffness in “elastic arteries” compared to “muscular arteries”. At the femoral artery migraine patients display the same tendency towards an increased arterial stiffness as the one observed at the brachial artery. Although this difference did not reach statistical significance, this was likely due to a lack of power.

The increased arterial stiffness in migraine patients might be related to an increased arterial tone compared to control subjects. The regulation of arterial tone is complex and involves autonomic, humoral and endothelial mechanisms [21]. Thus, an increased arterial stiffness in migraineurs could result from a higher sympathetic output. However, the conflicting evidence of the currently available data on autonomic nervous system function in migraine patients [26, 27] does not allow for definite conclusions to be drawn. As far as humoral factors are concerned, serotonin (5-HT) is the most likely candidate to be involved. It probably plays an important role in the pathogenesis of migraine which is associated with low levels of 5-HT between attacks and increased levels during attacks [28, 29]. However, given the biphasic vascular response to 5-HT depending on its plasma concentration [30], it may either increase or decrease vascular tone. Still, as within the physiological range the

vasodilator effects of 5-HT predominate [31], the lower plasma concentrations in migraineurs might translate into less vasodilatation and be responsible for an increased arterial stiffness. Finally, as far as endothelial mechanisms are concerned, Joannides et al. [32] recently reported that nitric oxide (NO) contributes to the regulation of mechanical properties of peripheral conduit arteries in human. They showed that a decreased NO production results in an unexpected increase in arterial compliance. Consequently, as in the present study migraine patients displayed a decreased compliance of the brachial artery this might be related to an increased production of NO. Moreover, as previous reports suggest that the NO-pathway is activated during the headache-free period [33], this hypothesis fits well with the current opinion on NO playing a pivotal role in the pathophysiology of migraine [34]. In contrast with the effects of NO on conduit arteries, at the level of resistance vessels an increased basal production of NO results in a decreased vascular tone [35] which could explain the tendency towards an increased flow at the temporal artery.

Apart from arterial tone, arterial stiffness also depends on vessel wall structure. As the present study suggests that the intima-media thickness of the brachial artery is increased in migraine patients this might contribute to an increased arterial stiffness. The observation that intima-media thickness tends to be increased in migraine patients is very intriguing but difficult to explain if the concept of an increased basal NO production is accepted. As NO has previously been shown to be a negative regulator of vascular smooth muscle cell proliferation [36] a decreased rather than an increased intima-media thickness would be expected.

It has recently been shown that the acute intake of both 5-HT_{1B/1D}-receptor agonists (de Hoon et al., *Chapter 6*) and ergot alkaloids [23, 37] results in a transient increase in arterial stiffness. Thus, it could be argued that the vascular changes observed in the present study might not only be related to migraine itself but also to the preceding use of anti-migraine drugs. However, with the exception of ergot alkaloids, most drugs used to abort an acute migraine attack are short-acting. Even for ergot alkaloids, a drug-free period of at least 3 days should be sufficiently long for functional vascular effects to subside [37, 38]. Although it cannot be excluded that the increased arterial stiffness is related to structural vessel wall changes due to the

repeated exposure to anti-migraine drugs, this is very unlikely. In addition, the repeated use of ergotamine has previously been shown not to affect intima-media thickness [23].

What are the consequences of the present findings? Firstly, as vascular changes are present during the interictal period, it is tempting to speculate that drugs normalising these changes might be useful in the prophylactic treatment of migraine. Interestingly, many of the currently available prophylactic anti-migraine drugs, including calcium antagonists and selective β -blockers, have previously been shown to decrease arterial stiffness [39] and might thus normalise the vascular changes which render migraine patients at increased risk for a new attack. Secondly, as the baseline vascular characteristics of migraine patients differ from those of non-migraineurs, migraine patients may display different cardiovascular responses to the administration of vasoactive substances in general and to anti-migraine drugs in particular. Thus, the cardiovascular effects of potential anti-migraine drugs should be carefully looked at both in healthy subjects and in migraine patients. Finally, although it remains to be established whether the observed vascular changes are a primary cause of migraine or secondary to it, they do support the contention that migraine is associated with vascular abnormalities.

In conclusion, the present study is the first to demonstrate that even during the interictal period migraine patients display both cranial and peripheral vascular changes, indicating an important role of the vasculature in the pathogenesis of migraine. Although the contribution of the trigeminovascular system in migraine headache is generally accepted, migraine should not be regarded as an isolated neurovascular problem but might be part of a more generalised vascular disorder.

3.5 References

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Chapter 3

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Chapter 4

Vascular Responsiveness to Nitric Oxide and Calcitonin Gene-Related Peptide in Migraine Patients

Abstract - Background: As both nitric oxide (NO) and calcitonin gene-related peptide (CGRP) are considered to contribute substantially to the pathophysiology of migraine, the present study investigated the vascular responsiveness to NO, CGRP and ischaemia in migraine patients. **Methods:** Ten migraine patients and ten matched control subjects received intra-brachial infusions of (i) serotonin, releasing endothelial NO; (ii) sodium nitroprusside, an exogenous NO-donor and (iii) CGRP, a potent vasodilating neuropeptide. Changes in forearm blood flow (FBF) were measured using venous occlusion plethysmography and expressed as the ratio in FBF in the infused over the control arm (FBF-ratio). Flow-mediated vasodilatation of the brachial artery, depending on the endogenous release of NO, was measured using ultrasound and expressed as percentage change in diameter versus baseline. Maximal forearm vasodilator potential was assessed by plethysmography after 13 minutes of ischaemia and expressed as minimal forearm vascular resistance. **Results:** Basal FBF-ratio (1.1 ± 0.1) did not differ between controls and migraine patients. A dose-dependent increase ($P < 0.001$) in FBF-ratio was induced by serotonin, nitroprusside and CGRP in control subjects (to 2.8 ± 0.3 , 6.7 ± 1.4 and 6.9 ± 1.2 at the highest dose, respectively) and migraine patients (2.5 ± 0.4 , 5.6 ± 0.8 and 6.5 ± 1.3 , respectively). FBF-ratios did not differ between both group. The peak increase in flow-mediated vasodilatation ($5.8 \pm 1\%$ versus $5.2 \pm 1\%$, respectively) and the minimal forearm vascular resistance (7.5 ± 0.8 versus 6.2 ± 0.6 arbitrary units, respectively) were comparable in control subjects and migraine patients.

Based on: J.N.J.M. de Hoon⁽¹⁾, P. Smits⁽²⁾, J. Troost⁽³⁾, H.A.J. Struijker-Boudier⁽¹⁾, L.M.A.B. Van Bortel⁽¹⁾. Vascular responsiveness to nitric oxide and calcitonin gene-related peptide in migraine patients. (submitted).

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Conclusions: Based on the forearm vascular responsiveness to NO, CGRP and ischaemia, migraine patients do not display generalised differences in vascular function compared to control subjects. In addition, no structural differences are present at the level of resistance vessels.

4.1 Introduction

The pathophysiology of migraine has been much debated over the past decades [1]. The concept of migraine being due to an inappropriate vasodilatation of cranial blood vessels as postulated by Graham and Wolff [2], has shifted towards a neurovascular theory regarding migraine as a complex disorder of the trigeminovascular system [3]. Although, this theory is generally accepted, the trigger responsible for activating this system and initiating migraine headache remains unknown.

One hypothesis, which has mainly been advocated by Olesen and co-workers [4-6], points to endothelial derived relaxing factor or nitric oxide (NO) as the triggering molecule for migraine attacks. They demonstrated that compared to healthy subjects, migraine patients suffer a more intense and more migraine-like headache after the administration of organic nitrates, acting as NO-donors, and histamine, stimulating the endogenous release of NO by the activation of endothelial H₁-receptors [7, 8]. Furthermore, an increased vasodilatation of the middle cerebral artery was observed in migraine patients compared to control subjects after the administration of organic nitrates [9]. Based on these data they concluded that migraine patients display an arterial supersensitivity to NO.

Apart from NO, calcitonin gene-related peptide (CGRP) has even more convincingly been shown to be a key-player in the pathophysiology of migraine. Indeed, Goadsby and colleagues [10] provided evidence that during migraine headache the trigeminovascular system is turned on and CGRP, a potent vasodilator neuropeptide, is released into the cranial circulation. However, the question remains which are the stimuli responsible for this release. Interestingly, based on animal data, the most important emerging hypothesis states that NO plays a regulatory role in neuropeptide release from perivascular sensory nerves [11, 12]. Consequently,

supersensitivity to NO might not only translate into an excessive vasodilatation by the direct action of NO on the blood vessel wall of migraine patients, but it might also translate into an excessive release of CGRP from perivascular nerves induced by NO.

The present study aims at a better understanding of the concept of supersensitivity to both endogenous and exogenous NO and the vascular reactivity to CGRP in migraine patients.

4.2 Subjects and methods

Subjects and subject selection

Ten migraine patients (as defined by the International Headache Society migraine criteria [13]) were compared to 10 healthy control subjects. Both groups were matched for gender, age, body mass index, blood pressure and cholesterol. Migraine patients were recruited from the neurology outpatient clinic and control subjects from among hospital and laboratory staff. Baseline characteristics of both groups are presented in Table 4.1. Based on medical history, physical examination and routine laboratory tests, all participants were in good health. Major exclusion criteria were: smoking, history of cardiovascular disease, arterial hypertension (blood pressure >160/95 mmHg), diabetes mellitus and hyperlipidaemia (total cholesterol >6.4 mmol/l). Except for oral contraceptives, no regular medication was allowed. After approval by the ethics committee of the academic hospital of Maastricht this research was carried out in accordance with the Declaration of Helsinki (1989). All subjects gave written informed consent to participate.

Study design

After screening, eligible subjects were invited to return to the hospital for 2 study periods. Before each period, they were asked not to use any drugs for at least 3 days and to abstain from caffeine or alcohol-containing beverages for at least 12 hours. All measurements were performed by one investigator in a quiet, temperature controlled room and with the subject lying comfortably in the supine position.

Table 4.1 Baseline characteristics at screening.

<i>Characteristic</i>	<i>Control subjects (n=10)</i>	<i>Migraine patients (n=10)</i>	<i>P-value</i>
Age (years)	35 ± 3	34 ± 2	0.82
Sex (male/female)	4 / 6	4 / 6	1.0
Body mass index (kg/m ²)	23 ± 1	24 ± 1	0.56
Cholesterol (mmol/l)	4.6 ± 0.1	4.8 ± 0.1	0.71
Systolic blood pressure (mmHg)	117 ± 3	115 ± 2	0.61
Diastolic blood pressure (mmHg)	67 ± 3	66 ± 2	0.67
Forearm volume (ml)	962 ± 67	987 ± 75	0.81
Brachial artery diameter (mm)*	4.35 ± 0.82	4.40 ± 0.81	0.88
Forearm blood flow at rest (ml/min)*	140 ± 58	230 ± 163	0.12
Forearm vascular resistance at rest*	48 ± 5	45 ± 5	0.68

Data presented as number or mean±SEM. *Baseline measurements during the second study period.

First study period

During this period, forearm vascular reactivity to serotonin (inducing an endogenous release of NO), sodium nitroprusside (SNP, an exogenous NO-donor) and CGRP was investigated using the “perfused forearm model” [14]. After local anesthesia (lidocaine 2%, B. Braun, Germany) the brachial artery of the non-dominant arm was cannulated with a 20-gauge catheter (Arterial cannula, Ohmeda, U.K.) for intra-arterial drug infusion (Graseby Syringe Pump 3200, Graseby Medical LTD, U.K.) and invasive blood pressure monitoring (monitor type 78201B, Hewlett Packard GmbH, Germany). Forearm arterial blood flow (FBF) was measured by ECG-triggered venous occlusion plethysmography using mercury-in-Silastic strain gauges (Periflow, Janssen Scientific Instruments, Belgium). Every 5 heart beats FBF was recorded simultaneously at both arms for 3 beats. Upper arm cuffs were intermittently and simultaneously inflated to a pressure of 45 mmHg. To ensure that FBF recordings referred predominantly to the forearm skeletal muscle resistance vessels perfusion, at least one minute before measurements were started the hand circulation was occluded by wrist cuffs inflated to 240 mmHg [15]. Heart rate, systolic and diastolic blood pressure were recorded with every FBF measurement. Mean arterial pressure (MAP) was calculated as the electronically integrated area under the brachial artery pulse-wave curve and averaged per FBF measurement.

Figure 4.1 Flow chart of the first study period.

After instrumentation an equilibration period of at least 30 minutes was allowed. After equilibration, basal FBF was recorded for 5 minutes during infusion of saline (NaCl 0.9%). Baseline measurements were followed by incremental infusions of serotonin (1, 3.3 and 10 $\text{ng}\cdot\text{min}^{-1}\cdot\text{dl}^{-1}$ forearm), SNP (0.04, 0.2 and 1 $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{dl}^{-1}$ forearm) and CGRP (3, 10 and 30 $\text{ng}\cdot\text{min}^{-1}\cdot\text{dl}^{-1}$ forearm) (Fig. 4.1). Each dose of serotonin and SNP was infused for 6 minutes; each dose of CGRP for 12 minutes. The mean of the measurements made during the final 2 minutes of each infusion period (i.e. at steady-state) was used in the analysis. After the highest dose of serotonin and SNP, saline was infused for at least 15 minutes to allow FBF to return to baseline. The rate of all intra-arterial infusions was kept constant at 100 $\mu\text{l}\cdot\text{min}^{-1}\cdot\text{dl}^{-1}$ forearm volume and the dosages were normalised to forearm volume measured by water displacement. Before each experiment, all solutions were freshly prepared from commercially available vials (human-CGRP and serotonin purchased from Clinalfa Switzerland) or from sterile stock solutions (SNP, provided by the hospital pharmacy). SNP was protected from light. In the analysis vascular responses to serotonin, SNP and CGRP, were expressed as the ratio in FBF in the infused over the control arm (FBF-ratio).

Second study period

During this period, after 15 minutes of supine rest, forearm vascular reactivity to ischaemia was investigated non-invasively. First, flow-mediated dilatation of the brachial artery of the right arm was measured using ultrasound (echo-tracking system). Thereafter, maximal post-ischaemic FBF of the left arm was measured using venous occlusion plethysmography. For a detailed description of how these measurements were performed and how the data were analysed, we refer to *Chapter 2*, sections 2.4 and 2.7. respectively. It should be mentioned however, that in the present study flow-mediated vasodilatation was measured every 30 seconds from 1 until 5 minutes after brachial artery reperfusion.

Statistical analysis

Comparison of baseline characteristics and non-serial parameters between both groups was done with the unpaired Student's *t*-test. The haemodynamic responses (FBF-ratio,

MAP and heart rate) to incremental infusions of serotonin, SNP and CGRP within each group of subjects as well as the difference in forearm vascular response (FBF-ratio) between migraine patients and control subjects, were evaluated by ANOVA for repeated measures. If ANOVA showed a significant change (within a group) or difference (between groups), a post hoc Student's *t*-test was performed to determine dose dependency. Unless otherwise stated, data are expressed as mean $\pm$ SEM; $P<0.05$ was considered statistically significant.

4.3 Results

Subject characteristics

Baseline characteristics of migraine patients and control subjects did not differ (Table 4.1). In both groups 2 subjects were on oral contraceptives. Migraine patients had been suffering from migraine for an average of 13 ± 1 years and had an attack frequency of 2.2 ± 0.4 each month. In most cases migraine headache was one-sided ($n=8$), pulsatile in nature ($n=7$) and accompanied by nausea or vomiting ($n=9$) and photo- or phonophobia ($n=10$). Only one patient regularly experienced migraine with aura. As an abortive drug sumatriptan ($n=7$) was most frequently used.

First study period

At baseline, FBF in the infused and control arm were similar within each group and did not differ between migraine patients and control subjects (1.8 ± 0.3 versus 1.7 ± 0.1 ml.min⁻¹.dl⁻¹ forearm, respectively). MAP slightly decreased ($P<0.001$) during SNP infusion in both migraine patients (from 87 ± 3 at baseline to 84 ± 3 mmHg at the highest dose) and control subjects (from 92 ± 3 to 88 ± 3 mmHg). In migraine patients this decrease in MAP was accompanied by a small increase ($P=0.001$) in heart rate (from 58 ± 3 to 60 ± 2 bpm). During the infusion of CGRP, a small increase ($P<0.001$) in heart rate was observed both in patients (from 58 ± 2 to 62 ± 3 bpm) and controls (from 61 ± 2 to 65 ± 2 bpm) whereas MAP did not change (89 ± 3 and 95 ± 3 mmHg at baseline, respectively). Although these changes in MAP and heart rate suggest a systemic vasodilator effects of SNP and CGRP, neither in migraine patients nor in

control subjects FBF in the control arm increased during any of the infusions, arguing against a systemic vasodilator effect.

A dose-dependent increase ($P<0.001$) in FBF-ratio was induced by serotonin, SNP and CGRP (Fig. 4.2) both in control subjects (from 1.1 ± 0.1 to 2.8 ± 0.3 , 6.7 ± 1.4 and 6.9 ± 1.2 at the highest dose, respectively) and in migraine patients (from 1.1 ± 0.1 to 2.5 ± 0.4 , 5.6 ± 0.8 and 6.5 ± 1.3 , respectively). There were no significant differences in the responses to any of the drugs between both populations.

Figure 4.2 Comparison of forearm blood flow ratio (FBF-ratio) between migraine patients (■, solid line) and control subjects (□, dashed line) during the intra-brachial infusion of serotonin (left top), sodium nitroprusside (SNP, left bottom) and calcitonin gene-related peptide (CGRP). FBF-ratio: ratio in forearm blood flow in the infused arm over the control arm. Data are presented as mean±SEM, n=10 in each group. (NS: not statistically significant).

Second study period

The reactive hyperaemic increase in blood flow induced by 5 minutes of forearm occlusion did not differ ($P=0.054$) between migraine patients (476 ± 53 %) and control subjects (608 ± 34 %). In response to this increase in flow, maximum brachial artery vasodilatation (Fig. 4.3) averaged 5.2 ± 1.0 % in migraine patients compared to 5.8 ± 1.0 % in control subjects ($P=0.64$). The time-course of the flow-mediated vasodilatation expressed as $AUC_{0 \rightarrow 5}$ did not differ between migraineurs and controls (370 ± 150 $\mu\text{m} \cdot \text{min}$ versus 570 ± 147 $\mu\text{m} \cdot \text{min}$, respectively, $P=0.35$).

Migraine patients and control subjects did not differ ($P=0.42$) with respect to basal FBF (2.2 ± 0.3 and 1.9 ± 0.2 $\text{ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$ forearm, respectively) or maximal post-ischaemic FBF (15.0 ± 1.4 and 13.0 ± 1.8 $\text{ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$ forearm, respectively) (Fig. 4.4).

Figure 4.3 Comparison of flow-mediated vasodilatation between migraine patients (■, solid line) and control subjects (□, dashed line). Diameter before occlusion ($t=0$) and from 45 seconds until 5 minutes after reperfusion. Data presented as mean $\pm$ SEM, $n=10$ in each group.

Figure 4.4 Comparison of baseline and maximal post-ischaemic forearm blood flow between migraine patients (hatched bars) and control subjects (open bars). Data are presented as mean $\pm$ SEM, $n=10$ in each group. (NS: not statistically significant).

Furthermore, forearm vascular resistance at baseline (44.9 ± 5.4 versus 48.0 ± 5.2 AU, respectively) and during reactive hyperaemia (6.2 ± 0.6 versus 7.5 ± 0.8 AU, respectively) was comparable ($P=0.68$ and 0.22 , respectively) in both groups. Time to reach maximal FBF or minimal forearm vascular resistance after reperfusion was similar ($P=0.62$) for migraine patients and control subjects (86 ± 8 and 81 ± 7 seconds, respectively).

4.4 Discussion

In vivo vascular research in migraine patients has been hampered by limited access to the cranial circulation. However, as migraine is associated with an increased incidence of Raynaud's syndrome [16] and with angina pectoris due to coronary artery vasospasm (i.e. Prinzmetal's angina) [17-19], it has been suggested to be part of a generalised vascular disorder [17]. Consequently, peripheral vascular research in migraine patients might offer an alternative approach to improve our understanding of the cranial vascular abnormalities associated with it.

The main finding of the present study is that neither for NO nor for CGRP an increased sensitivity could be demonstrated for the resistance vessels of the human forearm in migraine patients. As maximal post-ischaemic forearm blood flow was similar in both groups, there is no evidence suggesting structural differences of the resistance vessels either. In addition, flow-mediated vasodilatation did not differ between migraine patients and matched control subjects arguing against a supersensitivity to NO for conduit arteries as well. In summary, these data do not support the hypothesis of a systemic vascular supersensitivity to NO in migraine patients and do not support generalised functional vascular changes or structural changes of resistance vessels as a primary cause of the disease.

In the present study, the NO hypothesis of migraine was investigated by measuring the forearm vascular response to both the pharmacological and physiological-induced endogenous release of NO and to the administration of an exogenous NO-donor. The vascular response to the pharmacological release of NO was investigated by the intra-arterial infusion of serotonin. It has previously been

shown that at the dosages used in the present study, serotonin-induced vasodilatation of the human forearm is endothelium-dependent and accompanied by the release of NO [20, 21]. The vascular sensitivity to an exogenous source of NO was investigated by the infusion of sodium nitroprusside. Like nitrates, sodium nitroprusside is known to induce an endothelium-independent vasodilatation by the release of NO upon its degradation [22]. As both endothelium-dependent and endothelium-independent NO-induced vasodilatation were comparable between migraine patients and non-migraineurs, these findings argue against a vascular smooth muscle cell supersensitivity to NO in migraine patients.

In addition to the lack of functional differences, structural differences could not be demonstrated either. However, as venous occlusion plethysmography only measures flow changes at the level of resistance vessels, these findings do not allow us to conclude the absence of NO supersensitivity at the level of conduit arteries. Therefore, flow-mediated vasodilatation of the brachial artery was investigated. This non-invasive approach has been used frequently before to demonstrate changes in endothelial function [23-26]. It has also been shown that NO is responsible for the flow-mediated vasodilatation of human peripheral conduit arteries *in vivo* [27]. In the present study, neither the peak increase in brachial artery diameter nor the duration of flow-mediated vasodilatation was increased in migraine patients compared to control subjects. Consequently, at the level of the conduit artery of the human forearm our data do not support the hypothesis of supersensitivity to NO either.

What could be the explanations for the fact that the findings of the present study do not support the hypothesis of arterial supersensitivity in migraine patients as formulated by other investigators? Firstly, different vascular territories are involved. The forearm vasculature, which was investigated in the present study, might well behave differently from the cranial vasculature which is the territory of interest in migraine. Differences in receptor distribution and innervation could obviously result in a different pharmacology for both territories. Secondly, although investigated thoroughly, the earlier indications of increased arterial sensitivity to NO remain indirect [9]. Since intracranial diameter measurements cannot be obtained by current non-invasive techniques in human, vasodilatation of large intracranial arteries can only be estimated by simultaneous transcranial Doppler (TCD) and single positron

emission computed tomography (SPECT) measurements. Based on the observation that total cerebral blood flow (measured with SPECT) is not changed during nitroglycerine-induced headache while blood velocity (measured with TCD) is decreased, rather suggests vasodilatation than providing evidence for it.

Apart from the vascular smooth muscle cell, the endothelium has also been suggested to be a likely site of the primary abnormality in migraine [28]. However, the findings of the present study indicate that endothelial function is unaffected. The preservation of the vasodilatation resulting from both serotonin and shear-stress induced endothelial release of NO, makes it very unlikely for migraine to be part of a generalised “endotheliopathy”. Of course, a local, cerebral endothelial abnormality cannot be excluded. It has also been hypothesised that one of the early steps in the generation of migraine headache is the activation of endothelial 5-HT_{2B}-receptors on meningeal blood vessels inducing the release of NO [29]. Although serotonin does induce the endothelial release of NO [21], there is no evidence indicating an increased release in migraine patients.

Although we could not demonstrate an increased sensitivity to NO in terms of an increased vascular response, the migraine-like characteristics of headache induced by nitrates do make NO a likely candidate to be involved in the pathophysiology of migraine. Consequently, one might wonder whether supersensitivity of migraine patients to NO should be interpreted in terms of an increased sensitivity of perivascular nerves to NO instead of an increased sensitivity of the blood vessel wall itself. If so, the low density of CGRP containing perivascular nerve fibres in the peripheral circulation compared to the cranial circulation [30], may explain the lack of a difference in nitroprusside-induced changes in forearm blood flow between migraine patients and control subjects in the present study. According to this vision, in migraine patients NO might result in an over-activation of the trigeminovascular system and an excessive release of CGRP involved in neurogenic inflammation [3, 7]. Firstly, this hypothesis is supported by the observation that during a genuine migraine attack plasma levels of CGRP are increased in the cranial circulation [10]. Although in healthy volunteers, nitroglycerine-induced headache was not accompanied by an increase in CGRP plasma levels [31], this does not exclude a role for trigeminal supersensitivity to NO in migraine patients. Secondly, nitrates only induce typical

migraine headache in migraine patients after a surprisingly long latency period, suggesting that NO is involved in the initiation of a slow pathological process which eventually leads to the attack [6]. This pathological process could well be the activation of the trigeminovascular system. In addition, although nitrate-induced headache is accompanied by vasodilatation, vasodilatation as such is unlikely to be the cause of the headache. Not only because of the delay between the fast vasodilatation and the slow onset of migraine-like headache induced by nitrates, but also because other potent vasodilators including CGRP have not been reported to cause headache [32]. Finally, it should be noted that NO is involved in nociceptive processing and was recently shown to mediate vascular pain [33, 34]. Thus, it cannot be excluded that an increased sensitivity to NO in migraine patients merely reflects a lower threshold for the noxious effects of this molecule compared to non-migraineurs.

In summary, neither peripheral resistance vessels nor conduit arteries display any differences in their vascular response to NO and the release of it between migraine patients and control subjects. Likewise, the vascular response to CGRP is not altered in migraine patients. In addition to the lack of a generalised difference in vascular function, no structural difference at the level of resistance vessels is present in migraine either. As these data do not exclude local functional or structural vascular changes, it remains to be established whether trigeminovascular supersensitivity to NO, resulting in an excessive release of CGRP, contributes to the pathogenesis of migraine.

4.5 References

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“Headache has a mind of its own ...”

(Charles Darwin, 1809-1882)

Chapter 5

Mechanism of Action of the Vasodilator response to Calcitonin Gene-Related Peptide in Human

Abstract – Background: *In vitro* studies suggest that the vasodilator mechanism of action of calcitonin gene-related peptide (CGRP) involves various endothelium-dependent and endothelium-independent mechanisms. This study aimed at an *in vivo* analysis in humans of the contribution of nitric oxide (NO), prostaglandins, calcium-sensitive potassium channels (K^+_{Ca} -channels) and adenosine triphosphate sensitive-potassium channels (K^+_{ATP} -channels) to CGRP-induced vasodilatation. **Methods:** CGRP (3-10-30 ng.min⁻¹.dl⁻¹ forearm) was infused into the brachial artery of 40 healthy subjects. Forearm vascular responses were measured using venous occlusion plethysmography. First, dose-response curves were constructed during co-infusion of CGRP with placebo (NaCl 0.9%). After wash-out, in 5 subgroups (n=8 each), the infusions of CGRP were repeated with: placebo (time-control experiments), N^G-monomethyl-L-arginine (L-NMMA, NO-synthase inhibitor), indomethacin (cyclo-oxygenase inhibitor), tetra-ethyl-ammonium chloride (TEA, K^+_{Ca} -channel blocker) and glibenclamide (K^+_{ATP} -channel blocker), respectively. **Results:** CGRP induced a dose-dependent and reproducible decrease ($P<0.001$) in forearm vascular resistance. Compared to placebo, L-NMMA reduced ($P<0.001$) the decrease in forearm vascular resistance induced by CGRP (3 and 10 ng.min⁻¹.dl⁻¹ forearm). The absence of an inhibitory effect of L-NMMA on CGRP-induced vasodilatation at the highest dose of CGRP suggests that further mechanisms are involved. The vasodilator response to CGRP was not affected by co-infusion of either

Based on: Jan N.J.M. de Hoon⁽¹⁾, Peter Pickkers⁽²⁾, Paul Smits⁽²⁾, Harry A.J. Struijker-Boudier⁽¹⁾, Luc M.A.B. Van Bortel⁽¹⁾. Mechanism of action of the vasodilator response to calcitonin gene-related peptide in human. (submitted).

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indomethacin, TEA or glibenclamide.

Conclusions: The intra-brachial infusion of CGRP results in a dose-dependent and reproducible forearm vasodilator response. Based on the evidence presented, CGRP-induced vasodilatation is at least in part dependent on the release of NO and does not involve the release of prostaglandins or the activation of K^+_{Ca} or K^+_{ATP} -channels.

5.1 Introduction

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide which was first identified in 1981 by Rosenfeld and co-workers [1]. Its extensive distribution in the cardiovascular system suggests a potential role for CGRP in diverse physiological and pathophysiological processes, including: hypertension, congestive heart failure, myocardial ischaemia, Raynaud's syndrome, migraine and septic shock [2]. Although it is well established that CGRP exerts a potent vasodilating activity, its mechanism of action and contribution to local control of tissue perfusion remain a matter of debate [3].

CGRP is a neurotransmitter which is synthesised in primary sensory neurones of the dorsal root ganglia and trigeminal ganglion [4]. These neurones provide a dense perivascular network of CGRP-containing sensory nerves throughout the cardiovascular system. Activation of these nerves induces a nonadrenergic-noncholinergic (NANC) vasodilatation by the release of CGRP in a variety of vascular beds of different species [5]. CGRP exerts its vasodilating effects by interaction with CGRP-receptors which are present on both the vascular endothelium and vascular smooth muscle cells suggesting that its vascular actions involve endothelium-dependent as well as endothelium-independent mechanisms [2].

Depending on the species and tissue under investigation, at least three vasodilator mechanisms of action of CGRP have been reported. An endothelium-independent mechanism involving vascular smooth muscle cell adenylate cyclase activation [6-8]; an endothelium-dependent mechanism accompanied by the release of nitric oxide (NO) [9, 10] and the activation of adenosine triphosphate-sensitive potassium channels (K^+_{ATP} -channels) [11-13]. As both animal and human data on the

vasodilator mechanism of action of CGRP mainly comprise of *in vitro* research on isolated blood vessels, conflicting results might be related to data obtained under high, unphysiological concentrations of CGRP. In addition, as the mechanisms involved in the *in vivo* and *in vitro* vascular responses to CGRP are not necessarily the same, further research into the vasodilator mechanism of action of CGRP *in vivo* is warranted.

The present study is the first to investigate *in vivo* in human the vasodilator mechanism of action of (patho)physiological concentrations of CGRP. To this end, the perfused forearm technique was used and the contribution of endothelial mediators (NO and prostaglandins) and K⁺-channels to CGRP-induced vasodilatation was investigated.

5.2 Subjects and methods

Subjects

After approval from the local ethics committee, 40 non-smoking subjects gave written informed consent to participate in this study. Based on a medical history and clinical examination all subjects were in good health. Their demographic data are presented in Table 5.1. Before the start of each experiment, subjects were asked not to use any drug for at least 3 days and to abstain from caffeine or alcohol-containing beverages for at least 12 hours.

Study design

All experiments were performed in a single-blind fashion in a quiet, temperature-controlled room (23° to 24°C). Each experiment lasted 4-5 hours. After local anesthesia (lidocaine 2%, Astra Pharmaceuticals) the brachial artery of the non-dominant arm was cannulated with a 20-gauge catheter (Angiocath, Deseret Medical, Becton Dickinson) for intra-arterial drug infusion (automatic syringe infusion pump, type STC-521, Terumo) and blood pressure monitoring (monitor type 78353B, Hewlett Packard GmbH). All blood pressures mentioned were acquired from these intra-arterial measurements.

Table 5.1 Demographic data at baseline*.

	<i>CGRP with Placebo</i>	<i>CGRP with L-NMMA/SNP</i>	<i>CGRP with Indomethacin</i>	<i>CGRP with TEA</i>	<i>CGRP with Glibenclamide</i>
Number	8	8	8	8	8
Gender (male/female)	4/4	4/4	4/4	4/4	4/4
Age (years)	23 ± 3	24 ± 3	24 ± 4	23 ± 3	23 ± 3
Body mass index (kg/m ²)	21 ± 1	21 ± 2	21 ± 1	22 ± 2	22 ± 2
MAP (mmHg)	82 ± 6	82 ± 8	80 ± 5	81 ± 9	82 ± 7
Heart rate (bpm)	63 ± 11	61 ± 9	67 ± 5	61 ± 11	61 ± 4
Forearm volume (ml)	944 ± 184	963 ± 143	963 ± 163	981 ± 130	956 ± 230

*Data presented as mean±SD. Mean arterial pressure (MAP) and heart rate (beats per minute, bpm) are baseline values measured in supine position 30 minutes after brachial artery cannulation.

Forearm arterial blood flow (FBF) was measured by ECG-triggered venous occlusion plethysmography using mercury-in-Silastic strain gauges (Hokanson EC-4 strain gauge Plethysmograph, D.E.Hokanson Inc). FBF was measured 3 to 4 times per minute and recorded simultaneously at both arms. To ensure that recordings referred predominantly to the forearm skeletal muscle perfusion, at least one minute before FBF measurements were started the hand circulation was occluded by wrist cuffs [14]. Upper arm cuffs were intermittently and simultaneously inflated to a pressure of 45 mmHg using a rapid cuff inflator (Hokanson E-20). Heart rate, systolic and diastolic blood pressure were registered simultaneously with every FBF measurement. Mean arterial pressure (MAP) was calculated as the electronically integrated area under the brachial artery pulse-wave curve and averaged per FBF measurement.

Figure 5.1 Flow chart.

After instrumentation of both arms an equilibration period of at least 30 minutes was allowed. After equilibration, each experiment started with a 5-minute period of baseline measurements (baseline 1) during the infusion of placebo (NaCl 0.9%) (Fig. 5.1). Thereafter, in all experiments the first series of three increasing dosages of CGRP (3, 10 and 30 $\text{ng} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$ forearm) was infused together with placebo. After a wash-out period of 90 minutes baseline measurements were repeated (baseline 2) for 5 minutes. Subsequently, the second series of increasing dosages of CGRP was administered either during co-infusion of placebo (subgroup 1, $n=8$) or during co-

infusion of a specific inhibitor (subgroups 2 to 5, $n=8$ each). Before starting the second series of CGRP infusions the vasoactive effects of the inhibitor were evaluated. Therefore, measurements were taken just before (baseline 2) and 5 minutes after the start of the infusion of the inhibitor (15 minutes in case of indomethacin). In order to reach steady-state each dose of CGRP was infused for 12 minutes (pilot data not shown). The rate of all intra-arterial infusions was kept constant at $100 \mu\text{l} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$ forearm volume throughout the experiments and the dosages were normalised to forearm volume measured by water displacement.

Time-control experiments

In subgroup 1, both the first and the second series of CGRP infusions were co-infused with placebo. An antecubital vein of the infused arm was cannulated for the assessment of venous plasma levels of CGRP. Blood samples (8 ml) were drawn at the end of baseline 1 and at the end of each dose of CGRP during the first series of infusions.

The role of nitric oxide in CGRP-induced vasodilatation

In subgroup 2, during the second series of CGRP infusion the NO-synthase inhibitor N^G -monomethyl-L-arginine (L-NMMA, $0.2 \text{ mg} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$ forearm) was used to block the *de novo* synthesis of NO [15, 16]. The infusion of L-NMMA was accompanied by the continuous infusion of a low dose of the NO-donor sodium-nitroprusside (SNP, $0.02 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$ forearm) to “clamp” the basal NO-mediated vascular tone [17].

During these experiments, an antecubital vein of the infused arm was cannulated for assessment of the regional production of cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP). Therefore, both venous and arterial blood samples (4 ml) were drawn at the end of baseline 1 measurements and after the highest dose of CGRP.

The role of prostaglandins in CGRP-induced vasodilatation

In subgroup 3 indomethacin ($5 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$ forearm) was used as cyclo-oxygenase inhibitor to block the *de novo* synthesis of prostaglandins. To check for the inhibition of cyclo-oxygenase activity by indomethacin the *ex vivo* platelet thromboxane B_2

(TxB₂) production was assessed. Therefore, an antecubital vein of the infused arm was cannulated and venous blood samples (5 ml) were drawn just before and 15 minutes after the start of the indomethacin infusion (before starting the second series of CGRP infusions).

The role of potassium channels in CGRP-induced vasodilatation

Finally, it was investigated whether CGRP-induced vasodilatation is dependent on the activation of calcium-sensitive K⁺-channels (K⁺_{Ca}-channels, subgroup 4) and/or K⁺_{ATP}-channels (subgroup 5). Therefore, tetra-ethyl-ammonium chloride (TEA, 0.1 mg.min⁻¹.dl⁻¹ forearm) was used as non-selective K⁺_{Ca}-channel blocker [18] and glibenclamide (2 µg.min⁻¹.dl⁻¹ forearm) as K⁺_{ATP}-channel blocker [19], respectively.

Laboratory assessments

For the assessment of venous plasma concentrations of CGRP, after collection blood was immediately transferred into ice-cooled plastic tubes containing heparin (50 IU/ml blood) and aprotinin (500 KIU/ml blood). CGRP was measured in duplicate using a radioimmunoassay (RIA, Peninsula Laboratories Europe, LTD.). The detection limit was 1 pg/ml; intra- and inter-assay coefficients of variation (n=10) were 4.9% and 9.6%, respectively.

For the assessment of plasma concentrations of cGMP and cAMP, after collection blood was immediately transferred into pre-chilled EDTA-tubes. Cyclic GMP and cAMP levels were each measured in duplicate using commercially available RIA kits (IBL, Hamburg). For both cGMP and cAMP the detection limit was 0.05 pmol/ml. For cGMP intra- and inter-assay coefficients of variation (n=10) were 4.3% and 9.5%, respectively; for cAMP, 4.3% and 8.6%, respectively. The local forearm spillovers of cGMP and cAMP were calculated as the product of the arteriovenous difference in plasma concentration of cGMP or cAMP and the FBF and expressed as pmol.min⁻¹.dl⁻¹ forearm [20].

For the assessment of serum TxB₂ concentrations immediately after collection blood was transferred into glass tubes without additives and incubated at 37°C for 1 hour to allow blood clotting to take place. TxB₂ concentrations were determined in duplicate by a previously described RIA using ³H-TxB₂ as tracer [21].

Drugs

Human-CGRP (Clinalfa, 50 µg per vial) was dissolved in NaCl 0.9% to produce a stock solution of 500 ng/ml which was used no longer than 24 hours after preparation and stored at 4°C. Before each experiment, CGRP solutions (30, 100 and 300 ng/ml) were freshly prepared. L-NMMA acetate (Clinalfa, 30 mg per vial), indomethacin (Indocid PdA[®], Merck Sharp and Dohme, 1 mg per ampoule) and glibenclamide (Aventis Pharma, 2 mg per ampoule) were dissolved with NaCl 0.9% just before use. SNP (Nipride[®], Roche, 50 mg per ampoule), which was dissolved with glucose 5% and further diluted with NaCl 0.9%, was protected from light. TEA (1 mg/ml) was provided by the hospital pharmacy as a sterile solution.

Data analysis

Forearm vascular resistance (FVR) was calculated as MAP divided by simultaneously recorded FBF and expressed in arbitrary units (AU). Drug-induced changes in FVR were expressed as percentage change in FVR compared to the preceding FVR during placebo (i.e. baseline) or during inhibitor infusion without CGRP. Data are the mean of the measurements obtained during the last 2 minutes of each infusion period (i.e. at steady-state).

The vascular effects of CGRP during the first series of infusions were analysed by comparing the hemodynamic variables (FBF, FVR, MAP and heart rate) at baseline and at the three dose levels by ANOVA with repeated measures. Dose-response curves of the first and the second series of CGRP infusions were compared with a two-way, repeated measures ANOVA for paired data. If ANOVA showed a significant difference ($P < 0.05$), post hoc paired Student's *t*-tests (for pooled data) or Wilcoxon's matched-pairs signed-rank tests (for subgroup analyses) were performed to determine dose dependency. All data are expressed as mean $\pm$ SEM unless specified otherwise; $P < 0.05$ was considered statistically significant.

5.3 Results

Based on their demographic data (Table 5.1) the 5 subgroups were comparable.

Table 5.2 Pooled data (n=40) of local vascular and systemic hemodynamic effects of CGRP.

	Baseline 1	CGRP	CGRP	CGRP	Baseline 2
Dose CGRP (ng.min ⁻¹ .dl ⁻¹ forearm)	0	3	10	30	0
FBF _{exp} (ml.min ⁻¹ .dl ⁻¹ forearm)*	2.4 ± 0.2	5.1 ± 0.3‡	8.9 ± 0.5‡	13.1 ± 0.8‡	3.1 ± 0.2¶
FBF _{control} (ml.min ⁻¹ .dl ⁻¹ forearm)//	2.6 ± 0.3	2.5 ± 0.2	2.5 ± 0.2	2.2 ± 0.2§	2.0 ± 0.2¶
FVR _{exp} (arbitrary units)*	40.8 ± 2.5	18.8 ± 1.1‡	11.7 ± 1.3‡	7.5 ± 0.7‡	36.1 ± 3.2
FVR _{control} (arbitrary units)*	39.2 ± 2.6	40.9 ± 2.8	44.8 ± 3.4§	46.6 ± 3.6§	56.2 ± 4.7¶
MAP (mmHg)	81 ± 1	82 ± 1	81 ± 1	81 ± 1	86 ± 1¶
Heart rate (bpm)†	62 ± 1	62 ± 1	63 ± 1	64 ± 1§	61 ± 1

Data are presented as mean ± SEM.

*P<0.001, //P<0.01 and ‡P<0.05 by repeated measures ANOVA (over baseline 1 and three CGRP dosages).

‡P<0.001 (versus baseline 1 as well as versus previous CGRP infusion) and §P<0.01 (versus baseline 1) by paired Student's *t*-test.

¶P<0.01 versus baseline 1 by paired Student's *t*-test.

Vasodilator response to CGRP

The first dose-response curves of all experiments ($n=40$) were pooled to evaluate the vascular response to sequential infusions of CGRP with placebo (Table 5.2). CGRP induced a dose-dependent increase ($P<0.001$) in FBF of the experimental arm (FBF_{exp}). This increase was for all dosages significant ($P<0.001$) versus baseline as well as between dosages. FBF of the control arm (FBF_{control}) decreased ($P=0.007$) slightly while MAP did not change throughout the experiments. Although, after wash-out FBF_{exp} had not completely returned to pre-infusion levels ($P=0.003$), baseline 1 and baseline 2 did not differ in terms of forearm vascular resistance (FVR_{exp}).

During the infusion of the highest dose of CGRP, a small increase ($P=0.01$) in heart rate was observed (Table 5.2). A comparable minor increase in heart rate was seen during the infusion of the highest dose of CGRP for each of the subgroups described below, suggesting a systemic vasodilator effect. However, as neither MAP nor FVR_{control} decreased systematically, alterations in heart rate did not contribute significantly to changes in FVR_{exp}.

Time-control experiments (Fig. 5.2, top)

The first dose-response curve of CGRP during co-infusion of placebo resulted in a percentage fall in FVR_{exp} (51 ± 4 , 66 ± 5 and $75\pm5\%$, respectively) comparable ($P=0.9$) with that during the second dose-response curve (43 ± 5 , 68 ± 4 and $80\pm3\%$, respectively) obtained after a wash-out period of 90 minutes. Venous plasma levels of CGRP increased from 1.2 ± 0.1 pmol/l at baseline to 21.2 ± 2.5 , 37.0 ± 4.5 and 78.9 ± 15.6 pmol/l, respectively, at the end of each dose during the first series of CGRP infusions.

Figure 5.2 (opposite page) CGRP-induced decrease in forearm vascular resistance during co-infusion of placebo (■, solid line). *Top*, repeated co-infusion of CGRP and placebo (□, dashed line) after a 90 minutes wash-out period. *Middle*, co-infusion of CGRP and L-NMMA/sodium nitroprusside (□, dashed line). *Bottom*, co-infusion of CGRP and indomethacin (□, dashed line). *P*-values refer to statistical difference by repeated measures ANOVA between first and second dose-response curve. NS: not statistically significant. * $P<0.05$ by Wilcoxon's matched-pairs signed-rank test between individual dosages. Data presented as mean $\pm$ SEM, $n=8$ in each subgroup.

The role of nitric oxide in CGRP-induced vasodilatation (Fig. 5.2, middle)

The percentage fall in FVR_{exp} induced by CGRP during co-infusion of placebo (61 ± 3 , 78 ± 2 and $84 \pm 3\%$, respectively) was larger ($P < 0.001$) than during co-infusion of L-NMMA and SNP (26 ± 5 , 57 ± 4 and $81 \pm 2\%$, respectively), due to a decreased vascular response both during the infusion of 3 and 10 $ng \cdot min^{-1} \cdot dl^{-1}$ forearm of CGRP ($P = 0.012$ and 0.017 , respectively) with L-NMMA and SNP. Baseline FVR_{exp} (30 ± 4 AU) did not change during co-infusion of L-NMMA and SNP without CGRP (35 ± 4 AU).

Cyclic GMP spillover at baseline (4.6 ± 0.5 $pmol \cdot min^{-1} \cdot dl^{-1}$ forearm) tended to increase ($P = 0.07$) at the end of the infusion of the highest dose of CGRP (9.9 ± 2.1 $pmol \cdot min^{-1} \cdot dl^{-1}$ forearm). Cyclic AMP spillover (from 5.9 ± 1.2 to 7.6 ± 5.0 $pmol \cdot min^{-1} \cdot dl^{-1}$ forearm) did not change ($P = 0.67$).

The role of prostaglandins in CGRP-induced vasodilatation (Fig. 5.2, bottom)

The percentage fall in FVR_{exp} induced by CGRP during co-infusion of placebo (48 ± 6 , 74 ± 5 and $82 \pm 4\%$, respectively) did not differ ($P = 0.12$) from that during co-infusion of indomethacin (36 ± 3 , 67 ± 2 and $83 \pm 2\%$, respectively). Baseline FVR_{exp} (26 ± 3 AU) slightly increased ($P = 0.012$) during the infusion of indomethacin without CGRP (36 ± 4 AU). Since, $FVR_{control}$ (46 ± 7 versus 45 ± 5 AU) did not change this suggests a vasoconstrictor effect of indomethacin in the infused arm.

The *ex vivo* production of TxB_2 decreased ($P = 0.012$) from 235.0 ± 25.7 ng/ml before to 3.4 ± 0.9 ng/ml 15 minutes after the start of the infusion of indomethacin.

The role of K^+ -channels in CGRP-induced vasodilatation

In subgroup 4 (Fig. 5.3, top), the percentage fall in FVR_{exp} induced by CGRP during co-infusion of placebo (49 ± 6 , 66 ± 7 and $76 \pm 6\%$, respectively) was comparable ($P = 0.71$) to that during co-infusion of TEA (37 ± 2 , 64 ± 3 and $82 \pm 2\%$, respectively). In subgroup 5 (Fig. 5.3, bottom), the percentage fall in FVR_{exp} induced by CGRP during co-infusion of placebo (49 ± 5 , 64 ± 7 and $80 \pm 3\%$, respectively) was comparable ($P = 0.39$) to that during co-infusion of glibenclamide (36 ± 3 , 61 ± 3 and $81 \pm 2\%$, respectively). Compared to the preceding baseline, FVR_{exp} slightly increased (from 32 ± 3 to 38 ± 3 AU, $P = 0.025$) during the infusion of TEA without CGRP while it was

unaffected by glibenclamide (41 ± 7 versus 41 ± 5 AU).

Figure 5.3 CGRP-induced decrease in forearm vascular resistance during co-infusion of placebo (■, solid line). *Top*, co-infusion of CGRP with tetra-ethyl-ammonium chloride (□, dashed line). *Bottom*, co-infusion of CGRP with glibenclamide (□, dashed line). NS: not statistically significant by repeated measures ANOVA. Data presented as mean $\pm$ SEM, n=8 in each subgroup.

5.4 Discussion

The present study aimed to investigate *in vivo* in human the vasodilator mechanism of action of (patho)physiological concentrations of CGRP. Plasma levels of CGRP have been reported to range from 1 pmol/l in healthy subjects [2] to 110 pmol/l under pathological conditions (e.g. heart failure, migraine and cluster headache) [22, 23]. During our experiments, even at the highest dose of CGRP, venous plasma levels remained within this (patho)physiological range. Consequently, the conclusions drawn from our experiments can be regarded as clinically relevant.

The CGRP-induced dose-dependent increase in forearm blood flow observed in the present study is comparable to that previously reported by other investigators [9, 24]. Tachyphylaxis and period-effects as possible mechanisms responsible for decreased vasodilatation upon repeated exposure to CGRP, were excluded by time-control experiments. These experiments clearly demonstrate that repeated infusions of CGRP with a 90-minute interval result in comparable changes in forearm vascular resistance.

The role of nitric oxide in CGRP-induced vasodilatation

The results of the present study allow us to conclude that the vasodilator effect of CGRP on the resistance arteries of the human forearm is partly mediated by the release of NO. This conclusion is based on the observation that L-NMMA, a competitive inhibitor of NO-synthase, significantly reduces the vasodilator response to CGRP. To correct for the L-NMMA-induced decrease in forearm blood flow, resulting from inhibition of the basal endothelial release of NO [15], nitroprusside was co-infused with it. Addition of this exogenous NO-donor has previously been shown to compensate for the loss of the baseline release of NO from the endothelium and to provide a useful approach for “clamping” of the basal vascular tone both *in vitro* and *in vivo* [17, 25]. Involvement of the NO-pathway in CGRP-induced vasodilatation is further supported by our observation that cGMP spillover was doubled during the infusion of CGRP. Indeed, if CGRP exerts its vasodilator activity by the release of NO, an increased production of its second messenger cGMP, might be expected [16]. Although the increase in cGMP spillover did not reach statistical significance, it

should be noted that cGMP is produced at the intracellular level. Consequently, the increase in the extracellular concentration of cGMP merely reflects intracellular guanylate cyclase activation and is much smaller than the increase in forearm blood flow which is accompanied with it.

It could be argued that the CGRP-induced release of NO might have been triggered non-specifically by an increase in flow, and thus in shear stress, rather than by stimulation of endothelial CGRP-receptors. If so, L-NMMA would be expected to reduce CGRP-induced vasodilatation most prominently at the highest flow resulting in the highest shear stress. In the present study, however, during the highest flow CGRP-induced vasodilatation was not inhibited by L-NMMA. In addition, in previous studies using the same technique [17, 26], the infusion of L-NMMA inhibited the vasodilator effects of acetylcholine (an endothelium-dependent vasodilator) but not those of nitroprusside or verapamil (endothelium-independent vasodilators), despite achieving blood flow responses comparable to those achieved with CGRP. Both observations argue against a non-specific shear stress-induced release of NO during drug-induced changes in forearm blood flow.

Accepting the contribution of NO to CGRP-induced vasodilatation, the issue remains from which tissue NO originates. L-NMMA acts as a non-selective NO-synthase inhibitor inhibiting different isoforms of the enzyme in different tissues [16]. Although it is tempting to speculate that NO release results from the endothelial activation of NO-synthase, neuronal as well as other sources of NO cannot be excluded. Likewise, it remains to be resolved which subtype of CGRP-receptor is responsible for the CGRP-induced release of NO. As currently neither for the different CGRP-receptors nor for the different isoforms of NO-synthase selective antagonists are available for human use, it is not possible at this time to determine which subtype of each is involved.

The role of prostaglandins in CGRP-induced vasodilatation

In the present study, CGRP-induced vasodilatation was not affected by the co-infusion of indomethacin which is a reversible cyclo-oxygenase inhibitor interfering with prostaglandin synthesis. Since thromboxane B₂ formation, a marker for cyclo-oxygenase activity [21], was completely suppressed after the infusion of

indomethacin, the absence of an inhibitory effect of indomethacin on CGRP-induced vasodilatation cannot be attributed to ineffective dosing. Consequently, prostaglandins are unlikely to be involved in CGRP-induced vasodilatation.

The role of potassium channels in CGRP-induced vasodilatation

In the present study, neither TEA nor glibenclamide affected CGRP-induced vasodilatation, indicating that TEA and glibenclamide-sensitive K^+ -channels are probably not involved in CGRP-induced vasodilatation unless K^+ -channel blockade was incomplete.

The role of K^+_{Ca} -channels in CGRP-induced vasodilatation was investigated using TEA. Although charybdotoxin and iberiotoxin are more selective K^+_{Ca} -channel blockers, they are far too toxic for human use. TEA antagonises different types of K^+ -channels with varying degrees of potency [18], but it has been shown to selectively block single K^+_{Ca} -channels in arterial smooth muscle cells *in vitro* at concentrations comparable to those used in the present study [27]. In addition, TEA has previously been shown to inhibit the opening of K^+ -channels by hydrochlorothiazide in the forearm model at the same dose as the one used in the present study [28]. To investigate the role of K^+_{ATP} -channels in CGRP-induced vasodilatation glibenclamide was used. This drug has convincingly been shown to interact with vascular smooth muscle cell K^+_{ATP} -channel activity in the forearm model at a dose three times lower than the one used in the present study [19]. Therefore, the absence of an inhibitory effect of TEA and glibenclamide on CGRP-induced vasodilatation is unlikely to be due to ineffective dosing.

Limitations, unanswered questions and future perspectives

Previous *in vitro* studies on the mode of vasodilator action of CGRP gave conflicting results [6-8, 10-13, 29, 30]. As well as discrepancies due to inter-species and inter-tissue differences, differences in experimental conditions may be responsible for these conflicting results. As far as the forearm model is concerned, it could be argued that the administration of CGRP into the brachial artery lumen results in an exposure which is higher for the endothelium than for the underlying vascular smooth muscle cell. This approach might result in an overestimation of the contribution of endothelial

mechanisms compared to the physiological situation in which CGRP is released from perivascular nerves. Consequently, it cannot be excluded that in case of a perivascular release of CGRP non-endothelial mechanisms of action become more important. Therefore, our data do not entirely exclude a role for K^+ -channel activation by CGRP on the level of the vascular smooth muscle cell.

Although our data convincingly show that NO is involved in CGRP-induced vasodilatation of the human forearm, several questions remain. Firstly, as NO-synthase inhibition only partly attenuated CGRP-induced vasodilatation, which alternative mechanism might be involved? One of the mechanisms which has been suggested repeatedly is the activation of adenylate cyclase by the interaction of CGRP with G-protein-coupled CGRP-receptors on the vascular smooth muscle cell [6, 7, 31]. However, as selective adenylate cyclase inhibitors are not available for human use this mechanism cannot be investigated using the forearm model. As an alternative approach the cAMP spillover was assessed as an indirect measure of local intracellular adenylate cyclase activation. Possibly due to a lack of power, the limited increase in cAMP spillover did not allow us to conclude that adenylate cyclase activation took place during the infusion of CGRP. Likewise for cGMP, it should be noted that the measurement of extracellular cAMP might not be sufficiently sensitive to assess changes at the intracellular level.

Secondly, why did NO-synthase inhibition not affect CGRP-induced vasodilatation at higher dosages of CGRP, despite the apparent involvement of NO? One possible explanation is that L-NMMA did not fully inhibit the *de novo* generation of NO. Although this cannot be completely excluded, it is very unlikely in view of the high dose of L-NMMA which was used and which has been reported frequently to inhibit NO formation [15, 32]. Alternatively, NO may be bound to a carrier protein and stored in both the endothelium and the vascular smooth muscle cell [33]. The release of NO from intra-cellular stores would obviously not be inhibited by the administration of a NO-synthase blocker. To clarify the contribution of NO stores to NANC-vasodilatation in general and to CGRP-induced vasodilatation in particular, the inhibitory effect of NO-scavengers on CGRP-induced vasodilatation should be further investigated.

In summary, the results of the present study demonstrate for the first time *in vivo* in human that the vasodilator response to (patho)physiological concentrations of CGRP is at least in part dependent on the local release of NO. In addition, this vasodilator response appears not to be related to the release of prostaglandins or the activation of K^+_{Ca} -channels and K^+_{ATP} -channels. Obviously, a better understanding of the vasodilator mechanism of action of CGRP may have important implications for those pathophysiological conditions where increased levels of CGRP are involved. Under these conditions not only CGRP-antagonists but also selective NO-synthase inhibitors might offer an alternative pharmacological approach for future research and drug development.

Acknowledgements:

We are greatly indebted to Prof. Dr. J. Arnout (Centre for Thrombosis and Vascular Research, University of Leuven, Belgium) for performing TxB_2 assessments; to Dr. Paul Schiffers (Dept. of Pharmacology and Toxicology, Maastricht University) for performing CGRP, cGMP and cAMP assessments and to Aventis Pharma for supplying glibenclamide.

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Part III

Vascular Effects of Anti-Migraine Drugs

*He (Grant) soaked his feet in hot water
and put mustard plasters on the back of his neck.*

(Ulysses S. Grant, 1822-1885)

Chapter 6

Vascular Effects of 5-HT_{1B/1D}-Receptor Agonists in Migraine Patients

Abstract - Background: To investigate the vascular effects of selective 5-HT_{1B/1D}-receptor agonists. **Methods:** Sixteen migraine patients participated in a double-blind, placebo-controlled, 4-way cross-over study. After equipotent oral therapeutic dosages of sumatriptan (50 mg), rizatriptan (10 mg), zolmitriptan (2.5 mg) or placebo measurements were performed at the common carotid, brachial and temporal artery. Using ultrasound and applanation tonometry arterial vessel wall properties, blood flow and pressure waveforms were assessed. At the brachial artery flow-mediated vasodilatation (an endothelium-dependent process) was evaluated and blood pressures were recorded. **Results:** Mean arterial pressure, 91 ± 2 mmHg after placebo, increased ($P < 0.05$) by 4-6% after each triptan. Isobaric compliance of the brachial artery, 0.077 ± 0.010 mm²/kPa after placebo, decreased ($P < 0.01$) by 11 ± 8 , 11 ± 11 and $23 \pm 7\%$ after sumatriptan, rizatriptan and zolmitriptan, respectively. After zolmitriptan this decrease was larger ($P < 0.05$) than after sumatriptan. Isobaric compliance of the carotid artery, 1.31 ± 0.10 mm²/kPa after placebo, did not change. Each drug decreased ($P < 0.001$) both brachial and carotid artery diameter whereas temporal artery diameter, 1.35 ± 0.06 mm after placebo, only decreased after zolmitriptan (by $12 \pm 3\%$, $P < 0.001$). The resistance of the vascular bed distal of the temporal artery increased after both sumatriptan (by $26 \pm 11\%$, $P < 0.05$) and zolmitriptan (by $40 \pm 9\%$, $P = 0.001$). Flow-mediated vasodilatation did not differ between treatments.

Based on: Jan N.J.M. de Hoon⁽¹⁾, Jean M. Willigers⁽²⁾, Jaap Troost⁽³⁾, Harry A.J. Struijker-Boudier⁽¹⁾, Luc M.A.B. Van Bortel⁽¹⁾. Vascular Effects of 5-HT_{1B/1D}-Receptor Agonists in Migraine Patients. *Clinical Pharmacology and Therapeutics* (accepted for publication).

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Conclusions: Selective 5-HT_{1B/1D}-receptor agonists induce vasoconstriction and decrease the buffering capacity of conduit arteries. These effects are more pronounced at muscular compared to elastic arteries. Resistance is only increased at the temporal artery vascular bed, suggesting cranioselectivity for resistance vessels rather than for conduit arteries. Endothelial function is not differently affected by any of the triptans tested.

6.1 Introduction

The development of sumatriptan in the early 1990s was a major advance in the acute treatment of migraine [1]. It was the first selective 5-HT_{1B/1D}-receptor agonist to become available as an anti-migraine drug. Although sumatriptan is an effective and generally well tolerated drug, a major limitation for its use is the occurrence of chest symptoms suggestive of angina pectoris, which are reported in up to 15% of patients [2-5]. As electrocardiographic abnormalities are frequently absent, it is unlikely that these chest symptoms are always cardiac in origin. Nevertheless, the use of sumatriptan has been reported to be associated with both myocardial infarction and sudden death [6, 7]. Furthermore, its vasoconstrictor potency both on the coronary and systemic circulation has clearly been demonstrated [8-10] and rendered the drug contraindicated in patients with cardiovascular disease. Therefore, recent research aimed at the development of anti-migraine drugs with a safer cardiovascular profile and resulted in the introduction of second-generation “sumatriptan-like” drugs (referred to as triptans).

Most second-generation triptans, including rizatriptan and zolmitriptan, are selective 5-HT_{1B/1D}-receptor agonists characterised by an *in vitro* pharmacology comparable with that of sumatriptan in terms of receptor selectivity, potency and efficacy [11]. Their vasoconstrictor activity results from activation of the vascular smooth muscle cell 5-HT_{1B}-receptor subtype (previously known as 5-HT_{1DB}-receptor). This does not only apply for cranial vasoconstriction but also for coronary and peripheral vasoconstriction. Thus, from a pharmacological point of view, there is little reason to expect that second-generation triptans will display a cardiovascular profile profoundly different from that of sumatriptan. Moreover, *in vitro* data indicate that

many second-generation triptans contract both human coronary and cranial arteries with a potency and maximal effect comparable with that of sumatriptan [12-15]. However, as the clinical relevance of these *in vitro* data remains speculative, *in vivo* human research is needed to better define the cardiovascular profile of these drugs by comparing their vascular effects at clinically relevant plasma concentrations.

The aim of the present study was to compare the peripheral vascular effects of sumatriptan (reference drug) with those of zolmitriptan and rizatriptan (second-generation triptans) in migraine patients outside a migraine attack. Therefore, drug-induced changes in (i) large artery wall properties, (ii) vascular resistance and (iii) endothelial function were assessed non-invasively.

6.2 Subjects and methods

Subjects and subject selection

Sixteen eligible migraine patients (as defined by the International Headache Society migraine criteria [16]) were recruited from the neurology outpatient clinic. Based on medical history, physical examination, routine laboratory tests and electrocardiogram, all patients were in good health. Major exclusion criteria were: smoking, history of cardiovascular disease, arterial hypertension (blood pressure >160/95 mmHg), diabetes mellitus, hyperlipidaemia (total cholesterol >6.4 mmol/l) and pregnancy or lactation. Except for oral contraceptives, no regular medication was allowed. The study was approved by the ethics committee of the academic hospital of Maastricht and conducted in accordance with the Declaration of Helsinki (Hong Kong revision 1989). All patients gave written informed consent to participate.

Study design

After screening, patients were enrolled in a randomised, double-blind, placebo-controlled, 4-way cross-over study. Study periods were separated by a wash-out period of at least 1 week. During each period a single oral dose of an anti-migraine drug (sumatriptan 50 mg, rizatriptan 10 mg or zolmitriptan 2.5 mg) or placebo was

administered outside a migraine attack. Before each study period, patients were asked not to use any drugs for at least 3 days, to abstain from alcohol or caffeine containing beverages for at least 24 and 12 hours, respectively, and to remain fasted for at least 3 hours. Only the intake of water was allowed until 2 hours before drug intake. The study drug was taken with 150 ml of water under supervision of the investigator. After drug intake, patients remained fasting until all measurements had been performed.

Measurements

Measurements started 1.5 h after drug intake and were consecutively performed at the common carotid artery (an elastic artery providing cranial intracerebral and extracerebral perfusion), the superficial temporal artery (a muscular artery largely providing extracerebral meningeal perfusion) and the brachial artery (a peripheral muscular artery). Unless specified otherwise measurements were always performed right sided. They lasted approximately 1 hour and were all performed by one investigator in a quiet room after the patient had been lying in the supine position for at least 15 minutes.

First, systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were recorded at the left upper arm for 15 minutes every 3 minutes using a semi-automated oscillometric device (Dinamap[®] 950, Critikon Inc., Florida). The mean of these readings was used as the patient's post-drug haemodynamic parameters.

At the common carotid and brachial artery both pressure waveforms and vessel wall movements (i.e. arterial end diastolic diameter, D and diameter change, ΔD or distension) were recorded non-invasively using an applanation tonometer (Micro-tip[®] transducer, Millar Instruments Inc., Texas) [17] and an echo-tracking system (Wall Track System[®] combined with Scanner 350, Pie Medical, The Netherlands) [18], respectively. At the temporal artery only D was measured. In addition, using ultrasound centre stream velocity of the arterial blood flow was measured to calculate local blood flow and vascular resistance. Finally, flow-mediated vasodilatation of the brachial artery was recorded with the echo-tracking system at 45 seconds and 1.5, 2.5, 3.5 and 5 minutes after reperfusion and allowed us to calculate the area under the curve ($AUC_{1 \rightarrow 5}$) of the diameter-time curve. Flow-mediated vasodilatation was

measured using a protocol comparable with the one described by Celermajer and collaborators [19]

At the common carotid artery vessel wall movements and pressure waveforms were recorded ipsilateral and consecutively. At the brachial artery vessel wall movements (right) and pressure waveforms (left) were recorded contralateral and simultaneously. In the analysis, the local pulse pressures (ΔP) of the common carotid (ΔP_{CCA}) and brachial artery (ΔP_{BA}) as well as large artery wall properties were calculated [20]. Therefore, diameter-time recordings (ultrasound) and pressure-time recordings (applanation tonometry) were simultaneously analysed off-line using a custom made Matlab[®] script developed by the biophysics department of the university as previously reported [20]. By constructing diameter-pressure curves, distensibility coefficients (DC) and cross-sectional compliance (CC) were calculated over the operating pressure ΔP . In addition, to assure that differences in vessel wall properties between treatments resulted from drug-induced changes in the pressure-diameter relation rather than from changes in arterial pressure, isobaric vessel wall properties were calculated over a pre-defined pressure interval of 85 to 95 mmHg (DC_{iso} and CC_{iso}).

For a detailed description on all the measurements and the subsequent data analysis, we refer to the methods outlined in *Chapter 2*.

Statistical analysis

All vascular parameters obtained after each treatment were compared by ANOVA. In case of a significant difference ($P < 0.05$) between treatments, a post-hoc paired Student's *t*-test was performed to compare treatments individually. The difference between active treatment and placebo is expressed as percentage change versus placebo. Unless specified otherwise, all data are presented as mean $\pm$ SEM. $P < 0.05$ was considered statistically significant.

Table 6.1 Haemodynamic data (mean $\pm$ SEM): post-drug comparison between treatment groups (n=16 for each group).

	<i>Placebo</i>	<i>Sumatriptan</i>	<i>Rizatriptan</i>	<i>Zolmitriptan</i>	<i>P*</i>
SBP (mmHg)	115 $\pm$ 2	117 $\pm$ 2 †	120 $\pm$ 2 §	121 $\pm$ 2 ‡	<0.001
DBP (mmHg)	74 $\pm$ 2	77 $\pm$ 2 †	78 $\pm$ 2 ‡	77 $\pm$ 2 ‡	0.004
Heart rate (bpm)	57 $\pm$ 2	56 $\pm$ 2	57 $\pm$ 2	56 $\pm$ 1	NS
MAP (mmHg)	91 $\pm$ 2	95 $\pm$ 2 ‡	96 $\pm$ 2 §	96 $\pm$ 2 ‡	0.027
ΔP_{BA} (mmHg)	41 $\pm$ 1	40 $\pm$ 1 †	42 $\pm$ 1	44 $\pm$ 2	0.019
ΔP_{CCA} (mmHg)	38 $\pm$ 1	41 $\pm$ 2 †	43 $\pm$ 2 ‡	43 $\pm$ 2 ‡	0.002

Systolic (SBP), diastolic blood pressure (DBP) and heart rate (beats per minute, bpm); calculated mean arterial pressure (MAP), brachial artery pulse pressure (ΔP_{BA}) and common carotid artery pulse pressure (ΔP_{CCA}). **P*-value based on ANOVA for repeated measures. NS, not statistically significant. †*P*<0.05, ‡*P*<0.01 and §*P*<0.001 by paired Student's *t*-test of post-drug comparison versus placebo. †*P*<0.05 by paired Student's *t*-test of comparison versus zolmitriptan.

6.3 Results

All patients (3 males and 13 females) completed the study. Patients were aged 36 years (range, 23-55) and had a body mass index of 24 (range 19-29) kg/m². At screening blood pressure averaged 118 (range 102-132)/73 (range 58-89) mmHg. Total cholesterol was 5.1 (range 3.8-6.3) mmol/l.

Patients had been suffering from migraine for 18±2 years. One patient had a history of recurrent migraine with aura. Migraine attacks recurred with a frequency of 2.5±0.3 per month and lasted on average 1.8±0.1 days. In most cases headache was one-sided (n=13) and pulsatile in nature (n=9). In all patients it was accompanied by nausea or vomiting and both photophobia and phonophobia. As an abortive drug patients had used sumatriptan at least once before entering the study. Except for oral contraceptives (n=7), no other drugs were regularly used.

Haemodynamic data

Compared to placebo each active treatment increased DBP, MAP, SBP (except for sumatriptan) and ΔP_{CCA} (Table 6.1). Except for a smaller SBP ($P=0.027$) and ΔP_{BA} ($P=0.007$) after sumatriptan compared to zolmitriptan, the vasopressor effects did not differ between active treatments.

Vascular measurements

The elastic common carotid artery diameter was decreased by each active treatment ($P<0.001$ by ANOVA). Diameter, 6.93±0.14 mm after placebo, decreased to 6.65±0.16 mm after sumatriptan ($P=0.013$ versus placebo), 6.65±0.12 mm after rizatriptan ($P=0.001$) and 6.63±0.14 mm after zolmitriptan ($P=0.003$). This decrease did not differ between active treatments (Fig. 6.1, top). Drug intake did not affect distension (594±49 μ m after placebo) or distensibility; neither over operating pressure (DC) nor under isobaric conditions (DC_{iso}) (Table 6.2). Cross-sectional compliance decreased, while CC_{iso} did not change. Vascular resistance (703±26 kPa.l⁻¹.s after placebo) was unaffected by either triptan (Fig. 6.1, bottom).

Table 6.2 Post-drug comparison of mechanical arterial wall properties between treatment groups (n=16 for each group).

	<i>Placebo</i>	<i>Sumatriptan</i>	<i>Rizatriptan</i>	<i>Zolmitriptan</i>	<i>P*</i>
<i>Common carotid artery:</i>					
DC (10^{-3} /kPa)	36.6 ± 3.1	35.6 ± 2.5	34.5 ± 3.0	34.6 ± 2.0	NS
DC _{iso} (10^{-3} /kPa)	32.0 ± 2.8	33.6 ± 2.3	31.3 ± 2.0	33.1 ± 2.0	NS
CC (mm ² /kPa)	1.36 ± 0.10	1.22 ± 0.09†	1.19 ± 0.09‡	1.19 ± 0.07‡	0.013
CC _{iso} (mm ² /kPa)	1.31 ± 0.10	1.27 ± 0.10	1.16 ± 0.07	1.22 ± 0.07	NS
<i>Brachial artery:</i>					
DC (10^{-3} /kPa)	6.7 ± 0.7	6.7 ± 0.7 [‡]	6.6 ± 0.6 [‡]	5.4 ± 0.5 [†]	0.036
DC _{iso} (10^{-3} /kPa)	5.6 ± 0.6	5.9 ± 0.7	5.6 ± 0.5	4.9 ± 0.5	NS
CC (10^{-2} mm ² /kPa)	8.7 ± 0.9	7.1 ± 0.9§ [‡]	7.1 ± 0.8 [‡]	5.9 ± 0.6§	<0.001
CC _{iso} (10^{-2} mm ² /kPa)	7.7 ± 1.0	6.4 ± 0.8 [†] [‡]	6.2 ± 0.8	5.3 ± 0.6 [‡]	0.004

Distensibility coefficient (DC and DC_{iso}) and cross-sectional compliance (CC and CC_{iso}) over the operating pressure (ΔP) and over the pre-defined pressure interval of 85-95 mmHg (isobaric), respectively. * *P*-value based on ANOVA for repeated measures. NS, not statistically significant. † *P*<0.05, ‡ *P*<0.01 and § *P*<0.001 by paired Student's *t*-test versus placebo. [‡] *P*<0.05 by paired Student's *t*-test versus zolmitriptan.

Figure 6.1 Comparison of the decrease in diameter (top) and increase in vascular resistance (bottom) of the common carotid, brachial and temporal artery after intake of sumatriptan (open bars), rizatriptan (hatched bars) and zolmitriptan (closed bars). Data presented as percentage difference versus placebo (mean $\pm$ SEM); n=16 in each group. * $P<0.05$ and # $P<0.1$ versus placebo.

The muscular brachial artery diameter was decreased by each active treatment (for each drug $P<0.001$ versus placebo). Diameter, 4.16 ± 0.18 mm after placebo, decreased to 3.74 ± 0.15 mm after sumatriptan, 3.75 ± 0.15 mm after rizatriptan and

3.72±0.15 mm after zolmitriptan. This decrease did not differ between treatments (Fig. 6.1, top). After zolmitriptan DC was smaller compared to placebo ($P=0.025$), sumatriptan ($P=0.019$) and rizatriptan ($P=0.03$) (Table 6.2). Compared to placebo both CC and CC_{iso} decreased after sumatriptan ($P<0.001$ and $P=0.02$, respectively) and zolmitriptan ($P<0.001$ and $P=0.003$, respectively) while they tended to decrease after rizatriptan ($P=0.065$ and 0.08 , respectively). Zolmitriptan decreased brachial artery CC to a larger extent than sumatriptan ($P=0.014$) and rizatriptan ($P=0.04$) did. CC_{iso} was smaller after zolmitriptan compared to sumatriptan ($P=0.04$). Vascular resistance (9,730±1,017 kPa.l⁻¹.s after placebo) did not change by active treatment (Fig. 6.1, bottom).

The muscular temporal artery, diameter (1.35±0.06 mm after placebo) decreased ($P=0.012$ by ANOVA) and vascular resistance (30,738±3,374 kPa.l⁻¹.s after placebo) increased ($P=0.013$ by ANOVA) after active treatment (Fig. 6.1, top). Compared to placebo, only after zolmitriptan diameter (1.19±0.06 mm, $P<0.001$) was significantly smaller; after sumatriptan (1.26±0.05 mm) and rizatriptan (1.25±0.05 mm) it tended to be smaller ($P\leq 0.1$). Both after sumatriptan (37,007±4,264 kPa.l⁻¹.s, $P=0.04$) and zolmitriptan (41,186±4,091 kPa.l⁻¹.s, $P=0.001$) vascular resistance was increased compared to placebo. After rizatriptan (32,515±2,610 kPa.l⁻¹.s) the increase in resistance did not reach statistical significance and was smaller ($P=0.04$) compared to zolmitriptan (Fig. 6.1, bottom).

The increase in flow upon ischaemia at the brachial artery resulting in flow-mediated vasodilatation, did not differ between placebo (771%±53), sumatriptan (740±52%), rizatriptan (742±48%) and zolmitriptan (775±48%). Flow-mediated vasodilatation (Fig. 6.2) was comparable between placebo, sumatriptan, rizatriptan and zolmitriptan both in terms of maximal vasodilatation (5.5±1.0, 6.6±1.3, 5.8±0.9 and 6.2±1.2%, respectively) and in terms of AUC_{0→5} (340, 387, 296 and 310 min.µm, respectively).

Figure 6.2 Flow-mediated vasodilatation of the brachial artery after 5 minutes of forearm occlusion. Diameter before occlusion ($t=0$) and from 45 seconds until 5 minutes after reperfusion. Data are presented as mean $\pm$ SEM, $n=16$ in each group.

6.4 Discussion

Most studies investigating the vascular effects of anti-migraine drugs make use of isolated blood vessels and refer to *in vitro* data which are sometimes obtained under clinically irrelevant concentrations [12-15]. In contrast, the results of the present study refer to *in vivo* data obtained in migraine patients after the intake of equally effective acute therapeutic dosages of sumatriptan, rizatriptan and zolmitriptan [21-23]. Consequently, these data allow for a fair comparison between these drugs and can be expected to represent clinical reality. Moreover, as all measurements were performed within a time frame corresponding with the time to reach maximal plasma concentrations ($t_{\max}$) after oral drug intake [24-26], it is reasonable to assume that peak effects were observed. Still, a delayed onset of vascular effects, as previously suggested for eletriptan [27], cannot be excluded because the metabolism of both zolmitriptan and rizatriptan results in the formation of active metabolites [26, 28].

In accordance with previous reports sumatriptan, rizatriptan and zolmitriptan each induced a small increase in blood pressure [10, 25, 29]. The increase in mean arterial pressure is in line with previous research demonstrating an increased systemic vascular resistance after the administration of triptans [8, 9, 29]. Theoretically, the increase in mean arterial pressure could also have resulted from an increase in cardiac output due to an increase in stroke volume. Although cardiac output was not assessed, an increased stroke volume is suggested by the increase in carotid artery pulse pressure which was not accompanied by a decreased compliance. However, a change in cardiac output was not observed in previous research performing cardiac assessment after the administration of triptans [9, 27].

Arterial stiffness was assessed by investigating local pressure-diameter changes and calculating distensibility and cross-sectional compliance. Both distensibility, a measure of elasticity, and cross-sectional compliance, a measure of buffering capacity, are parameters which decrease with increasing arterial stiffness and refer to local mechanical vessel wall properties [30]. The present study demonstrates that each of the triptans investigated, and zolmitriptan most potently, decrease the buffering capacity of the brachial artery both at operating pressure and at isobaric values. At the common carotid artery the small decrease in buffering capacity at operating pressure can be interpreted as a passive phenomenon resulting from the increase in mean arterial pressure. Indeed, as the buffering capacity at isobaric values did not change the mechanical properties of the artery do not seem to be affected. In contrast with the common carotid artery, at the brachial artery isobaric compliance did decrease. This apparent difference in mechanical properties between the common carotid and the brachial artery after the intake of triptans, might be due to structural differences. As the common carotid artery is an elastic artery it has a higher proportion of elastic fibres and a smaller quantity of vascular smooth muscle cells [31]. Thus, a change in smooth muscle tone is likely to result in a small increase in stiffness of “elastic arteries” compared to “muscular arteries”. Taken together, these data demonstrate that the intake of both sumatriptan and second-generation triptans results in a comparable systemic vasopressor effect and a decreased buffering capacity of muscular arteries.

These vascular effects are regarded as undesirable and implicate that triptans should be used with caution in patients at increased risk for cardiovascular events.

Each of the active treatments decreased the diameter of both the common carotid and the brachial artery without affecting vascular resistance. As neither for the carotid nor for the brachial artery vasoconstriction was different between treatments this argues against an increased cranial selectivity of second-generation triptans compared to sumatriptan. At the temporal artery however, zolmitriptan most potently decreased arterial diameter and increased vascular resistance. As each of the triptans was equally effective in decreasing brachial artery diameter, these data do suggest an increased cranioselectivity for zolmitriptan. However, this does not translate into a more favourable cardiovascular profile as zolmitriptan also most potently affected brachial artery wall properties. Although it cannot be excluded that the changes at the temporal artery induced by sumatriptan and rizatriptan did not reach statistical significance due to a lack of power, these data do indicate that triptans can differently affect the same vascular territory. Obviously, these effects can be expected to be dose-dependent.

Thus within the cranial circulation selective 5-HT_{1B/1D}-receptor agonists, and most convincingly zolmitriptan, result in arterial and arteriolar vasoconstriction of the vascular territory supplied by the external carotid artery. Interestingly, studies on the pathophysiology of migraine have focussed on the dilatation and increased pulsations of the external carotid artery and its branches, including the temporal artery [32, 33]. Moreover, dilated meningeal blood vessels are considered the pain-producing structures in migraine [34, 35] and are largely perfused by the external carotid artery. Consequently, the vascular changes observed at the temporal artery might be useful surrogate markers indicative of anti-migraine activity.

Although cranial vasoconstriction is considered a prerequisite to yield a 5-HT_{1B/1D}-receptor agonist with anti-migraine activity [11], it is tempting to speculate that increasing vessel wall stiffness might be equally important. The pulsatile nature of migraine headache suggests that increased vascular pulsations are sensed by stretch receptors, resulting in the activation of perivascular nerve endings [34]. If so, increasing vessel wall stiffness *per se*, without obligatory inducing vasoconstriction, might sufficiently temper the activation of stretch receptors and render efficient anti-

migraine drugs with a reduced risk of coronary vasoconstriction. Besides selective 5-HT_{1B/1D} receptor agonists, also ergot alkaloids have previously been reported to decrease arterial compliance [36, 37], supporting the idea that decreasing the activation of perivascular nerves by increasing arterial stiffness might contribute to the efficacy of anti-migraine drugs.

Whether or not patients are at risk for unwanted coronary vasoconstriction after the exposure to 5-HT-receptor agonists has been related to endothelial function [38, 39]. As 5-HT_{1B/1D} receptor agonists interact with endothelial receptors mediating the release of vasodilator substances, including nitric oxide, their vascular effect is supposed to be the balanced result of vasoconstriction, mediated by smooth muscle receptors and vasodilatation, mediated by endothelial receptors [38]. Consequently, in patients with coronary artery disease, which has been associated with endothelial dysfunction [40], the vasoconstrictor effects of triptans might remain unopposed and render them at increased risk for untoward cardiovascular events. If the endothelium plays a pivotal role in providing a counteractive mechanism, the intake of triptans should not interfere with endothelial function. This was investigated by measuring flow-mediated vasodilatation of the brachial artery which has previously been shown to be dependent on the endothelial release of NO [41] and correlates well with coronary endothelial function [42]. In the present study, flow-mediated vasodilatation of the brachial artery did not differ between the triptans and placebo, arguing against an interference with endothelial function. However, as the brachial artery baseline diameter after the intake of a triptan was smaller than after placebo, these data have to be interpreted with caution. As previous studies have shown that flow-mediated vasodilatation dilates small arteries more than it does larger ones [43], placebo and active treatment groups cannot be directly compared [44]. Nevertheless, it can be concluded that none of the active treatments interfered differently with flow-mediated vasodilatation.

In summary, the data of the present study demonstrate that after the intake of equipotent therapeutic dosages, both sumatriptan and second-generation triptans induce vasoconstriction and decrease the buffering capacity of conduit arteries. These effects are more pronounced at muscular compared to elastic arteries. In contrast,

vascular resistance is only increased at the temporal artery vascular bed, suggesting cranioselectivity of triptans for resistance arteries rather than for conduit arteries. Zolmitriptan most potently affects the vascular territory of the temporal artery, but also induces the largest decrease in brachial artery buffering capacity. Endothelial function, as assessed by flow-mediated vasodilatation, is not differently affected by the triptans.

Acknowledgement:

This study was supported by AstraZeneca, Zoetermeer, The Netherlands.

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Chapter 7

Dihydroergotamine: Discrepancy Between Arterial, Arteriolar and Pharmacokinetic Data

Abstract – Background: To investigate the peripheral vascular effects and pharmacokinetics of dihydroergotamine (DHE) 0.5 mg after a single subcutaneous administration. **Methods:** A double-blind, placebo-controlled, two-way cross-over study was performed in 10 healthy male subjects. A wash-out period of 2 weeks separated the two study periods. During each period, just before and at regular time intervals after drug administration, vascular measurements were performed and venous blood samples drawn. At the brachial artery, vessel wall properties were assessed by ultrasound and applanation tonometry. Blood pressure and heart rate were recorded with an oscillometric device. Forearm blood flow was measured with venous occlusion plethysmography. For all parameter-time curves the area under the curve (AUC) was calculated. Differences in AUC after placebo and DHE (Δ AUC) were analysed and the time-course of the difference assessed. DHE plasma concentrations were fitted to a tri-exponential equation consistent with a two-compartment open model. **Results:** AUC for blood pressure, heart rate and forearm vascular resistance did not change after DHE. Brachial artery diameter and compliance decreased ($P < 0.01$); Δ AUC (95% confidence interval) equalled -8.81 mm.h ($-12.97/-4.65$) and $-0.98 \text{ mm}^2.\text{kPa}^{-1}.\text{h}$ ($-1.61/-0.34$), respectively. Diameter decreased ($P < 0.05$) from 1 until 24 h after DHE (peak decrease 9.7% at 10 h); compliance from 2 until 32 h (24.8% at 2 h). Time to reach maximum plasma concentration of DHE averaged $0.33 \pm 0.08 \text{ h}$ ($\pm$ SEM); terminal half-life was $5.63 \pm 1.15 \text{ h}$.

Based on: J.N.J.M. de Hoon, K.A. Poppe, H.H.W. Thijssen, H.A.J. Struijker-Boudier, L.M.A.B. Van Bortel. Dihydroergotamine: discrepancy between arterial, arteriolar and pharmacokinetic data. (submitted).

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Conclusions: As DHE decreases diameter and compliance of the brachial artery whereas forearm vascular resistance remains unchanged, it acts on conduit arteries without affecting resistance vessels. Furthermore, a discrepancy was demonstrated between the plasma concentrations of DHE which rapidly reach peak levels and quickly decline, and its long lasting vasoconstrictor activity.

7.1 Introduction

Migraine is a common and debilitating neurological disorder for which effective and safe treatment is needed. The ergot alkaloids ergotamine and dihydroergotamine (DHE) were the first compounds reported to be successful in the acute treatment of migraine [1]. Ergotamine was isolated in 1918 from the fungus *Claviceps purpurea* and reported effective as an anti-migraine drug in 1926. However, because of its unfavourable side effect profile the dihydrogenated derivative DHE was developed [2]. DHE differs from ergotamine in that it is less toxic, less emetic, devoid of uterotonic activity and it displays a decreased peripheral vasoconstrictor activity [2, 3]. In particular, DHE preferentially constricts venous capacitance vessels and arteriovenous anastomoses while being a much less potent arterial vasoconstrictor [4, 5]. However, it is not entirely clear how this vascular selectivity is obtained as both drugs are non-selective pharmacological agents interacting with serotonergic (5-HT₁ and 5-HT₂-receptors), adrenergic (α_1 and α_2 -receptors) and dopaminergic receptors (D₂-receptors) [2, 6].

The venoconstrictor activity of DHE has been investigated extensively whereas data on its arterial and arteriolar effects are limited. Both in animal [7] and in human [8-10] a discrepancy has been reported between the long lasting venoconstrictor activity of DHE and its plasma concentration profile showing rapidly increasing plasma levels which quickly decline. With respect to arterial effects, *in vitro* data show that ergotamine and DHE induce a sustained contraction of human arteries [11, 12]. For ergotamine both long lasting arterial contractions and a discrepancy between plasma concentrations and arterioconstrictor activity have also been reported in human [13]. In contrast, based on indirect measurements Andersen et al. [14] concluded that

DHE lacks peripheral arterial effects. Direct assessments of the arterial effects of DHE in man have never been performed.

These conflicting results between *in vitro* and *in vivo* data and the unexpected difference between the vascular effects of ergotamine and DHE, led us to investigate the vascular effects of DHE *in vivo* in human. Moreover, as the use of DHE in the acute treatment of migraine can be expected to increase because of the commercial availability of a nasal spray, a better knowledge of its vascular effects is warranted. In the present study, the peripheral vascular effects (arterial and arteriolar) of DHE over time and the pharmacokinetics of DHE were investigated simultaneously after the administration of a single subcutaneous injection of DHE to healthy subjects.

7.2 Subjects and methods

Subjects and study design

Ten non-smoking, male subjects (age 23 ± 2 years (mean $\pm$ SD), weight 72 ± 8 kg and height 182 ± 7 cm) entered and completed the study. Based on their medical history, clinical examination, routine laboratory tests and electrocardiogram all subjects were in good health. The study was approved by the ethics committee of the academic hospital of Maastricht and conducted in accordance with the Declaration of Helsinki (Hong Kong revision 1989). All patients gave written informed consent to participate.

A double-blind, randomised, placebo-controlled, two-way cross-over design was used. A wash-out period of at least 2 weeks separated the two study periods. During one period 0.5 mg dihydroergotamine mesylate (Dihydergot[®], Novartis Pharma, 1 mg/ml) was administered, during the other 0.5 cc NaCl 0.9% as placebo. Both were administered subcutaneously at the level of the thigh. On each occasion the drug was administered after overnight fasting and subjects remained fasting until 4 hours after drug administration. Before drug administration, subjects were asked to abstain from alcohol-containing beverages for at least 24 hours. Until all measurements had been performed (i.e. 32 hours after drug administration) alcohol and caffeine-containing beverages were not allowed.

Pharmacodynamic assessments

All measurements were performed by one investigator in a quiet room with the subject lying comfortably in the supine position for at least 15 minutes before measurements were started. Just before (0 h) and at 0.5, 1, 2, 4, 6, 8, 10, 24 and 32 hours after drug administration, pharmacodynamic assessments were performed. These consisted of non-invasive measurements of (i) vessel wall movements and pressure wave recordings of the brachial artery, (ii) forearm blood flow with and without wrist occlusion and (iii) blood pressure and heart rate.

Brachial artery wall properties

Using an echo-tracking system as described by Hoeks et al. [15] (Wall Track System[®] and Scanner 350, Pie Medical, The Netherlands) end diastolic diameter (D) and diameter changes (ΔD , distension) of the right brachial artery were measured. Using applanation tonometry (Micro-tip[®] transducer, Millar Instruments Inc., Texas) [16], pressure waveforms of the left brachial artery were recorded simultaneously with vessel wall movements. At each point in time at least 3 measurements were performed.

Both before and after vessel wall movement/pressure waveform recordings, diastolic blood pressure (DBP), systolic blood pressure (SBP) and heart rate were each measured 3 times with a 1-minute interval. These measurements were performed at the right upper arm using an oscillometric device (Dinamap[®], Critikon Inc., Florida). At each point in time the mean of these recordings was taken as the subject's reading.

In the analysis, mean arterial pressure (MAP), brachial artery pulse pressure (ΔP_{BA}) and large artery wall properties were calculated. Therefore, after pressure wave calibration, diameter-time recordings (ultrasound) and pressure-time recordings (applanation tonometry) were simultaneously analysed off-line using a custom made Matlab[®] script developed by the biophysics department of the university [17]. By constructing pressure-diameter curves, distensibility coefficients (DC) and cross-sectional compliance (CC) were calculated over the operating pressure (ΔP_{BA}) according to the equations outlined in *Chapter 2*. In addition, to assure that changes in vessel wall properties resulted directly from drug-induced changes rather than indirectly from changes in blood pressure, isobaric vessel wall properties were

calculated over a pre-defined pressure interval of 85 to 95 mmHg (isobaric distensibility, DC_{iso} and isobaric compliance, CC_{iso}). For a detailed description on all the measurements which were performed and the subsequent data analysis, we refer to the methods outlined in *Chapter 2*.

Forearm blood flow and vascular resistance

Using ECG-triggered venous occlusion plethysmography forearm blood flow (FBF) of the left forearm was measured with mercury-in-Silastic strain gauges (Periflow, Janssen Scientific Instruments, Belgium) [18]. Both before and after occlusion of the hand circulation, by inflation of a wrist cuff to 240 mmHg, FBF was recorded for 2 minutes. Because of instability of blood flow after wrist occlusion, hand circulation was occluded at least 1 minute before FBF measurements were repeated. FBF was recorded for 3 heart beats every 5 beats by intermittent inflation of an upper arm cuff to a pressure of 45 mmHg. FBF measurements obtained without occlusion of the hand circulation refer to total FBF (FBF_{total}). FBF recordings obtained during occlusion of the hand circulation refer predominantly to the forearm skeletal muscle perfusion (FBF_{muscle}) [19].

Total (FVR_{total}) and muscular forearm vascular resistance (FVR_{muscle}) were calculated as MAP divided by FBF_{total} and FBF_{muscle} , respectively, and expressed as arbitrary units (AU).

Pharmacokinetic assessments

Just before drug administration (0 h), at 10, 20, 30 and 45 minutes and at 1, 2, 3, 4, 6, 8, 10, 24 and 32 hours after drug administration, venous blood samples (8 ml) were collected in heparinised tubes from an antecubital vein of the left forearm. Within 2 hours after collection, plasma was separated by centrifugation (10 minutes at 1500 g), frozen at -20°C and stored at -80°C until analysis.

DHE plasma concentrations were measured in duplicate using a specific radioimmunoassay (RIA) as previously described [20]. The lower limit of quantification was determined to be 50 pg/ml. The slopes of the standard curves were consistent with correlation coefficients >0.995 . The variability of the calibration standards was less than 5%.

According to Schran et al. [21], after subcutaneous administration, at each point in time (t) DHE plasma concentration $C(t)$ can be described by a tri-exponential function: $C(t) = A.e^{-\alpha.t} + B.e^{-\beta.t} - (A+B).e^{-k_a.t}$ where A and B are coefficients referring to fictitious plasma concentrations of DHE at time zero and k_a , α and β are rate constants referring to the absorption of DHE and the rapid and slow phase of decline in plasma concentration, respectively. The equation was fitted to the experimental data to obtain the relevant pharmacokinetic constants of each subject. The pharmacokinetic software program MW/PHARM (Medi/Ware, Centre of Pharmacy, University of Groningen, The Netherlands) was used for the model fitting. Peak plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were determined from the plasma concentration-time curve of each subject. Half-lives of absorption ($t_{1/2,abs}$), distribution ($t_{1/2,\alpha}$) and elimination ($t_{1/2,\beta}$) were calculated as $0.693/k_a$, $0.693/\alpha$ and $0.693/\beta$, respectively. The total area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal method and extrapolation to infinity. Assuming complete absorption from the injection site, plasma clearance (CL) and volume of distribution (V_d) of DHE were calculated as $dose/AUC$ and CL/β , respectively.

Statistical analysis

All pharmacodynamic parameters were plotted as a function of time and the area under the parameter time-curves after placebo and after DHE were compared using a paired Student's t -test. The differences in AUC after placebo and DHE (ΔAUC) are presented as 95% confidence intervals (95%CI). In case of a significant difference ($P < 0.05$), post hoc paired Student's t -tests were performed to determine the time course of the difference. Data are presented as mean $\pm$ SEM.

7.3 Results

Six subjects reported that the subcutaneous injection of DHE was painful (mild to moderate). Two subject reported minor gastro-intestinal side effects (nausea, watery stools and flatulence) which were probably drug related and resolved spontaneously. After placebo no adverse events were reported.

Pharmacokinetic data

DHE (0.5 mg) was rapidly absorbed ($t_{1/2,abs}$ 0.11 ± 0.03 h) and reached a maximum plasma concentration of $1,419\pm88$ pg/ml (C_{max}) at 20 minutes ($t_{max} = 0.33\pm0.08$ h) after subcutaneous injection (Fig. 7.1A). The half-lives of distribution ($t_{1/2,\alpha}$) and elimination ($t_{1/2,\beta}$) averaged 0.92 ± 0.16 and 5.63 ± 1.15 h, respectively. Mean CL, V_{β} and AUC were 95 ± 9 l/h, 739 ± 114 l and 5825 ± 667 pg.ml⁻¹.h, respectively.

Haemodynamic parameters

Pre-drug SBP (112 ± 3 mmHg), DBP (59 ± 2 mmHg), MAP (80 ± 2 mmHg), pulse pressure (52 ± 2 mmHg) and heart rate (58 ± 2 bpm) were comparable during both study periods. The area under the parameter-time curves after placebo and DHE did not differ (Table 7.1). The time course of the haemodynamic parameters (Fig. 7.1B) was similar after placebo and DHE.

Brachial artery wall properties

Pre-drug diameter (4.698 ± 0.096 mm), distension (179 ± 14 μ m), DC_{iso} (9.39 ± 1.01 10^{-3} /kPa) and CC_{iso} (0.167 ± 0.016 mm²/kPa) did not differ between both study periods. After DHE, the AUC for diameter, distension and cross sectional compliance decreased (Table 7.1). Compared to placebo, diameter decreased ($P<0.05$) from 1 until 24 h after DHE (Fig. 7.1C); a maximum difference of 462 ± 60 μ m (9.7%, $P<0.001$) was measured 10 h after drug administration. Distension decreased from 2 until 8 h after DHE whereas CC_{iso} decreased from 2 until 32 h (Fig. 7.1D). Compared to placebo, a maximum difference in distension (34 ± 7 μ m or 17.5%, $P=0.002$) and CC_{iso} (3.91 ± 1.34 10^{-2} mm²/kPa or 24.8%, $P=0.017$) was observed 6 and 2 h after drug administration, respectively.

Forearm vascular resistance

Pre-drug FVR_{total} (15.0 ± 1.8 AU) and FVR_{muscle} (30.4 ± 3.1 AU) were similar during both study periods. The area under the parameter-time curves after placebo and DHE did not differ statistically (Table 7.1). The time course of the forearm vascular resistance was comparable after placebo and DHE (Fig. 7.1E).

Figure 7.1 *Top left:* the plasma-concentration time curve of dihydroergotamine (DHE) after a single subcutaneous administration of 0.5 mg. *Bottom left:* time course of systolic blood pressure (Δ, ▲), mean arterial pressure (○, ●) and pulse pressure (□, ■) after placebo (open symbols, dashed line) and DHE (closed symbols, solid line); Time course of brachial artery diameter (*top right*) and isobaric compliance (*middle right*) after placebo (□, dashed line) and DHE (■, solid line). *Bottom right:* time course of total forearm vascular resistance after placebo (□, dashed line) and DHE (■, solid line). Data are presented as mean±SEM (n=10). *Points in time with $P<0.05$ for difference between DHE and placebo; NS: no statistically significant difference.

Table 7.1 Pharmacodynamic data (n=10).

	ΔAUC (95%CI)	<i>P</i> -value*
<i>Haemodynamic parameters</i>		
Systolic blood pressure (mmHg.h)	90 (-11/191)	0.07
Diastolic blood pressure (mmHg.h)	47 (-40/133)	0.26
Mean arterial pressure (mmHg.h)	17 (-63/96)	0.65
Pulse pressure (mmHg.h)	43 (-23/110)	0.18
Heart rate (bpm.h)	31 (-71/134)	0.51
<i>Brachial artery wall properties</i>		
Diameter (mm.h)	-8.812 (-12.97/-4.650)	0.001
Distension ($\mu\text{m.h}$)	-790 (-1359/-222)	0.012
DC_{iso} ($10^{-3}.\text{kPa}^{-1}.\text{h}$)	-17.5 (-47.7/12.6)	0.22
CC_{iso} ($\text{mm}^2.\text{kPa}^{-1}.\text{h}$)	-0.98 (-1.61/-0.34)	0.007
<i>Forearm vascular resistance</i>		
FVR_{total} (AU.h)	-62 (-246/121)	0.46
FVR_{muscle} (AU.h)	52 (-94/199)	0.44

Pharmacodynamic data after the subcutaneous administration of 0.5 mg DHE. Data are presented as mean difference between the area under the parameter-time curve after placebo and after DHE (ΔAUC). 95%CI: 95% confidence interval. DC_{iso} (isobaric distensibility coefficient) and CC_{iso} (isobaric cross-sectional compliance) calculated within a pre-defined pressure interval of 85-95 mmHg. FVR_{total} and FVR_{muscle} : total and muscular forearm vascular resistance. **P*-value of treatment differences by paired Student's *t*-test.

7.4 Discussion

The vascular effects of ergot alkaloids depend on the dose and route of administration, the species and tissue under investigation and the pre-existing vascular tone [4]. In the present study both the arterial and arteriolar effects of DHE were investigated at the level of the forearm after the administration of 0.5 mg, which is regarded a clinically relevant dose and is being used in the acute treatment of migraine [22]. Following drug administration, both brachial artery diameter and cross sectional compliance, slowly decreased (from 1 to 2 hours after drug administration) and remained decreased for at least 24 hours. These data do not support the contention of DHE being a selective venoconstrictor and argue against DHE lacking peripheral arterial effects as reported by Andersen et al [14]. However, their data should be interpreted with caution. Andersen et al. assessed arterial effects indirectly by measuring changes in toe-arm systolic blood pressure gradients whereas the present data refer to direct measurements at the brachial artery. Consequently, regional differences between the upper and lower extremities in vascular reactivity to DHE might have contributed to these conflicting results. In addition, it should be noted that in contrast with the present study, the previous study was not placebo-controlled and did not feature a double-blind design.

In contrast with the effects of DHE on conduit arteries, at resistance vessels no drug-induced changes were observed in the present study. Neither total nor muscular forearm vascular resistance were affected by DHE and blood pressure did not change, indicating the absence of both local and systemic vasoconstriction of resistance vessels. These findings are in agreement with previous research demonstrating that DHE does not affect skeletal muscle perfusion [23]. Finally, although blood pressure is often reported to rise in response to DHE [4], the absence of a systemic vasopressor effect under experimental conditions similar to those of the present study is not exceptional [10, 24] and is likely due to the low dose of DHE administered. Taken together our data demonstrate that at the dose used, DHE induces a potent and long-lasting arterial vasoconstriction and decreases compliance without affecting resistance vessels of the forearm. These data illustrate the well known potential of vasoactive drugs to act selectively at one vascular territory without affecting the other and remind

of a complexity which should be kept in mind when the vascular effects of drugs are being investigated [25].

The pharmacokinetic parameters observed in the present study are comparable with those previously reported after the parenteral administration of DHE [21, 26, 27]. After subcutaneous administration DHE was quickly taken up into the systemic circulation and reached peak plasma concentrations within 30 minutes. Following absorption, DHE plasma levels rapidly declined in a biphasic fashion, suggesting fast tissue distribution into a peripheral compartment before being eliminated. Although the terminal half-life observed in the present study is in agreement with several previous reports [21, 27, 28], others disagree and report a much slower elimination [24]. Different results are probably related to different analytical assays used to determine DHE plasma concentrations and to different dosages of the drug. Because of the relative low dose of DHE administered in the present study and despite the use of a validated RIA, from 24 h after drug administration plasma levels were below the detection limit. Consequently, it cannot be excluded that terminal half-life was underestimated.

In accordance with its potent and long lasting vasoconstrictor activity reported by Aellig et al. [8, 10], the arterial effects of DHE observed in the present study are also characterised by a slow onset and long duration of action which correlates poorly with plasma levels. How can the discrepancy between the pharmacokinetics of DHE and its prolonged vascular activity be explained? The long terminal half-life of DHE as reported by some investigators [24] provides one explanation. Alternatively, the extensive metabolism of DHE results in the formation many active metabolites [29]. The main metabolite, 8'-hydroxy-dihydroergotamine, occurs in plasma in concentrations 5 to 7 times higher than the parent drug and has previously been shown to have potent and long lasting vasoconstrictor activity in man [30]. Although these arguments provide a possible explanation for the prolonged vascular activity of DHE *in vivo*, they do not explain the sustained vasoconstrictor activity of ergot alkaloids *in vitro* which persists even after repeated washing [11, 12]. Therefore, tight tissue binding resulting in slow diffusion from the receptor biophase is likely to contribute substantially to these effects. This is also suggested by the large volume of distribution. As ergotamine displays a comparable discrepancy between

pharmacokinetics and long lasting arterial vasoconstriction [13] as well as an increase in arterial stiffness [31], these properties seem to be a common characteristic of the ergot alkaloids as a class.

How do these vascular effects translate into the therapeutic use of DHE which is indicated in the treatment of orthostatic hypotension and acute migraine? In orthostatic hypotension the beneficial effects of DHE are believed to result from the constriction of venous capacitance vessels thereby promoting venous return and subsequently increasing cardiac output [4]. In migraine however, many mechanisms have been proposed, including constriction of large cranial extracerebral arteries and closure of arteriovenous anastomoses [2]. It is tempting to speculate that at the cranial arteries ergot alkaloids display the same long lasting vascular effects as at peripheral arteries. If so, this might explain why headache recurrence after the initially successful treatment of acute migraine is far less frequent after the use of DHE compared to the use of short acting 5-HT_{1B/1D}-receptor agonists such as sumatriptan [32].

In summary, DHE decreases brachial artery diameter and compliance while forearm vascular resistance remains unchanged. Consequently, DHE acts on large arteries without affecting resistance vessels. Furthermore, a discrepancy was demonstrated between the plasma concentrations of DHE which rapidly reach peak levels and quickly decline, and its long lasting vasoconstrictor activity.

Acknowledgements:

We are greatly indebted to G. Kalafsky and D. Katzgrau (Novartis Pharmaceuticals Corporation, Drug Metabolism and Pharmacokinetics, East Hannover, New Jersey, U.S.A.) for performing dihydroergotamine plasma concentration assessments.

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Part IV

Discussion & Synopsis

*"I suspect the Tartar sauce I had for lunch ...
... disagreed with me."*

(Sigmund Freud, 1856-1939)

Chapter 8

General Discussion and Conclusions

8.1 Interictal functional and structural arterial changes in migraine patients

The objective of the present thesis was to study migraine and its treatment by investigating migraine pathophysiology and acute anti-migraine drugs from a cardiovascular point of view (*Chapter 1*). Although migraine has repeatedly been associated with peripheral vascular disorders [1-3], structural and functional extracranial vascular research has hardly ever been performed in migraine patients. Therefore, our first aim was to make an inventory of cardiovascular properties of migraine patients outside a migraine attack.

As described in *Chapter 3*, it was demonstrated that compared to matched control subjects during the interictal period migraine patients display both cranial and peripheral arterial changes. The most striking functional and structural vascular differences between both populations were observed at the medium sized muscular brachial artery displaying an increased stiffness and a tendency towards an increased intima-media thickness in migraine patients. In addition, migraineurs showed a larger temporal artery diameter and tended to have an increased temporal artery blood flow compared to control subjects.

What might be the cause of the changed vascular properties of peripheral conduit arteries in migraine patients? The contribution of arterial tone to vessel wall stiffness was already extensively discussed in *Chapter 3*. However, as both functional (arterial stiffness) and structural (intima-media thickness) changes were observed, alternative explanations should be considered. As CGRP has previously been demonstrated to be involved in the pathogenesis of migraine headache [4], it is tempting to speculate about its putative contribution to interictal vascular changes as well. CGRP has a

physiological role in the regulation of vascular tone and was shown to inhibit proliferation of cultured smooth muscle cells [5, 6]. Consequently, a deficient basal release of CGRP could explain (i) an increased interictal arterial stiffness related to an increased vascular tone and (ii) intima-media thickening due to smooth muscle cell proliferation. This hypothesis is supported by the observation that migraine is associated with an increased incidence of Raynaud's Syndrome in which CGRP was shown to be deficient in digital cutaneous perivascular nerves [7, 8]. Although the interictal plasma concentration of CGRP in migraine patients is similar to that in control subjects [4], based on the wide physiological range of circulating plasma concentrations of CGRP and the short half-life of the peptide [9], a decreased basal release cannot be excluded. Likewise, a decreased basal CGRP release does not exclude a paroxysmal excessive release of it as observed during acute migraine [4, 10].

It remains speculative as to what extent the peripheral vascular changes in migraine patients parallel changes at the level of the intra-cranial arteries. If so, one might wonder whether increased arterial wall stiffness can be regarded as a defence mechanism against unwanted vasodilatation and is meant to prevent the perivascular stimulation of supersensitive stretch receptors activating the trigeminovascular system during the interictal period.

8.2 Migraine pathophysiology: a unifying hypothesis on CGRP and NO

One of the intriguing and unanswered questions in migraine pathophysiology relates to the trigger initiating a migraine attack [11]. Although it is generally accepted that migraine is a neurovascular disorder [12], it remains a matter of debate whether perivascular nerve activation is responsible for secondary vasodilatation or vice versa (*Chapter 1*). Regarding this issue NO and CGRP are considered to play a pivotal role. In order to better define the contribution of these molecules to the pathogenesis of migraine, the vascular responsiveness to both was further investigated in migraine patients (*Chapter 4*).

Olesen and collaborators provided evidence indicating that migraine patients display an arterial supersensitivity to NO [13, 14]. To improve our understanding of the concept of supersensitivity to NO, we compared the vascular responsiveness to both endogenous and exogenous NO between migraine patients and matched control subjects at the level of the forearm. The main finding was that neither for resistance arteries nor for conduit arteries the responsiveness to endogenous or exogenous NO was changed in migraine patients (*Chapter 4*). Likewise, the vascular responsiveness to CGRP did not differ between migraineurs and control subjects. Taken together these data do not support the hypothesis of a systemic vascular supersensitivity to NO in migraine patients and argue against a generalised change in vascular responsiveness as a primary cause of the disease. Obviously, the major limitation of our approach is the fact that the forearm vasculature might well behave differently compared to the cranial vasculature which is the territory of interest in acute migraine. As differences in receptor distribution and innervation could result in a different pharmacology for both territories our data do not exclude NO-supersensitivity at the cranial level. Alternatively, one might wonder whether supersensitivity to NO may be interpreted in terms of an increased sensitivity of perivascular nerves to NO instead of an increased sensitivity of the blood vessel wall itself (*vide infra*). If so, the lack of a difference in nitroprusside, 5-HT and flow-induced changes in forearm blood flow between migraine patients and control subjects in the present study may be explained by the low density of CGRP containing perivascular nerve fibres in the peripheral circulation compared to the cranial circulation [15].

As for CGRP, it is well established that this neuropeptide is a potent vasodilator involved in the pathogenesis of migraine [4, 10]. However, its mechanism of action remains a matter of debate [9, 15]. Depending on the species and tissue under investigation, at least three vasodilator mechanisms of action have been reported some of which are endothelium-independent [16-21] whereas others are endothelium-dependent [22, 23]. *Chapter 5*, presents the first study in man investigating the vasodilator mechanism of action of (patho)physiological concentrations of CGRP *in vivo*. To this end, the “perfused forearm model” was used and the contribution of endothelial mediators (NO and prostaglandins) and potassium channels (ATP and

calcium-sensitive K^+ -channels) to CGRP-induced vasodilatation was investigated. The results of this study allow to conclude that CGRP-induced vasodilatation of the resistance vessels of the human forearm is at least in part mediated by the release of NO whereas prostaglandins or K^+ -channels do not seem to contribute substantially.

The discrepancy between the results of the present study (*Chapter 5*) and some previously reported *in vitro* data might be related to (i) inter-species, (ii) experimental and (iii) inter-tissue differences. As far as inter-species differences are concerned, in relation to (patho)physiology, human data are obviously more relevant than animal data. With regard to experimental differences, most data on the vasodilator mechanism of action of CGRP refer to research on isolated blood vessels [16, 17, 19, 22, 23]. Consequently, conflicting results might be related to data obtained under clinically irrelevant concentrations of CGRP or might be due to differences between *in vivo* and *in vitro* responses [22]. Regarding the “perfused forearm model”, it must be recognised that the infusion of CGRP into the brachial artery lumen might result in an overestimation of the contribution of endothelial mechanisms compared to the physiological situation in which CGRP is released by perivascular nerves from the abluminal site. Finally, for inter-tissue differences, it is unknown to what extent the pharmacology of the forearm vasculature can be applied to the cranial vasculature implicating that extrapolation should be regarded as speculative.

Assuming that CGRP-induced vasodilatation is similar in peripheral and cranial vascular beds, what would be the implications of our findings in relation to migraine? Firstly, based on the observation that CGRP-induced vasodilatation in migraine patients is virtually identical to that induced in control subjects (*Chapter 4*), it is reasonable to expect that the vasodilator mechanism of action of CGRP is the same in both groups. Secondly, the fact that CGRP-induced vasodilatation is at least in part dependent on the release of NO (*Chapter 5*) whereas it has previously been shown that NO is involved in the regulation of neuropeptide release [24], suggests that the contribution of NO and CGRP to the pathophysiology of migraine might be reconciled in a unifying hypothesis. Indeed, Olesen’s hypothesis on arterial NO supersensitivity [13] may not have to be interpreted as an increased responsiveness to NO of the vessel wall *strictu sensu*, but rather as an over-activation of perivascular

nerves in response to a potentially noxious molecule. This over-activation would result in (i) a local process of neurogenic vasodilatation due to an excessive release of neuropeptides and (ii) the central transmission of a stimulus responsible for the sensation of pain. This hypothesis fits well with Moskowitz theory [25] of the trigeminovascular system playing a pivotal role in the pathogenesis of migraine and explains the increase in CGRP during a migraine attack as reported by Goadsby and

Fig. 8.1 Scheme illustrating a unifying hypothesis on the pathophysiology of migraine. An acute migraine attack is initiated by an unknown trigger releasing NO and/or CGRP which stimulate each others release and maintain a process of local vasodilatation. Based on the premise that this vicious circle should be interrupted in order to treat migraine, theoretical approaches for the future development of potential anti-migraine drugs are indicated.

collaborators [4]. Following our findings, CGRP would in turn stimulate the release of NO resulting in (i) a vasodilatation which might be interpreted as a defence mechanism of the brain to eliminate potentially noxious stimuli and (ii) a continuation of perivascular nerve activation, CGRP release and central pain transmission. This view is also supported by the fact that nitrates only induce typical migraine headache in migraine patients after a surprisingly long latency period [26], suggesting that NO is involved in the initiation of a slow pathological process which eventually leads to the attack [14]. In addition, although nitrate-induced headache is accompanied by vasodilatation, vasodilatation as such is unlikely to be the cause of the headache. Not only because of the delay between the fast vasodilatation and the slow onset of migraine-like headache induced by nitrates, but also because other potent vasodilators including CGRP have not been reported to cause headache [27].

In summary, it is suggested that migraine might be the consequence of a vicious circle resulting from interactions between NO and CGRP. Both a primary neurogenic or vascular event might cause the activation of this circle. However, once acute migraine is established, the stimulus initiating the onset of these neurovascular changes is only of secondary importance and treatment should be aimed at interrupting the process responsible for its continuation (Fig. 8.1).

8.3 Cardiovascular effects of anti-migraine drugs

In vitro data indicate that all currently available acute anti-migraine drugs contract both human coronary and cranial arteries with a potency and maximal effect comparable with that of sumatriptan [28-31]. For ergotamine, a non-selective compound, and sumatriptan, the first selective 5-HT_{1B/1D} agonist which became available for the acute treatment of migraine, this vasoconstrictor potency has been confirmed *in vivo* [32-36]. Moreover, the use of both drugs has been associated with both myocardial infarction and sudden death [37-39] and rendered their use contraindicated in patients at increased risk for cardiovascular events. Therefore, recent research aimed at the development of anti-migraine drugs with a safer cardiovascular

profile and resulted in the introduction of second-generation triptans. However, as the clinical relevance of the *in vitro* data on the vascular effects of these drugs remain speculative, *in vivo* human research was needed to better define their cardiovascular profile at clinically relevant plasma concentrations.

The research presented in *Chapters 6* and *7* aimed at investigating the vascular effects of the most frequently used commercially available acute anti-migraine drugs: dihydroergotamine (DHE), sumatriptan (reference drug) and the second generation triptans rizatriptan and zolmitriptan. The data of these studies demonstrate that after the intake of equipotent therapeutic dosages, both DHE and selective 5-HT_{1B/1D}-receptor agonists induce a comparable vasoconstriction (about 10%) and a similar decrease in compliance (about 20%) of the brachial artery without affecting resistance vessels of the human forearm. For the triptans, for which these effects were also investigated at the carotid and temporal artery, the changes in arterial diameter and compliance are more pronounced at muscular compared to elastic arteries. In contrast with their effects on conduit arteries, triptans only increase vascular resistance in the vascular bed distal of the temporal artery, suggesting cranioselectivity for resistance vessels rather than for conduit arteries. Although zolmitriptan most potently affects the vascular territory of the temporal artery, this does not necessarily translate into a more favourable cardiovascular profile as it also most potently affected brachial artery wall properties.

Taken together, these data demonstrate that the intake of both ergot alkaloids and selective 5-HT_{1B/1D}-receptor agonists results in vascular effects (vasoconstriction and increased arterial stiffness) which are regarded as undesirable. Therefore, all currently available acute anti-migraine drugs should be used with caution in patients at increased risk for cardiovascular events.

Although cranial vasoconstriction is considered a prerequisite to yield a 5-HT_{1B/1D}-receptor agonist with anti-migraine activity [40], it is tempting to speculate that increasing vessel wall stiffness might be equally important. The pulsatile nature of migraine headache suggests that increased vascular pulsations are sensed by stretch receptors, resulting in the activation of perivascular nerve endings and the release of CGRP [41]. If so, increasing vessel wall stiffness *per se*, without obligatorily inducing

vasoconstriction, might sufficiently temper the activation of stretch receptors and render efficient anti-migraine drugs with a reduced risk of coronary vasoconstriction. Interestingly, both selective 5-HT_{1B/1D} receptor agonists and ergot alkaloids decrease arterial compliance (*Chapters 6 and 7*) [42], supporting the idea that decreasing the activation of perivascular nerves by increasing arterial stiffness might contribute to the efficacy of anti-migraine drugs. If so, it is tempting to speculate that a decrease in brachial artery compliance might be a useful surrogate marker for anti-migraine efficacy as it can be measured non-invasively in the early development of new potential anti-migraine drugs. By selecting those compounds which efficiently increase arterial stiffness without causing vasoconstriction at clinically relevant plasma concentrations, the cardiovascular safety of future drugs might be increased.

8.4 From facts to future

What are the implications of the finding that migraine patients display interictal functional and structural vascular changes compared to matched control subjects? Firstly, this supports the hypothesis of migraine being part of a generalised vascular disorder and indicates that at present the importance of the role of the vasculature might well be underestimated in the pathogenesis of migraine. Secondly, it is tempting to speculate that drugs normalising the interictal increase in arterial stiffness in migraine patients might be useful prophylactic agents. Interestingly, many of the currently available prophylactic anti-migraine drugs, including calcium antagonists and selective β -blockers, have indeed been shown to decrease arterial stiffness [43]. Thirdly, as the baseline vascular characteristics of migraine patients differ from those of non-migraineurs, migraine patients may display a different response to the administration of vasoactive substances in general and to anti-migraine drugs in particular. Thus, the cardiovascular effects of potential anti-migraine drugs should be carefully looked at both in healthy subjects and in migraine patients.

Regarding the future development of anti-migraine drugs, there is little reason to expect that any selective 5-HT_{1B/1D}-receptor agonist will be devoid of undesirable

cardiovascular side effects. Due to their interaction with 5-HT_{1B}-receptors, which are negatively coupled with adenylate cyclase activity, vasoconstriction can be regarded as a class effect of the triptans. Consequently, new avenues should be explored to develop effective and yet cardiovascular safe anti-migraine drugs. A possible future approach might be based on the well established release of CGRP during migraine headache, the putative role of NO and our findings on the interaction between both. Therefore, the development of CGRP-receptor antagonists, NO-synthase inhibitors or NO-scavengers seems to provide the most rational approach to tackle the problem (Fig. 8.1). In this context the efficacy results of the first non-peptide CGRP-receptor antagonist BIBN4096SB, which is currently being evaluated in the acute treatment of migraine [44], are being awaited with great expectations.

Unfortunately however, many issues remain unresolved, some of which might be elegantly investigated using the “perfused forearm model”. Firstly, different CGRP-receptors have been identified and it is unsettled at present which receptor should be targeted to render an effective anti-migraine drug. Secondly, different constitutional and inducible isoforms of NO-synthase have been isolated but the isoform involved in CGRP-induced vasodilatation is unknown. Last but not least, as both CGRP and NO are involved in the regulation of vascular tone, it cannot be excluded that a compound interfering with either mediator will be bestowed with unwanted cardiovascular side effects. If so, history is likely to repeat itself and after the “triptan war” of the 20th century, the 21th century will become a new battlefield ...

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*A migraine is not a headache,
An anti-migraine drug is not a headache pill.*

(London-Underground, 2000)

Chapter 9

Summary - Samenvatting

9.1 Summary

After summarising the epidemiology, clinical features and diagnostic criteria of migraine, **Chapter 1** provides a historical overview on the pathophysiology of the disorder. Both the primary vascular and neuronal theory are discussed and reconciled in a unifying hypothesis regarding migraine as a neurovascular disease. The triggers which might be involved in initiating an acute migraine attack are summarised. Subsequently, a review is given of the currently available acute anti-migraine drugs. This review focuses on the mode of action and cardiovascular effects of these drugs and elaborates on recent research aimed at the development of safer compounds. Finally, the arguments suggesting a relation between migraine and cardiovascular disease in general are being commented on.

Chapter 2 discusses the methods for haemodynamic assessments used in this thesis. Ultrasound technology and venous occlusion plethysmography constituted the main techniques to assess large artery wall properties, tissue perfusion and endothelial function both in an invasive (the “perfused forearm model”) and in a non-invasive setting. For the assessment of large artery wall properties, a “novel” approach is out-lined consisting of: (i) the use of a robotic arm for fixing the ultrasound probe during measuring vessel wall movements, (ii) the calculation of local pulse pressures and (iii) the combined analysis of diameter-time and pressure-time recordings.

The objective of the present thesis is to study migraine and its treatment by performing human *in vivo* cardiovascular research on migraine pathophysiology and acute anti-migraine drugs. The specific **Aims** are: 1. to make an inventory of both the structural and functional cardiovascular properties of migraine patients during the interictal period, 2. to investigate the vasodilator mechanism of action of calcitonin gene-related peptide (CGRP) which is regarded to contribute substantially to the pathophysiology of migraine, and 3. to study the cardiovascular effects of therapeutic dosages of acute anti-migraine drugs in human.

It has frequently been suggested that migraine might be part of a more generalised vascular disorder. This hypothesis was challenged in **Chapter 3** by comparing the cardiovascular properties of migraine patients with or without aura during the interictal period with those of matched control subjects. Using ultrasound, large artery wall properties of the common carotid, temporal, brachial and femoral artery were measured and cardiac parameters were assessed. The most striking functional and structural vascular differences between both groups were observed at the medium sized brachial artery displaying an increased arterial stiffness and a tendency towards an increased intima-media thickness in migraine patients. In addition, migraineurs showed a larger temporal artery diameter and tended to have an increased temporal artery blood flow compared to control subjects. These data demonstrate that even outside the headache phase, migraine patients display vascular changes which extend beyond the cranial circulation pointing to the potential importance of the vasculature in the pathogenesis of migraine.

Nitric oxide (NO) and CGRP are probably involved in the pathophysiology of acute migraine headache. **Chapter 4** explores the hypothesis of migraine patients displaying a changed vascular responsiveness to NO, CGRP and ischaemia during the interictal period. Ten migraine patients and ten matched control subjects participated in this study in which both invasive (part I) and non-invasive (part II) techniques were applied. In *part*

I, the “perfused forearm model” was used and the vascular responsiveness to intra-brachial infusions of (i) serotonin, (ii) sodium nitroprusside, and (iii) CGRP were assessed. In *part II*, flow-mediated vasodilatation of the brachial artery and maximal forearm vasodilator potential were measured. Neither for NO nor for CGRP a changed vascular responsiveness was demonstrated at the level of resistance vessels of the human forearm in migraine patients. As maximal post-ischaemic forearm blood flow was similar in both groups, there is no evidence suggesting structural differences at the level of resistance vessels either. In addition, flow-mediated vasodilatation did not differ between migraine patients and matched control subjects arguing against a supersensitivity to NO for conduit arteries as well. Taken together, these data do not support the hypothesis of a systemic vascular supersensitivity to NO in migraine patients and do not support generalised *functional* vascular changes as a primary cause of the disease. In addition, no *structural* differences are present at the level of peripheral resistance vessels either.

Depending on the species and tissue under investigation *in vitro* studies show that the vasodilator mechanism of action of CGRP involves endothelium-dependent and/or endothelium-independent mechanisms. **Chapter 5** presents the first study to address the vasodilator mechanism of action of clinically relevant plasma concentrations of CGRP *in vivo* in human. It aimed at investigating the contribution of endothelial mediators (NO and prostaglandins) and K^+ -channels (calcium-sensitive potassium channels, K^+_{Ca} -channels and adenosine-5'-triphosphate sensitive-potassium channels, K^+_{ATP} -channels) to CGRP-induced vasodilatation. To this end, the “perfused forearm model” was used and the forearm vascular responses to increasing dosages of CGRP during co-infusion of either placebo (saline) or a selective inhibitor were investigated. The repeated co-infusion of CGRP with placebo (time-control experiments) demonstrated a dose-dependent and reproducible forearm vasodilator response to CGRP. Compared to placebo, the NO-synthase inhibitor N^G -monomethyl-L-arginine (L-NMMA) reduced the increase in forearm blood flow induced by CGRP. The absence of an inhibitory effect of L-NMMA on CGRP-induced vasodilatation at the highest dose of CGRP suggests that still other

mechanisms are involved. The vasodilator response to CGRP was not affected by co-infusion of the cyclo-oxygenase inhibitor indomethacin, the K^+_{Ca} -channel blocker tetraethyl-ammonium chloride or the K^+_{ATP} -channel blocker glibenclamide. Based on these findings it can be concluded that *in vivo* in human the vasodilator response to CGRP is at least in part dependent on the local release of NO whereas it appears not to be related to the release of prostaglandins or the activation of K^+_{Ca} and K^+_{ATP} -channels.

Chapter 6 compares the vascular effects of sumatriptan, the first selective 5-HT_{1B/1D}-receptor agonist used in the acute treatment of migraine, with those of the second-generation triptans rizatriptan and zolmitriptan in migraine patients outside a migraine attack. Sixteen migraine patients participated in a double-blind, placebo-controlled, 4-way cross-over study. After the oral intake of equipotent therapeutic dosages of sumatriptan (50 mg), rizatriptan (10 mg), zolmitriptan (2.5 mg) or placebo, measurements were performed at the common carotid, brachial and temporal artery. Triptan-induced changes in (i) large artery wall properties, (ii) tissue perfusion and (iii) endothelial function were assessed using ultrasound. Compared to placebo both sumatriptan and second-generation triptans caused a small blood pressure increase, induced arterial vasoconstriction and decreased the buffering capacity of conduit arteries. These effects were more pronounced at muscular compared to elastic arteries. In contrast with large artery wall properties, vascular resistance was only increased at the vascular bed distal of the temporal artery and suggests cranioselectivity of triptans for resistance vessels rather than for conduit arteries. Zolmitriptan most potently affected the vascular territory of the temporal artery, but also induced the largest decrease in brachial artery buffering capacity. Endothelial function, as assessed by flow-induced vasodilatation, did not differ between active treatments. Given the fact that the changes in large artery wall properties observed after the intake of sumatriptan and second-generation triptans were comparable, they can be regarded as a class effect of the triptans. Moreover, as these changes are generally considered as undesirable these data support the contention that triptans should be used with caution in patients at increased risk for cardiovascular events.

The research presented in **Chapter 7** was initiated because of the conflicting results between *in vitro* and *in vivo* data on the vascular effects of dihydroergotamine (DHE). Changes in brachial artery wall properties and forearm vascular resistance resulting from DHE (0.5 mg subcutaneously) were assessed by comparing parameter-time curves after placebo and DHE. In addition, DHE pharmacokinetics were assessed using a two-compartment open model with an absorption phase. The study was performed in 10 healthy, male volunteers and featured a randomised, double-blind, placebo-controlled, cross-over design. DHE induced no changes in blood pressure, heart rate and forearm vascular resistance. Brachial artery diameter and compliance decreased from 1 until 24 h and from 2 until 32 h after DHE, respectively. In contrast, time to reach maximum plasma concentration of DHE averaged only about 20 minutes. Consequently, at the level of the forearm, DHE acts on conduit arteries without affecting resistance vessels. In addition, a discrepancy was demonstrated between the plasma concentrations of DHE, which rapidly reach peak levels and quickly decline, and its long lasting arterial effects.

By reconciling the results presented in the individual chapters, in **Chapter 8** following hypotheses are discussed: (i) whether CGRP contributes to the interictal vascular changes observed in migraine patients, (ii) whether acute migraine results from a vicious circle driven by the actions of NO and CGRP, and (iii) whether drugs increasing arterial stiffness without causing vasoconstriction might be effective anti-migraine drugs with an improved cardiovascular safety profile. Finally, suggestions for future research and potential surrogate markers in the early development of both prophylactic and acute anti-migraine drugs are made.

9.2 Samenvatting

Naast een samenvatting van de epidemiologie, klinische kenmerken en diagnostische criteria van migraine, geeft **Hoofdstuk 1** een historisch overzicht van de pathofysiologie

van deze aandoening. Zowel de primair vasculaire als de primair neuronale theorie worden besproken en verenigd in een overkoepelende hypothese die migraine beschouwt als een neurovasculaire ziekte. De uitlokkende factoren die mogelijk betrokken zijn bij het ontstaan van een acute migraineaanval worden samengevat. Vervolgens wordt een overzicht gegeven van de thans beschikbare geneesmiddelen voor de acute behandeling van migraine. Hierbij wordt aandacht geschonken aan het werkingsmechanisme en de cardiovasculaire effecten van anti-migraine geneesmiddelen en wordt dieper ingegaan op recent onderzoek gericht op de ontwikkeling van veiliger geneesmiddelen. Tenslotte worden de argumenten besproken die er voor pleiten dat er mogelijk een verband bestaat tussen migraine en hart-vaatziekten.

Hoofdstuk 2 bespreekt de methoden voor het meten van hemodynamische effecten die in dit proefschrift gebruikt werden. Ultrageluid technieken en veneuze occlusie plethysmografie vormden de belangrijkste methoden voor het meten van vaatwand-eigenschappen, weefsel doorbloeding en endotheelfunctie en dit zowel in een invasieve (het “geperfundeerde onderarmsmodel”) als in een niet-invasieve benadering. Voor de meting van vaatwandeigenschappen van grote slagaders wordt een nieuwe aanpak beschreven bestaande uit: (i) het gebruik van een statief voor het stabilizeren van de ultrageluid probe tijdens het meten van de vaatwandbeweging, (ii) het berekenen van de lokale polsdruk en (iii) het gelijktijdig analyseren van diameter-tijd en druk-tijd registraties.

Het **Doel** van dit proefschrift bestaat erin om de pathofysiologie van migraine en de effecten van acute anti-migraine geneesmiddelen *in vivo* te bestuderen bij de mens met behulp van cardiovasculaire onderzoekstechnieken. De specifieke doelstellingen zijn de volgende: 1. het evalueren van zowel de functionele als structurele eigenschappen van het hart-vaatstelsel van migrainepatiënten tijdens de interictale periode, 2. het onderzoeken van het vaatverwijdende mechanisme van calcitonin gene-related peptide (CGRP) dat wellicht een belangrijke rol speelt in de pathofysiologie van migraine, en 3. het

bestuderen van de cardiovasculaire effecten van acute anti-migraine geneesmiddelen na de toediening van een therapeutische dosis.

Er wordt vaak gesuggereerd dat migraine deel uitmaakt van een meer veralgemeende vasculaire aandoening. Om deze hypothese te toetsen werden in **Hoofdstuk 3** de cardiovasculaire eigenschappen van migrainepatiënten, met of zonder aura, gedurende de interictale periode vergeleken met deze van een vergelijkbare groep controlepersonen. Met behulp van ultrageluid werden de vaatwandeigenschappen van de arteria carotis communis, arteria temporalis, arteria brachialis en arteria femoralis gemeten. De meest opvallende functionele en structurele verschillen tussen migrainepatiënten en controlepersonen werden vastgesteld ter hoogte van de arteria brachialis die een toegenomen stijfheid vertoonde evenals een tendens tot een toegenomen intima-media dikte. Bovendien hadden migrainepatiënten ter hoogte van de arteria temporalis een grotere diameter en een tendens tot toegenomen doorbloeding. Deze gegevens tonen aan dat migrainepatiënten, zelfs buiten een periode van hoofdpijn, vasculaire veranderingen vertonen die zich uitstrekken buiten het vaatgebied van het hoofd en wijzen op het potentiële belang van het vaatstelsel in de pathofysiologie van migraine.

Stikstofmonoxide (NO) en CGRP spelen wellicht een belangrijke rol in de pathofysiologie van migraine. **Hoofdstuk 4** toetst de hypothese die stelt dat migrainepatiënten tijdens de interictale periode een gewijzigde vasculaire gevoeligheid vertonen voor NO, CGRP en ischemie. Tien migrainepatiënten en 10 vergelijkbare controlepersonen namen deel aan deze studie waarin zowel invasieve (deel 1) als niet-invasieve (deel 2) technieken gebruikt werden. In *deel 1*, werd het “geperfundeerde onderarmsmodel” gebruikt om de vaatverwijding te bestuderen als gevolg van de intra-brachiale infusie van (i) serotonine, (ii) natrium nitroprusside en (iii) CGRP. In *deel 2*, werden de vaatverwijding van de arteria brachialis uitgelokt door toegenomen doorbloeding en de maximale onderarmsdoorbloeding gemeten. Noch voor NO, noch voor CGRP werd er een gewijzigde gevoeligheid vastgesteld ter hoogte van de

weerstandsvaten van de onderarm bij migrainepatiënten. Aangezien de maximale onderarmsdoorbloeding (na ischemie) vergelijkbaar was in beide groepen, is er evenmin een reden om aan te nemen dat er structurele verschillen zijn ter hoogte van de weerstandsvaten. Bovendien werd er geen verschil vastgesteld in de vaatverwijding van de arteria brachialis uitgelokt door een toename in doorbloeding, wat pleit tegen een overgevoeligheid voor NO ter hoogte van grote slagaders. Samengevat bevestigen deze gegevens niet de hypothese van een systemische vasculaire overgevoeligheid voor NO bij migrainepatiënten of van veralgemeende *functionele* veranderingen als primaire oorzaak van de aandoening. *Structurele* veranderingen op het niveau van perifere weerstandsvaten werden evenmin aangetoond.

Afhankelijk van de diersoort en het weefsel dat onderzocht wordt, toont *in vitro* onderzoek aan dat het vaatverwijdende mechanisme van CGRP zowel endotheel-afhankelijk als endotheel-onafhankelijk verloopt. **Hoofdstuk 5** beschrijft het eerste *in vivo* onderzoek bij de mens dat het vaatverwijdende mechanisme van CGRP bestudeert bij klinisch relevante plasmaconcentraties. De bijdragen van endotheliale mediators (NO en prostaglandines) en kalium-kanalen (calcium-gevoelige kalium-kanalen, K^+_{Ca} -kanalen en adenosine-5'-trifosfaat-gevoelige kalium-kanalen, K^+_{ATP} -kanalen) aan de vaatverwijding veroorzaakt door CGRP werden onderzocht. Met behulp van het "geperfundeerde onderarmsmodel" werd de onderarmsdoorbloeding tijdens de infusie van toenemende doses CGRP samen met placebo (fysiologisch zout) of met een selectieve inhibitor gemeten. De herhaalde infusie van CGRP samen met placebo (tijdscontrole-experimenten) toonde aan dat CGRP leidt tot een dosis-afhankelijke en reproduceerbare toename in onderarmsdoorbloeding. In vergelijking met placebo, verminderde de NO-synthase inhibitor N^G -monomethyl-L-arginine (L-NMMA) de toename in onderarmsdoorbloeding door CGRP. Het ontbreken van een remmend effect van L-NMMA op de vaatverwijding veroorzaakt door de hoogste dosis CGRP, suggereert dat nog andere mechanismen een rol spelen. Het vaatverwijdende effect van CGRP werd niet beïnvloed door de gelijktijdige infusie van de cyclo-oxygenaseremmer

indomethacine, de K^+_{Ca} -kanaalblokker tetra-ethyl-ammoniumchloride of de K^+_{ATP} -kanaalblokker glibenclamide. Op basis van deze bevindingen kan besloten worden dat *in vivo* bij de mens de vaatverwijding door CGRP ten minste gedeeltelijk berust op de lokale vrijstelling van NO en niet op de vrijstelling van prostaglandines of de activatie van K^+_{Ca} of K^+_{ATP} -kanalen.

Hoofdstuk 6 vergelijkt bij migrainepatiënten tijdens de interictale periode de vasculaire effecten van sumatriptan, de eerste selectieve 5-HT_{1B/1D}-receptor agonist voor de behandeling van een acute migraineaanval, met deze van de tweede-generatie triptanen rizatriptan en zolmitriptan. Zestien migrainepatiënten namen deel aan een dubbel-blind, placebo-gecontroleerd, 4-weg cross-over onderzoek. Na de orale inname van doses met een vergelijkbare klinische efficaciteit (sumatriptan 50 mg, rizatriptan 5 mg, zolmitriptan 2,5 mg) of placebo werden, met behulp van ultrageluid, metingen verricht ter hoogte van de arteria carotis communis, arteria temporalis en arteria brachialis. Veranderingen in (i) vaatwandeigenschappen, (ii) weefseldoorbloeding en (iii) endotheelfunctie ten gevolge van de inname van triptanen werden onderzocht. In vergelijking met placebo resulteerden zowel sumatriptan als de tweede-generatie triptanen in een kleine bloeddruktoename, een arteriële vaatvernauwing en een afname van het bufferend vermogen van grote slagaders. Deze effecten waren duidelijker aanwezig ter hoogte van musculaire dan ter hoogte van elastische vaten. In tegenstelling tot de vaatwandeigenschappen nam de weerstand uitsluitend toe ter hoogte van het vaatbed van de arteria temporalis. Dit suggereert een cranioselectiviteit van triptanen voor weerstandsvaten eerder dan voor grote slagaders. Zolmitriptan had de meest potente effecten op het vaatgebied van de arteria temporalis, maar veroorzaakte eveneens de meest uitgesproken afname in het bufferend vermogen van de arteria brachialis. De vaatverwijding van de arteria brachialis, die veroorzaakt wordt door een toename in doorbloeding en welke gebruikt werd als maat voor endotheelfunctie, was niet verschillend voor de onderzochte triptanen. Gegeven het feit dat de veranderingen in vaatwand-eigenschappen veroorzaakt door sumatriptan en tweede-generatie triptanen vergelijkbaar waren, kunnen zij beschouwd worden als een

klasse-effect van de triptanen. Aangezien deze vasculaire veranderingen algemeen beschouwd worden als ongunstig, dragen deze bevindingen bij tot de stelling dat triptanen met de nodige voorzichtigheid dienen gebruikt te worden bij patiënten met een verhoogd cardiovasculair risico.

Het onderzoek in **Hoofdstuk 7** werd uitgevoerd naar aanleiding van de discrepantie tussen de *in vivo* en *in vitro* resultaten van de vasculaire effecten van dihydroergotamine (DHE). Veranderingen in arteria brachialis vaatwand-eigenschappen en onderarmsweerstand ten gevolge van de toediening van DHE (0,5 mg subcutaan) werden gemeten door het vergelijken van het parameter-tijd verloop na placebo en DHE. Tevens werd de farmacokinetiek van DHE bepaald aan de hand van een open, twee-compartimentmodel met een absorptie fase. Aan dit dubbel-blind, placebo-gecontroleerd, cross-overonderzoek namen 10 gezonde mannelijke vrijwilligers deel. DHE veroorzaakte geen veranderingen in bloeddruk, hartfrequentie en onderarmsweerstand. De diameter en het bufferend vermogen van de arteria brachialis namen respectievelijk af van 1 tot 24 en van 2 tot 32 uur na DHE. Echter de maximale plasmaconcentratie van DHE werd gemiddeld reeds bereikt na 20 minuten. Bijgevolg heeft DHE ter hoogte van de onderarm een belangrijk effect op grote slagaders zonder de weerstandsvaten te beïnvloeden. Bovendien werd een discrepantie aangetoond tussen het plasmaconcentratie-verloop van DHE, dat snel piekwaarden bereikt en snel weer afneemt, en de langdurende arteriële effecten ervan.

In **Hoofdstuk 8** worden door het samenvoegen van de onderzoeksresultaten van de individuele hoofdstukken, volgende hypothesen besproken: (i) of CGRP bijdraagt tot de interictale veranderingen van het vaatstelsel zoals we die bij migrainepatiënten hebben vastgesteld, (ii) of een acute migraineaanval het resultaat is van een vicieuze cirkel die in stand gehouden wordt door de effecten van NO en CGRP, en (iii) of medicijnen die de stijfheid van de vaatwand van grote slagaders doen toenemen, zonder vaatvernauwing te veroorzaken, effectieve en veilige anti-migraine medicijnen zouden kunnen zijn.

Tenslotte worden suggesties gedaan in verband met toekomstig onderzoek en potentieel nuttige parameters in de vroege ontwikkeling van zowel preventieve als acute anti-migraine geneesmiddelen.

De weg is het doel.

(vrij naar de Koran)

Dankwoord – Acknowledgement

Toen ik in 1995 als “naïef” arts-onderzoeker de toenmalige Vakgroep Farmacologie binnenwandelde op een zonnige nazomerse ochtend, kon ik me onmogelijk de omvang inbeelden van de opdracht die me te wachten stond. Het doel was duidelijk -het behalen van een doctorstitel- maar de weg die daartoe moest leiden was bijzonder vaag. Nu, zowat 5 jaar later, ben ik me er meer dan ooit van bewust dat ik zonder de leiding, steun en hulp van een groot aantal mensen nooit in deze opdracht zou geslaagd zijn. Alhoewel namen noemen het risico inhoudt dat sommigen vergeten worden, wens ik toch enkele mensen speciaal te vermelden.

Mijn promotoren, Prof. dr. H. Struijker-Boudier en Prof. dr. J. Troost, en co-promotor, Dr. L. Van Bortel, dank ik voor hun vertrouwen en voor de kans die ze me gegeven hebben om me als klinisch farmacoloog te ontplooien. Harry, je aanwezigheid was zelden prominent maar werd zeer gewaardeerd op beslissende momenten. Luc, je hebt me niet alleen een vorming als wetenschapper gegeven maar tevens een voorbeeld van “*management beyond data-management*”. Ik hoop oprecht dat de voorbije jaren slechts het begin geweest zijn van een duurzame samenwerking in de toekomst.

Ing. J. Willigers wil ik bijzonder bedanken voor zijn zeer gewaardeerde hulp, gaande van het oplossen van de meest idiote technische problemen tot het uitwerken van gecompliceerde software. Jean, als technicus en als mens had ik me geen betere bio(psycho)fysicus kunnen bedenken.

Voor de onderzoeken beschreven in dit proefschrift, werd beroep gedaan op meer dan 200 vrijwilligers zonder wiens medewerking mijn werk onmogelijk zou zijn geweest. Yvonne, Lilian, Peter, Jacques en Leo zijn slechts enkele collega's die hun hart-

Acknowledgement - Dankwoord

vaatstelsel meermaals ter beschikking stelden van de wetenschap. Zij zijn er het levende bewijs van dat frequente blootstelling aan ultrageluid geen schadelijke effecten heeft. Ook Simone, Diana (zeer moedig), Lou en Peter van het laboratorium biochemie van het Akademisch Ziekenhuis Maastricht, behoorden tot de “vaste staf” (min of meer) gezonde controlepersonen. Onder de migrainepatiënten was het enthousiasme van enkele opvallende “slachtoffers” waaronder Loekie, Kim, Daniëlle, Peter en Erik een aangename stimulans in moeilijke perioden.

Prof. dr. P. Smits dank ik van harte voor het onderzoek dat op de afdeling Farmacologie en Toxicologie van de Katholieke Universiteit Nijmegen mocht plaatsvinden. Paul, dankzij jouw gastvrijheid en met de hulp van Peter Pickkers kreeg ik de kans om me op korte tijd de techniek van het onderarmsmodel eigen te maken.

De professoren P. Kitslaar, J. De Mey, P. Goadsby, A. Hoeks en P. Saxena ben ik zeer erkentelijk voor de beoordeling van mijn manuscript. In het bijzonder dank ik Arnold, voor de subtiele opmerking dat “*scares*” misschien wel hetzelfde klinkt als “*scarce*”, maar toch net iets anders betekent.

De onderzoeksassistentes van de divisie klinische farmacologie: Mirjam, Carla en Karin vormden een onvergetelijk trio dat ik dank voor de prettige samenwerking. Mirjam, de ochtenduren wil ik hierbij even buiten beschouwing laten.

Al waren de sociale kontakten met de meesten van de Vakgroep Farmacologie beperkt, toch zal ik velen herinneren als bijzondere collega's. Matthijs, gedoodverfd als vlaaienvreter -al was zijn BMI nauwelijks hoog genoeg om aan klinische studies te mogen deelnemen- herinner ik als mijn eerste kamergenoot met wie ik het beginnersleed kon delen. Gregorio, Chris en Jan als back-up providers van de laatste hoop in geval van “*a fatal error has occurred*” en verwante obstakels. Jo, als pendelcompagnon tussen Mol en Maastricht met wie ik inspirerende uren doorbracht in de auto. Mia en Els, als een

vrolijk duo secretaresses dat je problemen al opgelost had voor ze geformuleerd waren. Paul, als sportman steeds op zoek naar een nieuw record indoor-snelwandelen (enkel Jos vormt een ernstige concurrent), die een massa bio-analytische bepalingen verrichtte. Henk tenslotte, als senior farmacokineticus met wie zelfs negatieve interacties positief verliepen. Allen, bedankt voor je hulp en gezelschap.

I greatly appreciate the comments made on my thesis by Dr. P. Williams, Dr. M. Thomas and Dr. V. Libri from the clinical pharmacology unit of GlaxoWellcome in Greenford. Pauline, Malcolm and Vincenzo, each of you contributed to the final touch of this manuscript.

Mijn paranimfen, de dames P. Eekers en A. Maassen van den Brink, dank ik voor de zware verantwoordelijkheid die ze op zich hebben willen nemen om me moreel bij te staan tijdens de laatste hindernissen van mijn promotie-parcours. Patricia en Antoinette, na mijn promotie gaan we eens over andere dan hoofd-zaken brain-stormen (contradictio in terminis).

Mijn ouders dank ik voor de vrijheid die ze me gegeven hebben bij de keuze van mijn studies en hun steun tijdens mijn -in hun ogen- eeuwigdurende carrière als student. Ook mijn schoonouders dank ik voor hun interesse in mijn werk. Leo, bij het squashen kan ik je verslaan, maar voor de spelling van de Nederlandse taal sta ik graag de eer af aan jou.

Tenslotte, Ann. Elke uitspraak zou tekort doen aan jouw bijdrage tijdens mijn promotieperiode. Dat we samen van mijn doel onze weg konden maken, heb ik aan jou te danken ...

*"I took some cocaine,
watched the migraine vanish at once, ..."*

(Sigmund Freud, 1856-1939)

Curriculum vitae

Jan de Hoon was born on the 8th of May 1964 in Turnhout, Belgium. After finishing secondary school in 1982 (Sint Jozefcollege, Turnhout, Belgium) he studied chemistry at the university of Limburg (Limburgs Universitair Centrum, Diepenbeek, Belgium) and at the university of Antwerp (Universitaire Instelling Antwerpen, Antwerp, Belgium) where he obtained his scientific degree in chemical sciences on the 7th of July 1986 with the highest distinction. In the same year he was awarded by the Flemish Chemical Society for his research project entitled "Contrast enhancement in nuclear magnetic resonance imaging: the coupling of proteins to paramagnetic ions and their influence on relaxation time" which was performed at the department of organic chemistry under the supervision of Prof. dr. F.C. Alderweireldt at the university of Antwerp. From 1986 until 1992 he studied medicine at the university of Leuven (Katholieke Universiteit Leuven, Leuven, Belgium) and got his degree as a medical doctor on June 30th 1992 with the highest distinction. Thereafter, he started his internship for internal medicine at the University Hospital Gasthuisberg of Leuven and affiliated hospitals. From 1995 until 1999 he was part-time appointed as an assistant in internal medicine at the department of General Internal Medicine (head Prof. dr. H. Bobbaers) of the University Hospital. During the same period he worked part-time as a research assistant at the department of Pharmacology and Toxicology at the University of Maastricht in The Netherlands. Under the guidance of Prof. dr. H.A.J. Struijker-Boudier and dr. L.M.A.B. Van Bortel he worked on a project entitled "Migraine and anti-migraine drugs: focus on cardiovascular aspects". On the 1st of November 1998 he was registered as a clinical pharmacologist in The Netherlands and on the 16th of August 1999 as a specialist in general internal medicine in Belgium. Since August 1999 he is appointed as a supervising clinical research physician at the Centre for Clinical Pharmacology of the University Hospital Gasthuisberg in Leuven. He is currently detached to the clinical pharmacology unit of GlaxoWellcome at Greenford in the United Kingdom where he is involved with early drug development in general and with research on new anti-migraine drugs in particular. He is married to Ann Van Hoof who is due to have their first child in November.

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List of Abbreviations

A, B	fictitious plasma concentrations at time zero
α, β	rate constants referring to the rapid (α) and slow (β) phase of decline in plasma concentration, respectively
ACh	acetylcholine
ANOVA	analysis of variance
ATP	adenosine-5'-triphosphate
AU	arbitrary units
AUC	area under the curve
Δ AUC	change/difference in AUC
CAC α 1A	α_{1A} -subunit of a brain specific voltage gated P/Q-type calcium channel
cAMP	cyclic adenosine-5'-monophosphate
CC	cross-sectional compliance (over the operating pressure)
CC _{iso}	isobaric CC; CC over a pre-defined pressure interval
cGMP	cyclic guanosine-5'-monophosphate
CGRP	calcitonin gene-related peptide
95%CI	95% confidence interval
CL	plasma clearance
C _{max}	maximum plasma concentration
CSA	arterial end diastolic cross-sectional area
Δ CSA	change in arterial cross-sectional area during the cardiac cycle
CSD	cortical spreading depression
C(t)	plasma concentration at time t
CV	coefficient of variation
D	arterial end diastolic diameter
Δ D	change in arterial diameter during the cardiac cycle; distension
DBP	diastolic blood pressure
DC	distensibility coefficient (over the operating pressure)

Abbreviations

DC _{iso}	isobaric DC; DC over a pre-defined pressure interval
DHE	dihydroergotamine
dl	deciliter
ECG	electrocardiogram
EDRF	endothelium derived relaxing factor
EDTA	ethylene-diaminetetraacetic acid
eNOS	endothelial nitric oxide synthase
FBF	forearm blood flow
FBF _{control}	forearm blood flow in the control arm
FBF _{exp}	forearm blood flow in the experimental/infused arm
FBF-ratio	ratio in FBF in the infused over the control arm
fMRI	functional magnetic resonance imaging
FVR	forearm vascular resistance
FVR _{control}	forearm vascular resistance in the control arm
FVR _{exp}	forearm vascular resistance in the experimental/infused arm
FVR _{muscle}	muscular forearm vascular resistance
FVR _{total}	total forearm vascular resistance
h	hour
³ H	tritium
5-HIAA	5-hydroxyindole-3-acetic acid
5-HT	5-hydroxytryptamine; serotonin
IMT	intima-media thickness
IU	international units
k _a	rate constant referring to absorption
K ⁺ _{ATP} -channels	ATP-sensitive potassium channels
K ⁺ _{Ca} -channels	calcium-sensitive potassium channels
K _i	the concentration of competing ligand in a competition assay that would occupy 50% of the receptors if no radioligand were present
KIU	kallikrein inhibitor units
kPa	kilo pascal
l	liter

L-NMMA	N ^G -monomethyl-L-arginine
LogD _{pH7.4}	parameter referring to lipophilicity of drugs (see Table 1.3)
MAP	mean arterial pressure
mg, µg	milligram, microgram
MHz	mega hertz
min	minute
ml, µl	milliliter, microliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
n	number
NANC	nonadrenergic-noncholinergic
NAV	not available
ND	not determined
ng	nanogram
NK-receptor	neurokinin-receptor
NKA	neurokinin A
NMDA	N-methyl-D-aspartaat
NO	nitric oxide
NOS	NO-synthase
NOS III	identical to eNOS
NSAID	non-steroidal anti-inflammatory drug
<i>P</i>	level of significance
ΔP	pulse pressure
ΔP _{BA}	brachial artery ΔP
ΔP _{cal}	calculated ΔP
ΔP _{CCA}	common carotid artery ΔP
ΔP _{FA}	femoral artery ΔP
ΔP _{tono}	absolute ΔP as measured with tonometry
PET	positron emission tomography
pg	picogram
pK _i	the negative logarithm of K _i

Abbreviations

pmol	picomole
RC	repeatability coefficient (Bland-Altman)
rCBF	regional cerebral blood flow
RF	radio frequency
RIA	radioimmuno-assay
SBP	systolic blood pressure
SD	standard deviation
SEM	standard error of the mean
SNP	sodium nitroprusside
SP	substance P
SPECT	single positron emission computed tomography
SSOFRA	selective serotonin-1-F-receptor agonist
SVR	systemic vascular resistance
$t_{1/2,abs}$	absorption half-life
$t_{1/2,\alpha}$ and $t_{1/2,\beta}$	distribution and elimination half-life
$t_{1/2,z}$	terminal half-life
TCD	transcranial Doppler sonography
t_{max}	time to reach C_{max}
TEA	tetra-ethyl-ammonium chloride
TxB ₂	thromboxane B ₂
V _{β}	volume of distribution