

Involvement of calcitonin gene-related peptide in migraine: regional cerebral blood flow and blood flow velocity in migraine patients

L. H. Lassen · V. B. Jacobsen · P. A. Haderslev ·
B. Sperling · H. K. Iversen · J. Olesen · P. Tfelt-Hansen

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Abstract Calcitonin gene-related peptide (CGRP)-containing nerves are closely associated with cranial blood vessels. CGRP is the most potent vasodilator known in isolated cerebral blood vessels. CGRP can induce migraine attacks, and two selective CGRP receptor antagonists are effective in the treatment of migraine attacks. It is therefore important to investigate its mechanism of action in patients with migraine. We here investigate the effects of intravenous human alpha-CGRP (hαCGRP) on intracranial hemodynamics. In a double-blind, cross-over study, the effect of intravenous infusion of hαCGRP (2 μg/min) or placebo for 20 min was studied in 12 patients with migraine without aura outside attacks. Xenon-133 inhalation SPECT-determined regional cerebral blood flow (rCBF) and transcranial Doppler (TCD)-determined blood velocity (V_{mean}) in the middle cerebral artery (MCA), as well as the heart rate and blood pressure, were the outcome parameters. No change of rCBF was observed at the end of infusion [$1.2\% \pm 1.7$ with hαCGRP, vs. $-1.6\% \pm 3.1$ with placebo (mean \pm SD)] ($P = 0.43$). V_{mean} in MCA decreased to $13.5\% \pm 3.6$ with hαCGRP versus $0.6\% \pm 1.8$ with placebo ($P < 0.005$). Since rCBF was unchanged, this indicates a dilation of the MCA. hαCGRP induced a decrease in MAP (12%) ($P < 0.005$) and an increase in heart rate (58%) ($P < 0.0001$). CGRP dilates

cerebral arteries, but the effect is so small that it is unlikely to be the only mechanism of CGRP-induced migraine.

Keywords Human calcitonin gene-related peptide · Cerebral arteries · Transcranial Doppler · Regional cerebral blood flow · Migraine

Introduction

Based on animal research showing calcitonin gene-related peptide (CGRP) in perivascular nerves [1] and a strong vasodilator effect of CGRP on cerebral blood vessels [2], a role for CGRP in the pathogenesis of migraine pain was first suggested by reports of increased CGRP in external jugular venous blood during migraine attacks [3–5]. In one recent study, there was, however, no increase of CGRP [6]. More solid evidence was presented in a study which demonstrated that, CGRP infused intravenously in patients with migraine was able to induce a vascular type headache in great majority of patients and in some patients this headache fulfilled all diagnostic criteria for migraine without aura [7]. The final proof of the involvement of CGRP in migraine mechanisms was provided by two phase II clinical trials [8, 9], demonstrating significant efficacy of the specific CGRP antagonists, BIBN4096BS [10] and MK0974 [9]. Thus, CGRP is not only able to induce attacks, but it seems to be continuously important throughout the entire migraine attack. CGRP is one of the most potent dilators of isolated cerebral arteries known today [2, 11], but possible species differences make it difficult to predict the effect of CGRP in the human cerebral circulation. Although the effect of CGRP on the cerebral circulation has been studied in normal subjects [12], it is important to study it also in patients with

L. H. Lassen · V. B. Jacobsen · P. A. Haderslev ·
H. K. Iversen · J. Olesen · P. Tfelt-Hansen (✉)
Department of Neurology, Glostrup Hospital,
University of Copenhagen, 2600 Glostrup, Denmark
e-mail: ptha@glo.regionh.dk

B. Sperling
Department of Clinical Physiology and Nuclear Medicine,
Bispebjerg Hospital, Copenhagen, Denmark

migraine in whom migraine-like headache and migraine attacks are induced by CGRP [7]. The aim of the present study was therefore to investigate the cerebral hemodynamic effects of CGRP in patients with migraine outside of attack.

Patients and methods

Patients

Twelve patients with migraine (11 females, 1 male; mean age 39.5 years, range 31–47 years; mean weight 69.7 kg, range 51–89 kg) were included. All suffered from migraine without aura according to criteria of the International Headache Society [13]. The subjects were not allowed to take medication, coffee, tea, alcohol or tobacco for 12 h before the study, and they were not allowed to take a triptan 24 h or ergotamine 48 h before the study.

Exclusion criteria were as follows: use of any kind of daily medication including prophylactic headache therapy but excluding oral contraceptives; pregnancy or breastfeeding; excessive use of analgesics or alcohol; serious somatic or psychiatric disorders; ischaemic heart disease; a supine systemic blood pressure more than 160/90 or less than 110/75 mmHg at entry of study.

Patients were informed that they were free to withdraw at any time and all gave written informed consent. The study was approved by the local ethics committees of Copenhagen and Copenhagen County (KA 96054) and complied with the Declaration of Helsinki.

Design and procedure

The patients with migraine were studied outside of attacks. The study used a double-blind, placebo-controlled cross-over design. Subjects were randomized to receive 2 µg/min human α CGRP (h α CGRP; Clinalfa, Switzerland) or placebo (0.9% NaCl) infused intravenously for 20 min on 2 days separated by at least 1 week. The dose was chosen as the highest tolerated dose (because of blood pressure reduction) based on reports in the literature [14, 15]. Patients were randomly assigned by computer (Med. Stat®, version 2.12). Randomization and preparation of study drugs was done by medical staff, who were not involved in the study. Five patients started with placebo and seven with h α CGRP. This quota was chosen by the computer and not known before the study. The effectiveness of blinding was not estimated.

When subjects arrived at the laboratory, a cannula (Viggo Venflon®, 1.4 mm) was placed in the right cubital vein for h α CGRP/placebo infusion. Baseline values of mean maximal blood velocity (V_{mean}) in the middle

cerebral artery (MCA), regional cerebral blood flow (rCBF), blood pressure, heart rate and pCO₂ were recorded after 30 min of rest in the supine position in a quiet room. Then, h α CGRP or placebo was infused intravenously for 20 min by a volumetric pump (Braun Perfuser). V_{mean} , blood pressure, heart rate and end tidal pCO₂ were recorded every 10 min during and after infusion until 80 min after start of the h α CGRP infusion. rCBF was measured again during the last 5 min of infusion and at 75–80 min after start of h α CGRP/placebo infusion. At every recording, it was noticed if volunteers were flushing and information about headache presence, intensity (measured on a 0–10 scale) and characteristics were obtained before, during and after the infusions. The results concerning headache response have been published elsewhere [7].

Methods

rCBF was measured with a highly sensitive, brain-dedicated, fast-rotating, single photon emission computerized tomograph (Tomomatic 232). Each study lasted 4.5 min. A mixture of atmospheric air and ¹³³Xenon was rebreathed during the first 1.5 min through a closed system from a 4-L reservoir (740 Mbq/L). During the last 3 min, the ¹³³Xenon mixture was expired against atmospheric air. rCBF was recorded simultaneously in two slices positioned 50 and 90 mm above and parallel to the orbito-meatal plane (OM). Each slice was 16 mm thick and the distance between the centers of slices was 40 mm. The full width half maximum resolution of the instrument is about 16 mm in the horizontal plane. rCBF was calculated according to Celsis et al. [16].

A fixed matrix of regions of interest was superimposed on the rCBF picture. The shape and size were fitted to the outlines on the brain excluding extracranial flow. Regional mean values were calculated within the predefined regions of interest. The matrix was divided into regions of interest representing the hemispheric rCBF regions and the vascular territories of supply by the anterior-, middle- and posterior cerebral arteries. The maximum whole body radiation was approximately 0.6 mSV per rCBF measurement [17].

Time averaged mean of the maximal blood velocity (V_{mean}) in MCA at the usual headache side was measured with transcranial Doppler (TCD) ultrasonography (2 MHz, Multidop X Doppler: DWL, Sipplingen, Germany). A mean of four consecutive values of V_{mean} was taken (each representing the mean value of typically four to five heart beats automatically calculated by the computer). Positions of the measurements were reproduced from day to day by recording the position in relation to the angle and distance relative to the orbito-meatal line. Blood pressure and heart rate were measured with an automatic inflatable arm cuff

(Omega 1400, Invivo Research Laboratory, New York, USA). Simultaneously, with each rCBF and TCD measurement, the end expiratory $p\text{CO}_2$ was recorded by means of a capnograph (Datex OY, CD 101).

Statistics

Hemodynamic responses are given as mean and standard deviation (\pm SD). Differences in blood velocity, rCBF, blood pressure, heart rate and $p\text{CO}_2$ over time within the group were analyzed with analysis of variance (MANOVA, Statgraphics® 7.0), and changes were then located with a multiple range test (Confidence intervals, Statgraphics® 7.0).

Differences in the delta values (baseline—the last measurement during infusion and baseline—the last measurement during the study) in V_{mean} , rCBF, blood pressure, heart rate and $p\text{CO}_2$ between the effect of $\text{h}\alpha\text{CGRP}$ and the effect of placebo were tested with a paired t test (Statgraphics® 7.0). In all tests, $P < 0.05$ was considered statistically significant.

Results

During CGRP infusion, two volunteers experienced a substantial decrease in blood pressure (70/50 and 65/25, respectively) causing the infusion to be terminated ahead of time. Signs and symptoms were pallor, cold sweat, stomach ache, nausea and palpitation. The situation was restored after placing the volunteers in head-down tilt for 7 and 20 min, respectively. No rCBF and TCD measurements were taken during this time period, and therefore, results from these two volunteers were excluded from the calculations below.

Changes in end tidal $p\text{CO}_2$

End-tidal $p\text{CO}_2$ decreased significantly over time when volunteers were treated with $\text{h}\alpha\text{CGRP}$ ($P < 0.0001$) but not when treated with placebo ($P < 0.26$, MANOVA). The $p\text{CO}_2$ decrease at the last measurement during infusion was $-4.0\% \pm 0.8\%$ when treated with $\text{h}\alpha\text{CGRP}$ versus $0.7\% \pm 1.0\%$ when treated with placebo ($P < 0.05$, paired t test). The peak decrease in end-tidal $p\text{CO}_2$ induced by $\text{h}\alpha\text{CGRP}$ ($-6.8\% \pm 2.6\%$) occurred at 25 min, 5 min after end of infusion.

Transcranial Doppler measurements

The TCD examination was performed on the side of usual migraine. The following data for V_{mean} in MCA are corrected for changes in $p\text{CO}_2$ according to Markwalder et al. [18]:

$$V_{\text{mean(korr)}} = V_{\text{mean(meas)}} \exp\left(0.04\left(p\text{CO}_{2(\text{Basal})} - p\text{CO}_{2(n)}\right)\right).$$

V_{mean} changed significantly over time when patients were treated with $\text{h}\alpha\text{CGRP}$ ($P < 0.0001$, MANOVA), but not when treated with placebo ($P = 0.26$, MANOVA). At the last measurement during infusion, 15 min after its beginning, V_{mean} compared to baseline was $-13.5\% \pm 11.4$ when treated with $\text{h}\alpha\text{CGRP}$, versus $0.6\% \pm 5.6$ when treated with placebo ($P < 0.005$, paired t test). At the end of the in-hospital period (75 min after start of the infusion), there was no difference between the two groups ($P = 0.97$, paired t test) (Fig. 1, Table 1). There was a positive correlation between ΔV_{mean} and ΔMABP ($r = 0.703$, $P < 0.05$).

Regional cerebral blood flow

All data have been corrected for changes in $p\text{CO}_2$: $\text{rCBF}_{(\text{korr})} = \text{rCBF}_{(\text{meas})} \times \exp(0.04(p\text{CO}_{2(\text{Basal})} - p\text{CO}_{2(n)}))$. For comparison of V_{mean} and rCBF, we calculated rCBF in the territory of MCA, the artery used for TCD examination. Area-weighted data from the OM + 50 mm and OM + 90 mm were used for the results given below.

There was no change over time neither when patients were treated with $\text{h}\alpha\text{CGRP}$ ($P = 0.26$) nor when they were treated with placebo ($P = 0.88$, MANOVA). During infusion, rCBF compared to baseline was $+1.2\% \pm 5.5$ when treated with $\text{h}\alpha\text{CGRP}$ versus $-1.6\% \pm 9.8$ when treated with placebo. The difference between CGRP and placebo was not significant ($P = 0.43$, paired t test). Furthermore, no significant difference between CGRP and

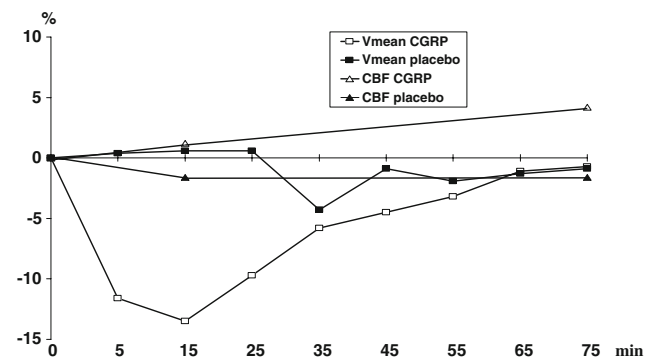


Fig. 1 The effect of $\text{h}\alpha\text{CGRP}$ (2 $\mu\text{g}/\text{min}$) for 20 min and the effect of placebo on the mean velocity (V_{mean}) in middle cerebral artery (*squares*) and the regional cerebral blood flow (rCBF) (*triangles*) in the territory of the middle cerebral artery (MCA) used for transcranial Doppler examination. X-axis: time from start of infusion (min), y-axis: changes in percent of baseline. V_{mean} (*squares*) decreased $-13.5\% \pm 3.6$ compared to baseline with $\text{h}\alpha\text{CGRP}$ treatment versus $0.6\% \pm 1.8$ with placebo treatment ($P < 0.0001$). After 75 min, there was no difference between the two groups ($P = 0.97$). No change of rCBF (*triangles*) was observed at the end of infusion ($P = 0.43$) or at the end of study period ($P = 0.12$)

Table 1 The maximum mean blood velocity (V_{mean}) in the middle cerebral artery (MCA)

Time (min)	V_{mean} in MCA (cm/s)					
	No pCO ₂ correction		Corrected for pCO ₂ changes			
	CGRP	Placebo	CGRP	Placebo	CGRP%	Placebo%
0	81.6 (10.7)	79.9 (12.3)	81.6 (10.7)	79.9 (12.3)	0	0
15–20	66.9 (13.5)*	79.5 (12.4)	70.7 (14.2)*	80.3 (12.7)	–13.5 (11.4)*	0.6 (5.6)
75–80	76.6 (9.9)	77.4 (12.7)	80.6 (11.3)	79.2 (13.6)	–0.7 (11.6)	–0.9 (6.4)

Values within parentheses represent the standard deviation (SD)

Data is shown with and without pCO₂ correction according to Markwalder et al. [18]

The pCO₂-corrected data is shown as absolute data and in percent change from baseline ($P < 0.001$)

* Significant changes compared with baseline

placebo was found at the end of the study period ($P = 0.12$, paired t test) (Fig. 1, Table 2).

The absolute rCBF data and the rCBF data with pCO₂ correction concerning the MCA area relevant for TCD examination are given in Table 2. There was also no significant difference in pCO₂-uncorrected rCBF between CGRP and placebo during ($P = 0.78$, paired t test) or after the infusion ($P = 0.357$, paired t test). There was a negative correlation between $\Delta rCBF_{\text{(MCA)}}$ and ΔMABP ($r = -0.654$, $P < 0.05$). rCBF during h α CGRP infusion was not significantly different in any territory or at any time point.

Blood pressure

The mean arterial blood pressure (MABP) decreased significantly over time when patients were treated with h α CGRP ($P < 0.0001$), but not when they were treated with placebo ($P = 0.48$, MANOVA). The maximal decrease occurred at the last measurement during infusion (20 min after start of infusion) and amounted to $-12.3\% \pm 8.1\%$ with h α CGRP treatment versus $+2.4\% \pm 5.9\%$ with placebo treatment ($P < 0.005$, paired t test). MABP had normalized at the end of the study period (80 min from

start of infusion) ($P = 0.49$) (Table 3). Both systolic and diastolic blood pressures decreased during infusion of CGRP compared to that during infusion of placebo ($P < 0.05$).

Heart rate

When patients were treated with h α CGRP, heart rate increased significantly over time ($P < 0.0001$, MANOVA), but not when they were treated with placebo ($P = 0.44$, MANOVA). The peak increase in heart rate after h α CGRP treatment occurred 20 min after start of infusion reaching $58.1\% \pm 22.7\%$ versus $1.9\% \pm 5.58\%$ with placebo treatment ($P < 0.0001$, paired t test). Heart rate after h α CGRP treatment was still different from that after placebo treatment at the end of the study period ($P < 0.005$, paired t test) (Table 3).

Other signs

Flushing after h α CGRP treatment was pronounced and appeared only in the face, neck and upper chest. It appeared from 10 min (time of first observation) after start of the

Table 2 Regional cerebral blood flow rCBF in the MCA territory recorded 50 and 90 mm above the orbitomeatal plane (OM)

Time (min)	Cerebral blood flow (ml blood/100 g/min)					
	MCA (OM 50 + OM 90) in the territory used for TCD examination					
	No pCO ₂ correction		Corrected for pCO ₂ changes			
	Absolute data		Absolute data		Data in % of baseline	
CGRP	Placebo	CGRP	Placebo	CGRP	Placebo	
0	70.8 (13.7)	67.7 (9.3)	70.8 (13.7)	67.7 (9.3)	0	0
15–20	66.8 (9.4)	65.3 (11.6)	71.1 (10.5)	66.7 (11.4)	1.2 (1.7)	–1.6 (3.1)
75–80	69.3 (7.9)	65.0 (9.8)	72.8 (8.7)	66.7 (10.4)	4.2 (3.0)	–1.6 (2.8)

Values within parentheses represent the standard deviation (SD)

Data is shown with and without pCO₂ correction according to Markwalder et al. [18]

The pCO₂-corrected data is shown as absolute data and in percent change from baseline ($P < 0.001$)

Table 3 The mean arterial blood pressure (MABP), systolic and diastolic blood pressure (mmHg) and heart rate (bpm) after CGRP and placebo treatment

Time (min)	Blood pressure (mmHg)						Heart rate (bpm)	
	MABP		Systolic BP		Diastolic BP		CGRP	Placebo
	CGRP	Placebo	CGRP	Placebo	CGRP	Placebo		
0	87.8 (9.6)	91.6 (9.5)	117.0 (11.1)	119.0 (12.6)	73.2 (9.5)	77.9 (9.4)	63.0 (6.0)	64.4 (7.2)
15	78.0 (7.5)*	90.0 (9.2)	110.7 (9.6)	117.2 (12.5)	61.7 (7.8)	76.4 (8.7)	92.9 (10.2)*	63.8 (6.8)
20	76.9 (10.1)*	93.5 (8.3)	112.2 (13.3)	123.9 (11.6)	59.3 (9.1)	78.3 (7.6)	98.9 (11.2)*	65.5 (7.1)
75	86.5 (9.2)	91.4 (8.7)	117.6 (12.0)	121.9 (13.4)	71.0 (8.9)	76.2 (7.2)	76.1 (7.0)*	62.9 (6.0)
80	85.3 (9.2)	91.3 (9.9)	116.4 (13.2)	123.3 (11.3)	69.7 (8.4)	75.3 (9.7)	75.3 (7.7)*	64.4 (5.3)

Values within parentheses represent \pm standard deviation (\pm SD)

* $P < 0.05$

infusion of h α CGRP in all patients until median 70 min after start of infusion (range 20–80 min). There was no flushing when the patients were treated with placebo. The median headache during infusion of h α CGRP was 1 versus 0 in the placebo-treated group ($P < 0.01$). During the following 11 h, all patients experienced headache after h α CGRP treatment versus the one after placebo treatment ($P < 0.001$). The median headache score was 4 after h α CGRP treatment and 0 after placebo treatment. For details, see [7].

Discussion

The main finding of the present study was that CGRP-infusion dilated the MCA in patients with migraine, while cerebral blood flow remained unchanged. The dose of 2 μ g/min of CGRP was chosen from the literature in which the same dose of 545 pmol/min was given in healthy volunteers without any reported adverse events [14]. In another study, up to 25 μ g CGRP was given as an intravenous bolus injection and all subjects had facial flushing [15]. The dose of 2 μ g/min for 20 min is probably the maximally tolerated dose of CGRP in patients with migraine, since it resulted in a substantial decrease of MABP and a marked increase in heart rate. Two subjects were near fainting and had to be withdrawn. In healthy volunteers, a dose of 1.5 μ g/min was used without adverse events apart from flushing and without any effect on mean blood pressure [12]. It remains uncertain whether the stronger circulatory response in the present study was exclusively due to the higher dose or whether patients with migraine are more sensitive to CGRP. After 20 min infusion at 1.5 μ g/min, the mean plasma level was 340 pmol/L in healthy volunteers [12]. It can be calculated from these results that the plasma level would be 450 pmol/L in our patients with migraine. The EC₅₀ for CGRP for the dilatatory effect on human pial arteries is 500 pmol/L [19]. The dose

of CGRP used in the present study thus resulted in a plasma level very near to the EC₅₀ for cerebral arteries in vitro.

The blinding of the present study can be criticized. Signs (increased heart rate) and symptoms (facial flushing and feeling of warmth) during CGRP infusion made it difficult to keep the study completely blinded. However, the present design is the best one available, because no technique is available to disguise such symptoms.

An interesting finding of the present study is the significant reduction of blood velocity (13.5% decrease) in the MCA, but rCBF remained unchanged during intravenous h α CGRP-infusion. A reduced velocity in an artery, with unchanged regional blood flow in its territory of supply, reflects dilation [20]. The relative diameter change can be roughly estimated from the relation: flow = mean velocity multiplied by the cross-sectional area of the artery ($2\pi r^2$). When the flow is constant, velocity is a function of the reciprocal value of r^2 and $V_a/V_b = r_b^2/r_a^2$ [20]. We can thus estimate from the change in mean velocity that the h α CGRP infusion caused a 7.5% increase in MCA diameter corresponding to 17% increase of its cross sectional area.

α CGRP infused in a considerably lower dose (0.6 μ g/min) did not change MCA velocity or rCBF in healthy volunteers [21], while the dose of 1.5 μ g/min in volunteers dilated the MCA (9% increase in diameter) to the same extent as in the present study [12]. The dilatation of MCA in the present study could, theoretically, be due to cerebrovascular autoregulation secondary to decreased blood pressure. However, conduction arteries like MCA do normally only autoregulate due to blood pressure changes to a much lesser extent than arterioles (for review, see [22]). A positive correlation ($r = 0.7$, $P < 0.05$) between changes in MCA and changes in MABP seems to support this possibility. However, this correlation could also reflect that MCA and systemic circulation vary in parallel. In healthy volunteers, using a slightly lower CGRP dose of 1.5 μ g/min, blood pressure was unchanged but MCA dilated to the

same extent as in the present study [12]. This strongly indicates that MCA dilation is a direct effect of CGRP.

In rat MCA, using the in vitro-pressurized arteriographic model, luminal CGRP was without any dilating effect, whereas abluminal CGRP dilated the artery [23]. This indicated that CGRP was unable to cross the blood–brain barrier in rat MCA. In contrast, both in patients with migraine (Fig. 1) and in healthy volunteers [12], a dilation of MCA was observed. Theoretically, CGRP could act on an endothelial receptor, but this is unlikely because the CGRP antagonist BIBN4096BS could not block the effect on MCA in man [12]. Furthermore, in man, the calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein (RAMP) are located mainly in the muscular layer of the human MCA [24]. In the endothelium, there was only a minor amount of CLR and largely absent RAMP1 [24]. There is thus no CGRP receptor (CLR plus RAMP1) on the endothelium. The present results with a dilation of MCA could indicate that the blood–brain barrier to CGRP in the MCA is less tight in man than in rats, possibly related to the large difference in size.

In healthy volunteers, dilation of MCA was accompanied by a modest (14%) increase in rCBF [12], whereas in the present study, in patients with migraine, a 5% increase in rCBF was not statistically significant (Fig. 1). The discrepancy may be due to random variation. Altered cerebrovascular reactivity in patients with migraine compared to controls is another possibility. The decrease of mean MABP from 88 to 77 mmHg observed in the present study could also be a factor if autoregulation was disturbed by CGRP.

In patients with subarachnoid hemorrhage, a CGRP-infusion concomitant with intravenous fluid to correct any drop in blood pressure resulted in a dilation of the MCA on the vasospasm side [25]. Blood pressure was unchanged while heart rate and cardiac output were increased and total peripheral resistance was decreased [25]. In two studies in man [14, 15], an increase in noradrenaline in plasma was observed after CGRP. We found in the present study a decrease in mean blood pressure and an increase in heart rate as would be expected after administration of a potent vasodilator such as CGRP.

In conclusion, the potent endogenous migraine-inducing molecule CGRP resulted in a dilation of MCA and unchanged rCBF. CGRP can thus most likely cross the blood–brain barrier to some extent in the large human cerebral arteries. The vasodilator effect on these arteries is in our opinion, however, so small that it is unlikely to be the only mechanism of CGRP-induced migraine observed in our patients with migraine [7].

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Conflicts of interest None of the authors have any conflict of interest in connection with this work.

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