Publications on pituitary adenylate cyclase-activating polypeptide-38 (PACAP38)


*First evidence on PACAP triggering migraine*


*First review paper suggesting the PACAP receptor as a novel target*


PACAP38 induces migraine-like attacks in patients with migraine without aura

Henrik Winther Schytz, Steffen Birk, Troels Wienecke, Christina Kruuse, Jes Olesen and Messoud Ashina

Experimental studies have shown that infusion of vasoactive neurotransmitters may trigger headache or migraine-like attacks in man. Pituitary adenylate cyclase activating peptide-38 (PACAP38) is a strong vasodilator found in trigeminal sensory and parasympathetic perivascular nerve fibers. We therefore hypothesized that infusion of PACAP38 would cause headache in healthy subjects and migraine-like attacks in migraine patients. Twelve healthy subjects and 12 migraine patients were examined in two separate studies. All subjects were allocated to receive 10 pmol/kg/min PACAP38 and placebo in a randomized, double-blind crossover study design. Headache was scored on a verbal rating scale (VRS) during hospital (0–2 h) and post-hospital (2–12 h) phases. Mean blood flow velocity in the middle cerebral artery (VMCA) by transcranial Doppler (TCD) and diameter of the superficial temporal artery (STA) by high resolution ultrasonography were recorded during hospital phase in migraineurs. PACAP38 infusion caused headache in all healthy subjects and 11 out of 12 migraine patients. Seven migraine patients experienced migraine-like attacks after PACAP38 and none after placebo (P = 0.016). Most of attacks (6 out of 7) occurred during the post-hospital phase [mean time 6 h (range 2–11)]. Two healthy subjects reported migraine-like attacks after PACAP38 during the hospital phase and none during the post-hospital phase. In the hospital phase, the area under the curve (AUC) for headache score was larger during PACAP38 infusion compared to placebo in healthy subjects (P = 0.005) and tended to be larger in migraineurs (P = 0.066). In the post-hospital phase, the AUC for headache was larger after PACAP38 infusion compared to placebo in both healthy subjects (P = 0.005) and migraine patients (P = 0.013). In migraine patients, PACAP38 caused a peak decrease of 16.1% in VMCA and a 37.5% increase in STA diameter at 20 min after start of infusion. In conclusion, PACAP38 infusion caused headache and vasodilatation in both healthy subjects and migraine patients. In migraine sufferers, PACAP38 caused delayed migraine-like attacks. The findings stimulate further investigation of the neuronal and vascular mechanisms of PACAP38.

Keywords: PACAP38; migraine without aura; transcranial Doppler; cerebral vessels; parasympathetic nervous system

Abbreviations: PACAP38 = Pituitary adenylate cyclase activating peptide-38; STA = superficial temporal artery; TCD = transcranial Doppler; VIP = vasoactive intestinal polypeptide; VRS = verbal rating scale
Introduction

It is still a matter of intense debate if vasodilatation contributes to migraine pain per se or is just an epiphenomenon during migraine attacks (May and Goadsby, 1999; Welch, 2003; Olesen and Goadsby, 2006; Schoonman et al., 2008). In the last 15 years we have systematically investigated both the migraine eliciting and hemodynamic effects of different vasoactive neurotransmitters found in perivascular nerve fibers (Thomsen, 1994; Lassen et al., 2002; Rahmann et al., 2008). The studies have shown, with one exemption of sildenafil (Kruuse et al., 2003), that all known inducers of migraine-like attacks dilate cephalic vessels (Thomsen, 1994; Lassen et al., 1995, 2002; Kruuse et al., 2006). However, we also observed that vasodilatation is not a sufficient factor for induction of migraine-like attacks (Rahmann et al., 2008). Given that cephalic vessels might also be dilated during a migraine attack (Iversen et al., 1990; Friberg et al., 1991), simultaneous recording of headache and vasodilatation in migraine patients after infusion of neuropeptides are of key importance.

Cephalic vessels are innervated by sensory, parasympathetic and sympathetic nerve fibers (Edvinsson et al., 2001; Jansen-Olesen et al., 2004; Edvinsson and Uddman, 2005). We have recently shown that infusion of the parasympathetic neuropeptide vasoactive intestinal polypeptide (VIP) induces a marked dilatation of cephalic arteries (Hansen et al., 2006; Rahmann et al., 2008). However, VIP induces only mild headache and no migraine-like attacks (Rahmann et al., 2008). Pituitary adenylate cyclase activating peptide-38 (PACAP38) is a 38 amino-acid neuropeptide (Miyata et al., 1989) belonging to the same secretin/glucagon superfamily as VIP. Both VIP and PACAP activate the VPAC1 (Hosoya et al., 1993) and VPAC2 (Lutz et al., 1993) receptors, but interestingly a third receptor, PAC1, is selectively activated by PACAP. Infusion of PACAP38, which is the most prominent form (Arimura and Shioda, 1995), induced vasodilatation in healthy subjects of a similar magnitude but longer lasting than VIP (Birk et al., 2007). The headache characteristics after PACAP38 have not been previously described in healthy subjects or migraine patients. Given that PACAP is found in perivascular nerve fibers implicated in migraine pathogenesis, we hypothesized that PACAP38 infusion would induce headache in healthy subjects and migraine-like attacks in migraine patients. In addition, we aimed to describe the dilatation of cephalic vessels in migrainers after PACAP38 infusion. We therefore performed two double-blind placebo controlled crossover studies to record possible headache and migraine eliciting effects of PACAP38 in healthy subjects and migraine patients without aura.

Methods

The study consisted of two separate studies. In the first study we recruited 12 otherwise healthy patients diagnosed with migraine without aura (MO) according to the International Headache Society (IHS). One of the migraineurs had previously been participating in a similar study with infusion of VIP (Rahmann et al., 2008). Exclusion criteria were as in the healthy study with the exception of the migraine diagnosis. The Ethics Committee of the County of Copenhagen approved both studies (healthy subjects: KA02139 and migraine patients: KA20060087). In addition, the migraine study was approved by the Danish Medicines Agency (Eudract nr: 2006-003774-94), which was monitored by Copenhagen University Hospital GCP-unit and registered at Clinicaltrials.gov (ID: NCT00380263). All subjects gave informed consent to participate, and both studies were undertaken in accordance with the Helsinki Declaration of 1964, as revised in Edinburgh in 2000.

Experimental design

In a double-blind, placebo-controlled, crossover design, the subjects were in a balanced order randomly allocated to receive 10 pmol/kg/min PACAP38 (Calbiochem®, Darmstadt, Germany) or placebo (isotonic saline) over 20 min on 2 days separated by a least 1 week. Before the experiment each subject underwent a general physical examination. All subjects reported to the laboratory 08:30 h headache free. The experiment was postponed, if the subject had a migraine attack within 5 days or tension type headache 48 h before the start of the study. The intake of coffee, tea, cocoa or other methylxanthine-containing foods or beverages was not allowed for the last 8 h prior the study. All procedures were performed in a quiet room at a temperature of 25°C. The subjects were placed in the supine position and a venous catheter was inserted into the right antecubital vein for drug infusion. The subject then rested for at least 30 min before baseline measurements of blood pressure, heart rate (HR) and ECG were performed, and the infusion started using a time and volume controlled infusion pump. Headache intensity, 

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Headache and migraine-like attack criteria

Headache intensity was recorded repeatedly on a verbal rating scale (VRS) from 0 to 10 [0, no headache; 1, a very mild headache (including a feeling of pressing or throbbing—pre-pain); 10, worst imaginable headache] (Iversen et al., 1989). Headache characteristics and associated symptoms were also recorded to determine the quality and type of the headache.

Headache induced experimentally by infusion of a neuropeptide can not fulfill strict IHS criteria for migraine without aura (IHS, 2004). First, the migraine-like attacks reported are induced by pharmacological substances and can therefore not be spontaneous, though they often phenotypically mimic spontaneous migraine attack in the majority of patients (Thomsen, 1994; Lassen et al., 2002). Secondly, most...
spontaneous migraine attacks develop in a matter of hours and often go through a phase where they phenomenologically only fulfill the criteria for tension-type headache before the headache gets worse, becomes unilateral and has the associated symptoms required for migraine. Thirdly, patients in experimental provocation studies cannot be denied treatment of the induced attacks and often treat before all migraine criteria are fulfilled. Based on these circumstances we have used the following two criteria for a migraine-like attack induced 0–12 h after infusion of an experimental drug:

Migraine-like attack attacks fulfilling either (1) or (2):

1. Headache fulfilling criteria C (Moderate to severe pain intensity is considered ≥4 on VRS.) and D for migraine without aura (IHS, 2004).
2. Headache described as mimicking usual migraine attack and treated with a triptan.

Middle cerebral artery blood flow velocity

\[ V_{MCA} \] was recorded bilaterally with transcranial Doppler (TCD) with hand-held 2 MHz probes (Multidop X; DWL, Sippelingen, Germany). Fixed probes were avoided, because they may cause discomfort and even headache (Thomsen, 1995). Four recordings were taken and averaged at each time point. One recording is a time-averaged mean over 4 s or approximately four cardiac cycles. Identification of the MCA were done as previously described (Thomsen and Iversen, 1993). Every TCD recording was performed by the same trained physician (HWS). End-tidal CO2 (\( P_{e\text{CO}_2} \)) was recorded simultaneously with TCD recordings using an open mask that caused no respiratory change in MCA diameter can be estimated as:

\[
\Delta d = (\sqrt{(V_{MCA1}/V_{MCA2})} - 1) \times 100
\]

Diameter of the superficial temporal artery

Diameter of the frontal branch of the superficial temporal artery (STA) was measured by a high resolution ultrasonography unit (Dermascan C; Cortex Technology, Hadsund, Denmark: 20 MHz, bandwidth 15 MHz) as previously described (Iversen et al., 1990; Kruuse et al., 2003).

Vital signs

HR and blood pressure were measured every 10 min using an auto-inflatable cuff (ProPac Encore; Welch Allyn Protocol). ECG (Cardiofax V; Nihon-Koden, Shinju-ku, Tokyo, Japan) was monitored on a LCD screen and recorded on paper every 10 min.

Data analysis and statistics

All values are presented as mean values ± SD, except headache scores which are presented as median values. Peak mean vascular variables are presented with 95% confidence intervals. Baseline was defined as \( T_0 \) before the start of infusion of each dose. We calculated median peak headache score and median time to peak headache after PACAP38 and placebo.

The mean plasma half life of PACAP38 has been shown to be 3.5 min (Birk et al., 2007). We therefore defined an immediate phase as 0–30 min, a post-infusion phase from 30 to 90 min after start of infusion and a post-hospital phase 2–12 h after start of infusion. The two studies were analyzed separately and no direct statistical analysis was applied for comparing the healthy subjects and migraine patients.

In the healthy subject study the primary end-points were: the difference in incidence of headache or migraine-like attacks between PACAP38 and placebo; the difference in area under the curve (AUC) for headache score during infusion (0–30 min), post-infusion (30–90 min) and post-hospital phase (2–12 h) between PACAP38 and placebo.

In the migraine patient study the primary end-points were: the difference in incidence of headache or migraine-like attacks between PACAP38 and placebo; the difference in AUC for headache score during infusion (0–30 min), post-infusion (30–90 min) and post-hospital phase (2–12 h) between PACAP38 and placebo.

Incidence of headache, migraine-like attacks and adverse events were analyzed as binary categorical data with McNemar test. We calculated AUC according to the trapezium rule (Matthews et al., 1990) to obtain a summary measure and to analyze the differences in response between PACAP38 and placebo. Baseline was subtracted before calculating AUC to reduce variation between sessions within subject. Analysis of AUC values were performed with a paired two-way t-test, except headache scores where data were tested with Wilcoxon signed rank test. We tested for period and carry-over effects for all baseline variables with Mann-Whitney test and independent t-test.

Furthermore, to explore possible changes in MAP, systolic and diastolic blood pressure, we analyzed for changes over time for each treatment day separately with univariate analysis of variance (ANOVA) with the fixed factors volunteer and time. To reduce mass error.

For the vascular variables investigated in migraine patients we found no differences at baseline (Table 1), except for MAP, which, for unknown reasons, was higher on the placebo day (\( P=0.031 \)) due to a higher diastolic blood pressure (\( P=0.042 \)). There was no carry-over or period effect for baseline values of MCA, STA, MAP, HR or \( P_{e\text{CO}_2} \).

Results

Twelve healthy subjects (7F/5M, mean age 25, range 20–31) and 12 migraine patients (11F/1M, mean age 31, range 20–47 years) completed the study on both study days. The migraine patients had an attack frequency ranging from 1 to 4 attacks per month. There were missing data on \( V_{MCA} \), MAP, HR and \( P_{e\text{CO}_2} \) values on the placebo day of one migraine patients due to a technical error.

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Headache in healthy

All healthy subjects experienced headache (0–12 h) after PACAP38 infusion compared to three after placebo (McNemar test, \(P = 0.002\)) (Fig. 1). Two subjects reported a migraine-like attack occurring 10 and 50 min after start of PACAP38 infusion compared to zero subjects after placebo (McNemar test, \(P = 0.500\)) (Table 2). The median peak headache score was 3.5 (range 2–4) after PACAP38 and 0 (range 0–1) after placebo. The median time to peak headache was 5.5 h after PACAP38. Four subjects took paracetamol and three of these responded well to the medication.

During the hospital phase the AUC was larger after PACAP38 compared to placebo both during the immediate (AUC0–30 min, \(P = 0.005\)) and the post-infusion period (AUC30–90 min, \(P = 0.008\)). During the post-hospital phase we found that AUC was larger after PACAP38 compared to placebo (AUC0–12 h, \(P = 0.005\)).

Headache in migraine patients

In total 11 migraine patients experienced headache (0–12 h) after PACAP38 infusion compared to three after placebo (McNemar test, \(P = 0.021\)) (Fig. 1). Seven migraine patients reported migraine-like attacks after start of PACAP38 infusion compared to zero after placebo (McNemar test, \(P = 0.016\)) (Table 3). One of the migraine-like attacks occurred during the hospital phase 50 min after start of PACAP38 infusion, whereas six attacks occurred during the post-hospital phase (mean 6 h (range 2–11 h)). In two out of seven migraine-like attacks the subjects took a triptan and described headache as mimicking a usual migraine attack before all headache characteristics for a usual migraine attack were fulfilled. The median peak headache score was 2.5 (range 0–10) after PACAP38 and 0 (range 0–4) after placebo. The median time to peak headache occurred at a median of 4.0 h (range 0–12).

During the hospital phase AUC was larger after PACAP38 compared to placebo during the post-infusion phase (AUC30–90 min, \(P = 0.009\)), though it only tended to increase during the immediate phase (AUC0–30 min, \(P = 0.066\)). In the post-hospital phase AUC was larger after PACAP38 compared to placebo (AUC0–12 h, \(P = 0.013\)).

Middle cerebral artery

During the hospital phase \(V_{MCA}\) corrected for \(P_{etCO2}\) decreased after PACAP38 compared to placebo both during the immediate (AUC0–30 min, \(P = 0.001\)) and the post-infusion phase (AUC30–90 min, \(P = 0.007\)) (Figs 2 and 3).

PACAP38 decreased \(P_{etCO2}\) compared to placebo during the immediate (AUC0–30 min, \(P = 0.001\)) and post-infusion phases (AUC30–90 min, \(P = 0.005\)). After correcting \(V_{MCA}\) for \(P_{etCO2}\) changes, the diameter of MCA was calculated to dilate 9.5% (95% CI: 6.3–12.7) compared to baseline at T20 after onset of PACAP38 infusion. The peaks of all vascular data after PACAP38 are shown in Table 4.

**STA**

There was a significant increase in STA diameter on PACAP compared to placebo during the immediate (AUC0–30 min, \(P < 0.001\)) and post-infusion phases (AUC30–90 min, \(P < 0.001\)). (Figs 3 and 4).
HR and mean arterial blood pressure

We found an increase in HR on PACAP38 compared to placebo during both the immediate (AUC0–30 min, \(P<0.001\)) and the post-infusion phases (AUC30–90 min, \(P<0.001\)) (Fig. 3 and Table 4).

No difference in MAP was found between PACAP38 and placebo during the immediate (AUC0–30 min, \(P=0.366\)) and the post-infusion (AUC30–90 min, \(P=0.104\)) phases. In addition, no difference in systolic blood pressure was found between PACAP38 and placebo during the immediate (AUC0–30 min, \(P=0.056\)) and the post-infusion phases (AUC30–90 min, \(P=0.056\)).

ANOVA showed that systolic blood pressure increased over time during the infusion of placebo (\(P=0.051\)) and the post-infusion phases (AUC30–90 min, \(P=0.001\)). In addition, diastolic blood pressure decreased over time after PACAP38 (\(P=0.001\)) and completely terminated the headache after 2.5 h.

Adverse events

Adverse events were recorded and reported during immediate and post-infusion phases in both studies (Table 5). Heat sensation, palpitations and flushing were more often reported on PACAP38 than on placebo in both healthy subjects and migraine patients. One migraine patient experienced uncontrolled crying during PACAP38 infusion. The patient described this episode as very peculiar, since no pain or shift in emotions was experienced. All subjects experienced flushing, especially on the face and trunk, which lasted up to 24 h. One migraine patient described facial puffing 24 h after PACAP38 infusion and also right sided headache (VRS 2), which was very uncomfortable. An oral antihistamine (8 mg acrivastin) almost completely abolished the facial puffing and completely terminated the headache after 2.5 h.

Discussion

The major finding of the present study was that PACAP38 induced mild to moderate headache in both healthy subjects and migraine patients. Furthermore, 50% of the migraine patients reported migraine-like attacks several hours (mean 6 h) after start of PACAP38 infusion. Increased flushing, palpitations and heat sensation were more often reported after PACAP38 than after placebo as a sign of autonomic activation. The adverse events after

### Table 2 Headache characteristics after PACAP38 in healthy subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Peak headache (duration)</th>
<th>Headache characteristics</th>
<th>Associated symptoms</th>
<th>Migraine-like attack (onset)</th>
<th>Treatment (time/efficacy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a 10 min (10–60 min)</td>
<td>Bilat/1/throb/yes</td>
<td>+/−/−</td>
<td>Yes (30 min)</td>
<td></td>
</tr>
<tr>
<td>b 6 h (5–12 h)</td>
<td></td>
<td>Bilat/3/pres/yes</td>
<td>+/+</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>a 10 min (10–50 min)</td>
<td>Bilat/1/pres/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>b 5 h (4–6 h)</td>
<td></td>
<td>Bilat/2/throb/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>a 20 min (20–50 min)</td>
<td>Bilat/1/pres/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>b 5 h (4–9 h)</td>
<td></td>
<td>Bilat/3/pres/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>a 60 min (10 min to 12 h)</td>
<td>Bilat/3/pres/no</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>b 7 h (10 min to 12 h)</td>
<td></td>
<td>Bilat/4/pres/no</td>
<td>−/−/+</td>
<td>No</td>
<td>Paracetamol (8 h)/yes</td>
</tr>
<tr>
<td>5</td>
<td>a 10 min (10 min to 8 h)</td>
<td>Bilat/1/pres/no</td>
<td>−/−/−</td>
<td>No</td>
<td>Paracetamol (4 h)/yes</td>
</tr>
<tr>
<td>b 3 h (10 min to 8 h)</td>
<td></td>
<td>Bilat/4/pres/no</td>
<td>−/−/+</td>
<td>No</td>
<td>Paracetamol (8 h)/yes</td>
</tr>
<tr>
<td>6</td>
<td>a 30 min (20 min to 11 h)</td>
<td>Bilat/2/pres/no</td>
<td>−/−/−</td>
<td>No</td>
<td>Paracetamol (2 h)/no</td>
</tr>
<tr>
<td>b 2 h (20 min to 11 h)</td>
<td></td>
<td>Bilat/1/pres/yes</td>
<td>+/−/−</td>
<td>No</td>
<td>Paracetamol (2 h)/no</td>
</tr>
<tr>
<td>7</td>
<td>a 80 min (10 min to 12 h)</td>
<td>Bilat/4/throb/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>b 2 h (10 min to 12 h)</td>
<td></td>
<td>Bilat/2/pres/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>a None</td>
<td>Bilat/4/pres/no</td>
<td>+/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>b 6 h (5–10 h)</td>
<td></td>
<td>Bilat/3/throb/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>a None</td>
<td>Bilat/3/paracetamol/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>b 6 h (4–12 h)</td>
<td></td>
<td>Bilat/3/throb/no</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>a 10 min (10–30 min)</td>
<td>Bilat/1/pres/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>b 9 h (3–5 h)</td>
<td></td>
<td>Bilat/4/throb/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>a 10 min (10 min to 12 h)</td>
<td>Bilat/1/throb/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>b 7 h (10 min to 12 h)</td>
<td></td>
<td>Bilat/2/throb/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>a 40 min (10–60 min)</td>
<td>Bilat/4/throb/yes</td>
<td>+/−/−</td>
<td>Yes (30 min)</td>
<td></td>
</tr>
<tr>
<td>b 4 h (3–12 h)</td>
<td></td>
<td>Bilat/3/pres/yes</td>
<td>−/−/−</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

a = PACAP38 day 0–90 min; b = PACAP38 day 2–12 h; c = placebo day 0–90 min; d = placebo day 2–12 h. Headache characteristics: localisation/intensity/quality (throb = throbbing; pres = pressing)/aggravation by movement. Associated symptoms: nausea/photophobia/phonophobia.
PACAP38 infusion may have compromised blinding, since they could be clearly noticed by both the investigator and subject after infusion of the experimental drug. The adverse events were however caused by the physiologic response to PACAP38 and could not be avoided. Though imperfect, the present double-blind approach is therefore the best possible way of coping with methodological error.

Distribution of PACAP38 relevant for headache

PACAP immunoreactive nerve fibers surrounding cerebral vessels have been demonstrated in the cat (Uddman et al., 1993; Jansen-Olesen et al., 1994) and rat (Edvinsson et al., 2001). PACAP have been identified in human sensory (Tajti et al., 1999), sympathetic

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**Table 3** Headache characteristics after PACAP38 in migraine patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Peak headache (duration)</th>
<th>Headache characteristics</th>
<th>Associated symptoms</th>
<th>Mimics migraine attack (onset)</th>
<th>Treatment (time/efficacy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 min (10–50 min)</td>
<td>Bilat/2/throb/yes</td>
<td>+/-/-</td>
<td>Yes</td>
<td>Yes (20 min)</td>
</tr>
<tr>
<td>b</td>
<td>3 h (3–12 h)</td>
<td>Bilat/2/pres/yes</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>50 min (50 min to 12 h)</td>
<td>Left/1/pres/no</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>12 h (50 min to 12 h)</td>
<td>Left/3/pres/no</td>
<td>+/-/-</td>
<td>Yes</td>
<td>Yes (11 h)</td>
</tr>
<tr>
<td>3</td>
<td>80 min (80 min to 10 h)</td>
<td>Bilat/5/pres/no</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>7 h (80 min to 10 h)</td>
<td>Bilat/7/pres/yes</td>
<td>+/-/-</td>
<td>Yes</td>
<td>Yes (2 h)</td>
</tr>
<tr>
<td>4</td>
<td>70 min (70 min to 12 h)</td>
<td>Left/1/pres/no</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>6 h (70 min to 12 h)</td>
<td>Left/5/pres/yes</td>
<td>+/-/+</td>
<td>Yes</td>
<td>Yes (6 h)</td>
</tr>
<tr>
<td>5</td>
<td>50 min (50–70 min)</td>
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<td>+/-/-</td>
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<td>b</td>
<td>4 h (90 min to 12 h)</td>
<td>Bilat/10/pres/yes</td>
<td>+/-/+</td>
<td>Yes</td>
<td>Yes (3 h)</td>
</tr>
<tr>
<td>7</td>
<td>50 min (50–60 min)</td>
<td>Bilat/1/throb/yes</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>9 h (6–12 h)</td>
<td>Bilat/2/pres/yes</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>10 min (10–30 min)</td>
<td>Bilat/1/throb/no</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>40 min (40 min to 3 h)</td>
<td>Left/1/throb/no</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>2 h (40 min to 3 h)</td>
<td>Left/1/throb/no</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>70 min (20 min to 7 h)</td>
<td>Bilat/2/pres/yes</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>4 h (20 min to 7 h)</td>
<td>Bilat/3/throb/yes</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>60 min (20 min to 12 h)</td>
<td>Bilat/2/pres/yes</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>2 h (20 min to 12 h)</td>
<td>Bilat/1/pres/yes</td>
<td>+/-/-</td>
<td>Yes</td>
<td>Yes (11 h)</td>
</tr>
<tr>
<td>12</td>
<td>40 min (40–50 min)</td>
<td>Bilat/1/pres/no</td>
<td>+/-/-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>b</td>
<td>5 h (4–12 h)</td>
<td>Bilat/4/throb/yes</td>
<td>+/-/+</td>
<td>Yes</td>
<td>Yes (4 h)</td>
</tr>
</tbody>
</table>

*a = PACAP38 day 0–90 min; b = PACAP38 day 2–12 h; c = placebo day 0–90 min; d = placebo day 2–12 h. Headache characteristics: localisation/intensity/quality (throb = throbbing; pres = pressing)/aggravation by movement. Associated symptoms: nausea/photophobia/phonophobia. Migraine-like attack according to criteria described in method section.

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Fig. 2 Individual and mean flow velocities in the middle cerebral arteries (VMCA) assessed by transcranial doppler ultrasonography. Thick lines show mean values. Infusion of PACAP38 (filled square) in migraine patients resulted in a decrease in VMCA during the infusion ($P = 0.001$) and post-infusion ($P = 0.005$) period compared with placebo (open square).
and parasympathetic (Uddman et al., 1999) ganglia and in the trigeminal nucleus caudalis (Uddman et al., 2002). PACAP receptors are found in human smooth muscle cells of cerebral arteries (Knutsson and Edvinsson, 2002), sensory, parasympathetic and in sympathetic ganglia with perivascular nerve fiber projections (Knutsson and Edvinsson, 2002). Hence, PACAP can be defined as a sensory, a parasympathetic and a sympathetic neuropeptide, which can modulate both vessels and nerve fibers through its receptors. The headache induced by PACAP38 could thus originate from several anatomical locations.

**PACAP induced vasodilatation in comparison with VIP**

PACAP38 elicits vasodilatation in both animal (Uddman et al., 1993; Jansen-Olesen et al., 1994; Seki et al., 1995; Dalsgaard et al., 2003; Boni et al., 2008) and human (Jansen-Olesen et al., 2004; Birk et al., 2007) cerebral arteries. Animal studies have suggested that the VPAC₁ receptor is primarily responsible for vasodilatation (Fahrenkrug et al., 2000; Boni et al., 2008), but the precise receptor mechanism of PACAP-induced vasodilatation is not yet fully understood and may thus vary between species and from tissue to tissue.

The mean peak decrease in \( V_{MCA} \) in the healthy volunteers after PACAP38 was, as previously published (Birk et al., 2007), −12.8% (95% CI: −21.2 to −4.4), which is close to the −16.1% (95% CI: −18.2 to −10.6) mean peak decrease found in the migraine sufferers (Table 4). In comparison, infusion of the parasympathetic neuropeptide VIP caused a −14.4% (95% CI: −17.4 to −11.4) (Hansen et al., 2006) decrease in healthy subjects and −16.3% (95% CI: −19.9 to −12.6) (Rahmann et al., 2008) in migraine patients. Thus, it seems that migraine patients are not hypersensitive to the activation of the shared VIP/PACAP VPAC₁ receptor that most likely is responsible for vasodilatation (Fahrenkrug et al., 2000; Boni et al., 2008). Furthermore, maximal vasodilatation in all vessels peaked 20 min after start of infusion and then very slowly declined while the median time to peak headache score in migraine patients occurred at a median of 4.0 h after start of infusion. This indicates that vasodilatation per se is not causative for induction of migraine-like attacks in the post-hospital phase. However, we cannot exclude that a secondary vasodilatation occurred during the post-hospital phase or that the initial and long-lasting vasodilatation induced a cascade of events leading to the delayed headache. Interestingly, VIP infusion did not induce migraine-like attacks (Rahmann et al., 2008) and only mild headache (Hansen et al., 2006). Therefore, the shared VIP/PACAP receptors do not seem to be causative for induction of migraine-like attacks after PACAP38 infusion. Instead the PAC₁ receptor and the physiological processes initiated by its activation might be the primary target for the difference in headache sensitivity after VIP and PACAP38 infusion.

**PACAP induced headache**

Both healthy subjects and migraine patients in the present study appeared to experience headache with almost the same intensity (Fig. 1). However, the main difference between the healthy group and the migraine group was that no healthy subjects experienced delayed migraine-like attacks in the post-hospital phase after PACAP38 compared to six migraine patients. Obviously, the headache experienced by healthy subjects cannot mimic a usual migraine attack and they do not use triptans. Still, by excluding the two migraine patients, who only described the headache as mimicking usual migraine attack and treated it with a triptan, it appears that PACAP38 induced more migraine-like attacks in the

![Fig. 3 Hemodynamic and respiratory changes from baseline in migraine patients after PACAP38 in comparison with median headache score on a VRS.](http://brain.oxfordjournals.org/)

**Table 4 Mean peak hemodynamic variables 0–90 min after PACAP38 infusion in healthy subjects and migraine patients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Peak time after onset of PACAP38 (min)</th>
<th>Mean change from baseline after PACAP38 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy subjects</td>
<td>Migraine patients</td>
</tr>
<tr>
<td>MCA diameter</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Velocity in MCA</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>STA diameter</td>
<td>nm</td>
<td>30</td>
</tr>
<tr>
<td>Heart rate</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

MCA = middle cerebral artery; STA = superficial temporal artery; nm = not measured in study. Data from healthy subjects have previously been published elsewhere (Birk et al., 2007).
post-hospital phase among migraine patients compared to healthy subjects (4 versus 0 attacks).

The peak median headache score after PACAP38 was 2.5, which is less than compared to other neuropeptides known to induce migraine attacks (Table 6). This difference may be attributed to a more frequent intake of triptans compared to previous studies (Thomsen, 1994; Lassen et al., 2002). Hence, Lassen et al. reported two migrainers taking sumatriptan, while the study by Thomsen et al. took place before triptans were widely available. Interestingly, all studies report a similar median time to peak headache score, which indicate that the neuropeptides all induce changes, which take hours to manifest. The migraine patients developed migraine-like attacks after a mean of 6 h after PACAP38. Given that the plasma half-life of PACAP38 is only 3.5 min (Birk et al., 2007), it seems most likely that PACAP38 acts as a initiator of a cascade of events that eventually lead to a migraine-like attack. In the following we would like to discuss the possible cascade of events.

### Possible mechanisms behind PACAP38 induced migraine-like attacks

In principle, at least three different mechanisms could be involved in PACAP38’s induction of migraine-like attacks: (i) Sensitization of peripheral sensory trigeminal fibers; (ii) mast cell degranulation secondarily causing activation of peripheral sensory trigeminal fibers; (iii) facilitation of pain by direct sensitization of central, second order trigeminal neurons.

Activation of VPAC and PAC1 receptors elevates cellular cyclic adenosine monophosphate (cAMP) (Dickson et al., 2006). Calcitonin gene-related peptide (CGRP) and cilostazol also increase cAMP and cause a delayed headache that develops hours after

---

**Table 5** Number of healthy subjects and migraine patients reporting adverse events after PACAP38 and placebo infusion from 0 to 90 min

<table>
<thead>
<tr>
<th>Healthy subjects</th>
<th>Placebo</th>
<th>PACAP38</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms (0–90 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat sensation</td>
<td>4</td>
<td>11</td>
<td>0.016</td>
</tr>
<tr>
<td>Palpitation</td>
<td>0</td>
<td>11</td>
<td>0.001</td>
</tr>
<tr>
<td>Flushing</td>
<td>0</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nausea</td>
<td>1</td>
<td>5</td>
<td>0.0125</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>10</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Migraine patients</th>
<th>Placebo</th>
<th>PACAP38</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms (0–90 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat sensation</td>
<td>0</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Palpitation</td>
<td>1</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>Flushing</td>
<td>1</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>2</td>
<td>0.500</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>11</td>
<td>0.002</td>
</tr>
</tbody>
</table>

PACAP38 and placebo compared with McNemar test.

**Table 6** Median peak headache score and median time to peak headache score of migraine patients in the present study using PACAP38 and previous studies of GTN and CGRP (Lassen et al., 2002; Thomsen, 1994)

<table>
<thead>
<tr>
<th></th>
<th>Median peak headache score</th>
<th>Median time (h) to peak headache score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PACAP38</td>
<td>2.5 (0–10)</td>
<td>4 (0–12)</td>
</tr>
<tr>
<td>GTN</td>
<td>5.5 (0–10)</td>
<td>5.5 (3–10)</td>
</tr>
<tr>
<td>CGRP</td>
<td>4 (1–6)</td>
<td>5 (1–12)</td>
</tr>
</tbody>
</table>
the experimental drugs are administrated (Lassen et al., 2002; Birk et al., 2006). Animal models in both rat and guinea pig (Ingram and Williams, 1996) have shown that trigeminal neurons can be sensitized through elevation of cAMP. Interestingly, approximately half of healthy subjects and migraine patients in the present study reported a throbbing headache quality, which has been related to sensitization of meningeal nociceptors (Strassman et al., 1996; Burstyn et al., 1998). At present we do not know if activation of VPAC or PAC1 receptors can actually mediate sensitization of trigeminal neurons. PACAP38 has been reported to stimulate adenylyl cyclase activity at least 1000 times greater than that of VIP in cultured neural cells (Miyata et al., 1989). Furthermore VIP only induces a minimal headache and no migraine-like headache in migrainers (Hansen et al., 2006; Rahmann et al., 2008). It is possible that the VPAC receptors, in contrast to the PACAP selective PAC1 receptor, are not involved in sensitization of trigeminal neurons.

Degranulation of mast cells may be involved in migraine pathophysiology (Theoharides et al., 2005). Mast cells surround cerebral (Edvinsson et al., 1976) and dural vessels (Ottosson and Edvinsson, 1997; Rozniecki et al., 1999) in close apposition to both parasympathetic and sensory nerve fibers (Rozniecki et al., 1999). Furthermore, mast cell degranulation caused neuronal activation in C-fibers innervating the dura mater (Levy et al., 2007). PACAP and VIP injected intradermally in healthy subjects caused a rapid flare, which became erythematous after 5 min (Warren et al., 1992). Maximum increase in skin blood flow occurred 15 min after PACAP injection and the increase lasted approximately 6h compared to only 2h after VIP injection. Furthermore, VIP only induces a 10% increase in degranulation of histamine from dural mast cells. PACAP38 has a higher uptake rate into the brain compared to VIP (Dogrukol-Ak et al., 2004) and CNS represents a relatively protected space for PACAP38 in comparison with its degradation in blood (Dogrukol-Ak et al., 2004). If headache/migraine-like attack is caused by noiception within the brain, these pharmacokinetic findings could explain why PACAP38 might be more potent than VIP in causing headache/migraine-like attack either directly or via mast cell degranulation. It would be highly relevant to compare the potency of PACAP and VIP in degranulating mast cells in future experimental models.

Experimental animal models have proposed that PACAP might have a role in central pain transmission (Hashimoto et al., 2006). Capsaicin can elevate PACAP in cerebrospinal fluid (Zhang et al., 1997) suggesting that PACAP might be released from activated C-fibers in the spinal cord. Furthermore, the PAC1 receptor antagonist, PACAP 6-38, effectively attenuates nociception in animal models of chronic inflammatory and persistent pain (Davis-Taber et al., 2008; Ohsawa et al., 2002) after intrathecal administration. In PACAP— gene knockout mice inflammatory pain disappears (Mabuchi et al., 2004), and PACAP38 promotes late-onset, transcriptional-dependent, activity dependent central sensitization (Ji et al., 2003; Mabuchi et al., 2004), which takes hours to manifest and lasts for prolonged hours or days. Based on these data, it would be plausible to suggest that exogenously administrated PACAP38 could have a facilitatory effect on second-order trigeminal neurons contributing to headache/migraine.

**Conclusion**

PACAP38 induced both headache and migraine-like attacks. Dilatation of cephalic arteries seems unlikely to be the direct cause of the migraine-like attacks occurring in the delayed phase after PACAP38 infusion. Possible mechanisms of PACAP38 induction of headache and migraine-like attacks are peripheral sensitization of trigeminal sensory neurons, mast cell degranulation secondarily leading to activation of peripheral sensory trigeminal fibers and facilitation of pain by sensitization of central second order trigeminal neurons. Regardless of this, the effect is probably mediated by PAC1 receptor activation. Therefore a PAC1 receptor antagonist might be a future target for the treatment of migraine.

**Acknowledgements**

The authors wish to thank lab technicians Winnie Grønning Nielsen, Kirsten Brunsgaard and Lene Elkjær for their excellent and dedicated assistance. The study was supported by the Mauritsen La Fontaine Foundation, the Cool Sorption Foundation and the Lundbeck Foundation via the Lundbeck Foundation Center for Neurovascular Signalling (LUCENS).

**References**


The PACAP Receptor: A Novel Target for Migraine Treatment

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Summary: The origin of migraine pain has not yet been clarified, but accumulating data point to neuropeptides present in the perivascular space of cranial vessels as important mediators of nociceptive input during migraine attacks. Pituitary adenylate cyclase-activating polypeptide (PACAP) is present in sensory trigeminal neurons and may modulate nociception at different levels of the nervous system. Human experimental studies have shown that PACAP-38 infusion induces marked dilatation of extracerebral vessels and delayed migraine-like attacks in migraine patients. PACAP selectively activates the PAC1 receptor, which suggests a possible signaling pathway implicated in migraine pain. This review summarizes the current evidence supporting the involvement of PACAP in migraine pathophysiology and the PAC1 receptor as a possible novel target for migraine treatment. Key Words: Migraine, vasodilatation, mast-cell degranulation, trigeminal nociceptive system, human experimental headache models, drug targets.

INTRODUCTION

Migraine patients experience intense head pain during attacks, which results in disability and high socioeconomic costs. Thus, clinical research to discover new specific drug targets for migraine is highly needed. The origin of pain during migraine attacks is still not fully elucidated. Activation of peripheral trigeminal nociceptors in the perivascular space of cranial arteries probably generates input that leads to the experience of migraine pain. In support of this view, it has been shown that signaling molecules, such as nitric oxide (NO) and calcitonin gene-related peptide (CGRP), found in nerve fibers surrounding cranial arteries, induce migraine-like attacks indistinguishable from spontaneous migraine attacks. Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide present in the perivascular space of cranial arteries. Recent studies point to the involvement of PACAP in migraine pain. Here we review the evidence on how PACAP might be implicated in specific receptor activation during migraine attacks and discuss how the PAC1 receptor could be a novel target for migraine treatment.

PACAP STRUCTURE, DISTRIBUTION, AND RECEPTORS

PACAP was originally isolated in the late 1980s from an ovine hypothalamus extract on the basis of its ability to stimulate cAMP formation in rat pituitary cells. PACAP belongs to the vasoactive intestinal polypeptide (VIP)–secretin–growth hormone–glucagon superfamily and is found as a 38-amino-acid peptide (PACAP-38) and a truncated 27-amino-acid peptide (PACAP-27). PACAP-38 is the predominant peptide and represents more than 90% of the total PACAP content in most tissues, including the CNS. PACAP has been identified in human sensory and parasympathetic ganglia, as well as in second-order neurons of the trigeminal nucleus caudalis (TNC). The N-terminal 28 amino acids of PACAP-38 share 68% homology with VIP, and the two related peptides are colocalized in rat parasympathetic ganglia.

PACAP is a neuropeptide present in the perivascular space of cranial arteries. Recent studies point to the involvement of PACAP in migraine pain. Here we review the evidence on how PACAP might be implicated in specific receptor activation during migraine attacks and discuss how the PAC1 receptor could be a novel target for migraine treatment.

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sory, parasympathetic, and sympathetic ganglia with perivascular nerve fiber projections. 24

In this review we will describe the possible role of PACAP in nociceptive processing in the peripheral and central nervous system relevant for migraine pain.

The peripheral actions of PACAP

Stimulation of the superior sagittal sinus causes a 2.6-fold increase in PACAP plasma concentrations in the external jugular vein in cats. 25 Whether this is caused by PACAP released from trigeminal sensory or parasympathetic perivascular fibers is unknown. Human experimental studies suggest that parasympathetic activation is pronociceptive, in that migraine pain is reduced after anesthetic blocking of the parasympathetic sphenopalatine ganglion. 26, 27 Because parasympathetic and trigeminal fibers are closely related in the perivascular space, 28 it is possible that PACAP released from either system could lead to modulation of sensory input in trigeminal neurons.

Dilatation of cranial vessels might contribute to pain during migraine attacks. 29, 30 PACAP-38 dilates both animal 31–33 and human 34 cerebral arteries; however, only VPAC 1 receptor antagonists inhibit PACAP-38-induced dilation in the rat middle cerebral artery (MCA) 35 and middle meningeal artery (MMA). 33 In the human coronary artery, PACAP-induced dilatation is not changed by PAC 1 receptor antagonism. 36 These data indicate that activation of the PAC 1 receptor does not contribute to extracranial or intracranial vasodilatation.

During recent years, mast cell degranulation has been suggested to be involved in migraine pathophysiology. 37, 38 The evidence is based primarily on studies showing that plasma histamine levels are elevated during migraine attacks in a subpopulation of migraine patients, 39 and that histamine induces migraine-like attacks following intravenous infusion. 40 Furthermore, mast cell degranulation causes activation of meningeal nociceptors in the rat dura mater. 41, 42 VPAC 2 receptors, but not VPAC 1, are expressed on human mast cells; 43 to date, no studies have investigated the expression of PAC 1 receptors on mast cells. In human skin, PACAP-38 and VIP degranulate mast cells and cause histamine release in vitro. 44 VIP seems to be the more potent than PACAP-38 in degranulating mast cells in vitro. 44 Furthermore, VIP releases a relatively small proportion (10%) of histamine from human dural mast cells, compared with CGRP (10% vs 32%). 45 Thus, it seems unlikely (although it remains to be investigated) that PACAP can induce sufficient mast cell degranulation in the perivascular space of cranial arteries to result in nociceptive input.

Stimulation of both VPAC 1,2 and PAC 1 receptors elevates cAMP. 46 but in cultured neural cells PACAP-38 stimulates adenylate cyclase activity at least 1000 times more than VIP does. 11 Thus, it is possible that PACAP via the PAC 1 receptor could elevate cAMP in peripheral trigeminal nociceptors, resulting in nociception. In fact, animal models in both rat 47 and guinea pig 48 have shown trigeminal neurons to be sensitized through elevation of cAMP. Recently, Akerman and Goadsby 49 also showed that VPAC 1 and PAC 1 receptor inhibition blocked neuronal firing of second-order trigeminal neurons elicited by activation of the parasympathetic superior salivatory nucleus projecting to the perivascular space. Nonetheless, it has not yet been directly demonstrated if VPAC or

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**FIG. 1.** Highlight of the principal intracellular signaling pathways activated by VPAC 1, VPAC 2, and PAC 1 receptors. Upon activation, all three receptors are capable of causing downstream production of cAMP. In addition, the three receptors can also activate phospholipase C (PLC), leading to an increase in Ca ++. AC = adenylate cyclase; CaM = calmodulin; DAG = diacylglycerol; ER = endoplasmic reticulum; Gαq/11 = Gαq, G-family proteins; Ins(1,4,5)P 3 = inositol 1,4,5-trisphosphate; PACAP = pituitary adenylate cyclase-activating polypeptide; PIP 2 = phosphatidylinositol 3,4-bisphosphate; PKC = protein kinase C; VIP = vasoactive intestinal polypeptide; α, β, γ = subtypes of G-protein. Reproduced from Dickinson et al.,23 with permission from Elsevier.
PAC1 receptors mediate activation or sensitization of trigeminal nociceptors.

The central actions of PACAP

PACAP immunoreactivity is present in the human TNC, but is also found in cell bodies of the brain stem locus coeruleus, which projects to the TNC and is reported to be activated during spontaneous migraine attacks. Animal experimental models have proposed that PACAP might have a role in central pain transmission. Capsaicin elevates PACAP in rat cerebrospinal fluid in vivo, suggesting that PACAP may be released from activated C-fibers in the spinal cord. In PACAP gene knockout mice, inflammatory pain disappears and PACAP promotes the functional coupling of neuronal NO-synthase to NMDA receptors. This leads to NO production in superficial layers of the dorsal horn in the spinal cord and late-onset, transcriptional- and activity-dependent central sensitization. PAC1 receptor knockout mice have a decreased response in nociceptive behavior after a formalin test, which is a model of inflammatory nociception. PAC1 receptor antagonism also effectively attenuated nociception in inflammatory models of pain in rats and mice after intrathecal administration. These data suggest that activation of the PAC1 receptor could lead to modulation of nociceptive input in the second-order neurons.

HUMAN PACAP MODEL OF MIGRAINE

The headache eliciting and vasodilatory effect in cranial arteries after infusion of PACAP-38 was examined in 12 healthy volunteers and 12 patients with migraine without aura in a randomized double-blind crossover study. PACAP-38 infusion caused headache in all 12 healthy subjects and in 11 of the 12 migraine patients. The headache peaked 4–5 hours after the end of infusion (FIG. 2). The most important finding of the study was that 58% of the migraine patients experienced migraine-like attacks after PACAP-38 infusion; most attacks occurred several hours after the end of infusion. PACAP-38 also induced pronounced dilatation of intra- and

![FIG. 2. Individual and median headache scores on a verbal rating scale (VRS) from time 0–90 minutes and 2–12 hours after PACAP-38 infusion in healthy subjects (top panel) and migraine patients (bottom panel). Note break in scale for time axis. Thick red lines indicate median headache scores. Double-ended arrows mark infusion time. Adapted from Schytz et al. with permission.](image-url)
extracranial arteries, with maximum at 20 minutes after the start of infusion, which remained sustained throughout the 90-minute recording period (FIG. 3).

VIP, given in exactly the same quantity as PACAP-38 (200 pmol/kg), has been studied in similar studies.60,61 These studies showed that the systemic administration of VIP induces only a very mild and short-lasting immediate headache in both healthy subjects60 and migraineurs.61 Despite marked immediate vasodilatation, no migraine sufferer reported delayed migraine-like attacks after VIP. Given that VIP infusion does not cause migraine, the shared VPAC1 and VPAC2 receptors seem unlikely to be involved in PACAP-38-induced migraine. Thus, migraine induction by PACAP-38 might be caused by selective activation of the PAC1 receptor.

A recent study attempted to explore pronociceptive properties of PACAP-38 and VIP in humans, using a skin model of acute pain.62 Pain intensities after VIP and PACAP-38 were mild and limited to a short time, approximately 100 seconds after injection. VIP caused more neurogenic inflammation and mast cell degranulation than did PACAP-38, as reflected in changes in blood skin flow and wheal. Thus, flow and wheal are mediated via the VPAC receptors.

**HOW MIGHT PACAP-38 INDUCE MIGRAINE?**

At present, there is no firm evidence implicating the PAC1 receptor in migraine pathophysiology, and no PAC1 receptor antagonist is available for human use to test this hypothesis. Nonetheless, the ability of PACAP-38 to induce migraine, in contrast to VIP, strongly points to PAC1 receptor activation as a possible mediator of migraine. Experimental data noted in this review suggest that vasodilatation,10,33,35,36 mast cell degranulation,44,62 and neurogenic inflammation62 are induced by the VPAC receptors and therefore do not seem important in PACAP-38-induced migraine. Instead, PACAP-38 might modulate the PAC1 receptors at the second-order trigeminal neurons. After intravenous PACAP-38 administration, however, only 0.053% passes the blood–brain barrier (BBB) after 5 minutes via a saturable mechanism in mice.63 This suggests that activation of PAC1 receptors within the BBB is unlikely to mediate PACAP-38-induced migraine.

The most likely explanation for migraine development after administration of PACAP-38 seems to be modulation of dural or extracranial trigeminal nociceptors outside of the BBB. Thus, intracellular cAMP increase in dural nociceptors following PAC1 activation could be a necessary link in the cascade of events that leads to migraine development. Indeed, the headache-inducing effect of cilostazol, which is known to increase intracellular cAMP, has been tested; 92% of the healthy subjects developed headache, including 18% who had migraine-like features, such as pulsating pain quality and aggravation by physical activity.64 PACAP-38 and CGRP share the cAMP intracellular signaling pathway, and CGRP also does not pass the BBB freely.55 Intravenous infusion of CGRP induces migraine-like attacks occurring several hours after the end of the infusion, just as PACAP-38 induced migraine-like attacks, and the CGRP receptor antagonist telcagepant is effective for the acute treatment of migraine.66 Even though PACAP-38 most likely induces migraine through peripheral modulation, it is possible that a PAC1 receptor antagonist permeable to
the BBB would have a synergetic dual effect at both first-order and second-order trigeminal neurons. A PAC1 receptor antagonist is likely to be devoid of vascular side effects, which would be beneficial to migraine patients with ischemic vascular comorbidity.

Future studies should elucidate the possible pronociceptive effects of the PAC1 receptor. The development of a PAC1 receptor antagonist that can be tested in human clinical research would be extremely beneficial for our understanding of migraine mechanisms and possibilities for new treatments.

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