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Premonitory and nonheadache symptoms induced by CGRP and PACAP38 in patients with migraine

Song Guo, Anne L.H. Vollesen, Jes Olesen, Messoud Ashina*

Abstract

Migraine attacks are often preceded by premonitory symptoms (PS) that may be triggered pharmacologically. We investigated the incidence of PS after administration of calcitonin gene-related peptide (CGRP) or pituitary adenylate cyclase-activating peptide-38 (PACAP38) in patients with migraine without aura (MO) who reported and did not report migraine-like attacks induced by these pharmacological triggers. In addition, we investigated the association between PS and familial predisposition for migraine. In our study, MO patients received continuous intravenous infusion of α-CGRP (n = 40) and PACAP38 (n = 32) for 20 minutes. Premonitory and nonheadache symptoms were recorded by a self-administered questionnaire. Information on familial predisposition was obtained by telephone interview of first-degree relatives using a validated semistructured questionnaire. Twenty-five of 40 patients (63%) developed a migraine-like attack after CGRP infusion and 23 of 32 patients (72%) developed an attack after PACAP38 infusion. Only 2 patients (9%) with a CGRP-induced migraine-like attack reported PS, whereas 11 patients (48%) reported PS after PACAP38. Patients who developed a migraine-like attack did not report more PS than did patients with no attack after CGRP ($P = 0.519$) or PACAP38 ($P = 0.103$). Additionally, we found no difference in PS between patients with familial predisposition of migraine (75%) and patients with no family predisposition (56%) ($P = 0.101$). In conclusion, CGRP did not induce PS, whereas PACAP38 induced PS in 48% of patients. However, CGRP and PACAP38 did not induce more PS in patients who developed an attack compared with those who did *not* develop an attack.

Keywords: Migraine, Premonitory, Calcitonin gene-related peptide, Pituitary adenylate cyclase-activating peptide-38, Familial

1. Introduction

Migraine is a complex disorder characterized by multiphasic events including an initial premonitory phase with premonitory symptoms (PS) reported by 7% to 88% of patients with migraine.[6,18,30,38,46] According to the International Classification of Headache Disorders (ICHD-3β) PS occur hours to a day or 2 before a migraine attack with or without aura (The International Classification of Headache Disorders, third edition [beta version], 2013). One prospective electronic diary study reported that PS reliably occurred in more than 82% of selected patients, who reported having PS[21] and that patients were able to correctly predict their migraine attacks in 72% of the cases when experiencing PS. The most common PS in these patients were unusual fatigue, neck stiffness, yawning, mood swings, hunger, and poor concentration, which may suggest activation of deep brain structures in migraine[21,32] involving the neurotransmitter dopamine[3,14] and the hypothalamus.[5] Furthermore, intravenous infusion of glyceryl trinitrate (GTN), a pharmacological trigger of migraine, was reported to induce PS in patients with migraine and showed activation of the hypothalamus.[1,31] Because PS are the first symptoms before a migraine attack, investigating the underlying mechanisms of PS is crucial to elucidate how a migraine attack begins. Additionally, it represents a window of opportunity for early acute migraine therapies. Further understanding of the pathophysiology of the premonitory phase is therefore highly warranted. Calcitonin gene-related peptide (CGRP) and pituitary adenylate cyclase-activating peptide-38 (PACAP38) are pharmacological triggers used to study migraine pathophysiology,[29,39] and to date, no studies have investigated the effects of these migraine triggers on PS. We hypothesized that CGRP or PACAP38 induces PS.

In this study, we (1) investigated the incidence of PS after intravenous infusion of CGRP or PACAP38 in patients with migraine without aura (MO) and (2) compared the incidence of nonheadache symptoms in patients who reported migraine-like attacks after CGRP or PACAP38 with the incidence of these symptoms in patients who did not develop attack. In addition, we investigated the association between PS and familial predisposition for migraine.

2. Methods

The data for this study were collected during 2 recently published studies in which we examined the association between incidence of PACAP38 and CGRP-induced migraine-like attacks and familial predisposition (family load) of migraine.[22,24] Because we had the family history of the patients, we investigated the association between PS and family load in this study.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Danish Headache Center, Department of Neurology, Rigshospitalet, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

**Corresponding author. Address: Danish Headache Center and Department of Neurology, Rigshospitalet, Faculty of Health and Medical Sciences, University of Copenhagen, Nordre Ringvej 57, 2600 Glostrup, Copenhagen, Denmark. Tel.: (+45) 38 63 33 85. E-mail address: ashina@dadlnet.dk (M. Ashina).*

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The patients were recruited from a cohort of 1010 unrelated MO patients from the Danish Headache Center who were genotyped for the 12 identified single-nucleotide polymorphisms associated with migraine.[9] Diagnosis of MO was obtained by telephone interview based on a validated semistructured questionnaire[19,20] according to the latest International Classification of Headache Disorders (ICHD-3β) (The International Classification of Headache Disorders, third edition [beta version], 2013). Data on the history of migraine in the patient's first-degree relatives (parents, siblings, and children) were also obtained by telephone interview after the proband had completed the study. Patients identified with ≥2 first-degree relatives with migraine were defined as having a high family load, whereas patients identified with ≤1 first-degree relative with migraine were defined as having a low family load.

2.1. Design

In total, 72 MO patients received 20 minutes of continuous intravenous infusion of 1.5 μg/min human α-CGRP (n = 40) or 10 pmol·kg^{-1}·min^{-1} human PACAP38 (n = 32). The doses were used as in previous provocation experiments.[8,12,25,26,39] α-CGRP has a molecular weight of 3789.3 g/mol and was purchased from Calbiochem, Merck4Biosciences, Darmstadt, Germany, whereas PACAP38 has a molecular weight of 4534.3 g/mol and was purchased from Bachem AG, Bubendorf, Switzerland. Both the examiners and the MO patients were blinded to family load of the patients.

To be included in the study, women of childbearing potential had to report using adequate contraception. Exclusion criteria were any other type of headache (except episodic tension-type headache ≤8 days per month); intake of any daily medication over the last 10 days (including migraine preventives) serious somatic or psychiatric diseases. A full medical examination and electrocardiography were performed on the day of the study. We informed patients that CGRP or PACAP38 might induce headache in some individuals, but the timing or the characteristics of headache was not discussed.

The study was approved by the Regional Committee on Health Research Ethics of Copenhagen (H-2-2011-141 and H-2-2013-033) and the Danish Data Protection Agency, and was conducted according to the Helsinki II declaration of 1964, as revised in 2008. The study was also registered at ClinicalTrials.gov (NCT01924052 and NCT01841827). All participants gave their written informed consent before inclusion.

2.2. Experimental protocol

All patients arrived in a nonfasting state at the clinic between 8:30 and 10:30 AM. They had to be without any kind of headache or intake of analgesics 48 hours before the study day. A venous catheter (Venflon) was inserted into an antecubital vein for the administration of CGRP or PACAP38. During the study, patients were in the supine position in quiet surroundings. After 15 minutes of rest, we collected data on nonheadache symptoms during baseline (at 10 minutes before start of infusion, T_{-10}, and at the time of infusion start, T_0) and started the infusion using an infusion pump (Braun Perfusor, Melsungen, Germany).

2.3. Premonitory and nonheadache symptoms

Based on previous provocation studies of PS using GTN,[1,31] pharmacologically triggered PS was defined as "nonheadache symptoms before the onset of headache." Thus, patients who develop a headache not fulfilling the criteria of a migraine-like attack after CGRP or PACAP38 can have PS. Accordingly, nonheadache symptoms reported during or after the headache phase in patients who developed a migraine-like attack did not fulfill our PS criteria. Therefore, we defined 3 phases (premonitory, headache, and postdrome) in which the incidence of premonitory or nonheadache symptoms was investigated (**Fig. 1**). The premonitory phase occurs before the onset of headache; the headache phase occurs during headache including migraine; and the postdrome phase occurs after the end of headache.

The following nonheadache symptoms were recorded using a questionnaire: fatigue (unusual tiredness), yawning, stiff neck, hunger (food craving), poor concentration, mood swings (depression/irritable/emotional), nausea, photophobia, and phonophobia. These symptoms were chosen because a prospective electronic diary study in a selected group of patients with PS showed that they were the most common PS.[21] During this study, we recorded the nonheadache symptoms at T_{-10} and then every 10 minutes up to 120 minutes after the start of infusion. After discharge from the hospital study setting, the patients were carefully instructed to continue recording these nonheadache symptoms using a self-administered questionnaire every hour until 12 hours after the start of infusion or until they went to bed.

In addition, we also applied the strict International Headache Society (IHS) definition for a PS: "Nonheadache symptoms preceding and forewarning of a migraine attack by 2 to 48 hours, occurring before the onset of pain in migraine without aura."[44]

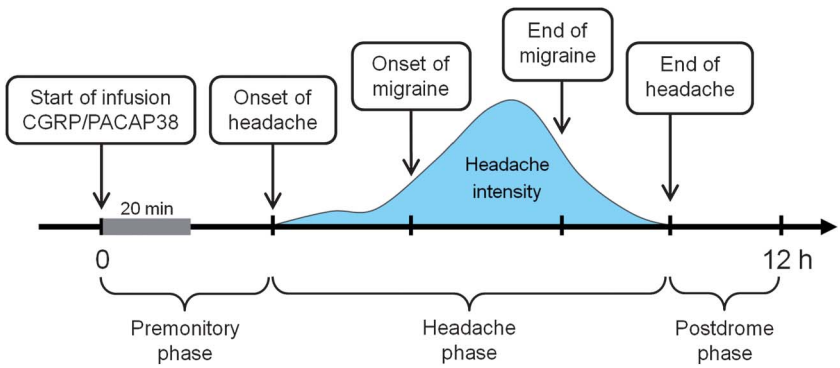

Figure 1. Experimental day (0-12 hours) was divided into 3 phases: premonitory phase (before onset of headache), headache phase (between onsets of headache to the end of headache), and postdrome phase (from headache resolution). The headache phase includes migraine for those patients who developed a migraine-like attack after PACAP38 or CGRP infusion, which was given for 20 minutes.

This definition is different from our definition of pharmacologically triggered PS mentioned earlier.

2.4. Migraine-like attacks and questionnaire

We recorded migraine-like attacks using a validated questionnaire.[27] After discharge from the study hospital setting, the patients were carefully instructed to continue recording their headache by a self-administered questionnaire every hour until 12 hours after the start of infusion or until they went to bed. This self-administered questionnaire was identical to the one used by the investigators during in-hospital phase of the study and measured headache characteristics and associated symptoms according to the IHS criteria (The International Classification of Headache Disorders, third edition [beta version], 2013). The questionnaire also included questions concerning adverse events and asked patients whether the reported headache mimicked their spontaneous migraine attacks. The patients were allowed to take their usual acute migraine medication at any time during the in- and out-hospital phase.

2.5. Statistical analysis

Clinical characteristics are presented as mean and range. Because this study used data from 2 previous studies, no power calculation of sample size were performed.

Primary endpoint: We tested the difference in incidence of nonheadache symptoms (fatigue, yawning, stiff neck, hunger, poor concentration, mood swings, nausea, photophobia, and phonophobia) between 2 groups of patients (reported migraine attacks vs reported *no* migraine attacks) after CGRP or PACAP38 during the predefined phases and for the entire study period (0–12 hours). Tests were applied for CGRP and PACAP38 as separate groups.

Secondary endpoints: We investigated the difference in incidence of nonheadache symptoms between patients with migraine with high family load vs patients with low family load. The incidences of nonheadache symptoms were analyzed as categorical data with the Fisher exact test.

We also tested the difference in area under the curve (AUC) for headache intensity over the 12-hour observation period for patients who *did* and did *not* develop a migraine attack after CGRP or PACAP38. We calculated AUC according to the trapezium rule[33] to obtain a summary measure to analyze the differences in response between the 2 groups of patients. Differences in AUC for headache intensity scores were tested using the nonparametric Mann–Whitney *U* test.

The *P* values were not adjusted for multiple testing. All analyses were performed with SPSS Statistics version 19 for Windows (Chicago, IL). $P < 0.05$ was considered the level of significance.

3. Results

We collected data on nonheadache symptoms from 40 patients who received CGRP and 32 patients who received PACAP38 infusion. Migraine responses after PACAP38 and CGRP infusion and family history have been reported in 2 previously published studies.[22,24] Briefly, 23 of 32 (72%) patients developed a migraine-like attack after PACAP38 infusion and 25 of 40 (63%) patients developed a migraine-like attack after CGRP. Clinical characteristics of the patients with migraine are shown in **Table 1**.

There was a significant difference in the AUC for headache intensity over the 12-hour observation period for patients who did and did not develop a migraine attack after CGRP or PACAP38 (**Fig. 2**). We found no differences in headache localization (frontal, temporal, occipital, and vertex) between the 2 groups for both CGRP and PACAP38 ($P > 0.05$).

3.1. Premonitory symptoms and nonheadache symptoms induced by CGRP

We found no statistical difference in incidence of any nonheadache symptoms during the premonitory, headache, or postdrome phase after CGRP between the 2 groups (attack vs *no* attack) (**Fig. 3**). In the premonitory phase, only 2 of 25 patients (9%) reported nonheadache symptoms before CGRP-induced migraine-like attacks, which fulfilled our criteria of PS, whereas among those who did not develop a migraine-like attack, none reported PS ($P = 0.519$). None of the patients in this study reported nonheadache symptoms that fulfilled the strict IHS definition of PS because all symptoms were reported less than 2 hours before onset of headache. Because some patients still had headache at the end of the study period, postdrome phase was only registered in 11 patients who developed an attack and in 9 patients who did not develop an attack.

For the entire recording period of the study (0–12 hours), patients who developed a migraine-like attack after CGRP reported significantly more nausea ($P = 0.008$), photophobia ($P = 0.003$), and phonophobia ($P = 0.001$) than did patients who did not report attacks (**Table 2**). We found no difference in other nonheadache symptoms between these 2 groups. Fatigue

Table 1

Clinical characteristics of the patients with migraine who did and did not report a migraine-like attack after CGRP or PACAP38.

	CGRP (n = 40)			PACAP38 (n = 32)		
	Attacks (n = 25)	No attacks (n = 15)	*P*	Attacks (n = 23)	No attacks (n = 9)	*P*
Females/males	24/1	12/3	0.139*	20/3	6/3	0.314*
Age in years, mean (range)	46 (27-61)	44 (19-65)	0.621†	47 (25-59)	50 (32-60)	0.462†
Weight in kg, mean (range)	70 (50-90)	68 (53-91)	0.502†	74 (54-94)	67 (53-85)	0.184†
Migraine attack frequency in d/mo, median (range)	5 (1-9)	3 (1-8)	0.219‡	3 (1-8)	1 (1-5)	0.139‡
Participants on prophylactic migraine medication (% and n)	40 (10)	33 (5)	0.746*	43 (10)	56 (5)	0.699*

* *P* value: Fisher exact test.
† *P* value: unpaired *t* test.
‡ *P* value: Mann–Whitney *U* test.

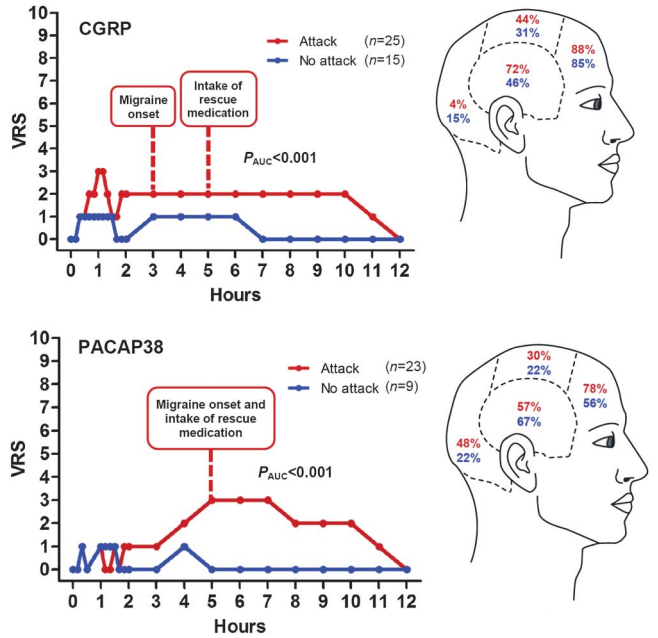

Figure 2. Median headache intensity by the verbal rating scale (VRS 0-10) and headache localization for patients who *did* (●/red %) and *did not* (●/blue %) report migraine attacks after CGRP or PACAP38 over registration period (0–12 hours). There was a significant difference in the AUC between the 2 groups for both peptides ($AUC_{0\text{-}12\,h}$: $P < 0.001$). Median time to onset of migraine attacks was 3 hours (range 0.17–12 hours) after CGRP and 5 hours after PACAP38 (range 2–10 hours). Median time to intake of rescue medication was 5 hours for both CGRP (range 0.5–12 hours) and PACAP38 (range 2–10 hours). For both peptides, the median time to onset and end of headache was 0.33 and 12 hours, respectively.

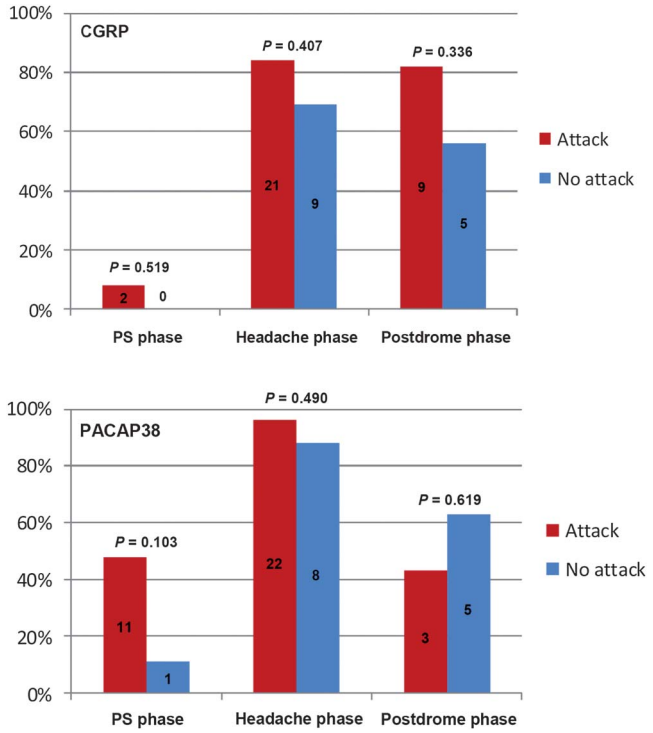

Figure 3. Incidence (%) of any nonheadache symptoms during the premonitory (PS), headache, and postdrome phase after CGRP (attack: n = 25, no attack: n = 13) or PACAP38 (attack: n = 23, no attack: n = 9). Patients who did not develop a headache nor had a postdrome phase after CGRP or PACAP38 were not included in the figure. Migraine associated nonheadache symptoms (nausea, photophobia, and phonophobia) were excluded during the headache phase. The number of patients is shown in the columns.

(84%), photophobia (72%), and nausea (68%) were the most common nonheadache symptoms reported by patients with attacks after CGRP.

Figure 4 displays more detailed descriptions of nonheadache symptoms reported by patients receiving CGRP. Information provided in the table includes the occurrence of nonheadache symptoms reported by each patient during the 3 phases, the onset of head pain or migraine attack, and the usual PS of the patients before their spontaneous migraine-like attacks.

3.2. Premonitory symptoms and nonheadache symptoms induced by PACAP38

We found no difference in the incidence of any nonheadache symptoms during the premonitory, headache, or postdrome phase after PACAP38 between the 2 groups (attack vs *no* attack) (**Fig. 3**). In the premonitory phase, 11 of 23 patients (48%) reported PS symptoms according to our definition before PACAP38 induced migraine-like attacks, whereas only one patient (11%) reported PS among those who did *not* develop a migraine attack ($P = 0.103$). Seven of 11 patients with induced PS experienced PS before their spontaneous attacks. However, we found no correlation between the induced PS and the usual PS of the patients (supplementary material, available online at http://links.lww.com/PAIN/A337). Two patients reported a PS that fulfilled the IHS definition as they reported nonheadache symptoms at least 2 hours before the onset of headache. Their PS corresponded only to some extent to their usual PS, and the time to onset of PS in these 2 patients was 20 and 30 minutes after infusion, respectively. More detailed descriptions of the premonitory and nonheadache symptoms for all patients can be found in the supplementary materials (available online at http://links.lww.com/PAIN/A337).

For the entire study recording period (0-12 hours), we found no difference in the incidence of nonheadache symptoms between the 2 groups (**Table 2**). Fatigue (91%), nausea (74%), and yawning (65%) were the most common nonheadache symptoms reported by patients who reported migraine-like attacks after PACAP38.

Figure 5 displays more detailed descriptions of nonheadache symptoms reported by patients receiving PACAP38. Information provided in the table includes the occurrence of nonheadache symptoms reported by each patient during the 3 phases, the onset of head pain or migraine attack, and the usual PS of the patients before their spontaneous migraine-like attacks.

3.3. Premonitory and nonheadache symptoms according to familial predisposition

In 71 of 72 patients, the family history of migraine was assessed by telephone interview, because one was adopted with unknown relatives. Twenty-eight patients had a high family load of migraine, whereas 43 had a low family load.

Retrospective assessment of PS showed that 21 of 28 (75%) patients with high family load reported to have PS before their spontaneous migraine attacks, whereas 24 of 43 (56%) with low family load reported to have PS ($P = 0.101$). In addition, we found no difference in the incidence of nonheadache symptoms induced by CGRP or PACAP38 between patients with high family load and patients with low family load (Table 3, supplementary materials, available online at http://links.lww.com/PAIN/A337).

Table 2

Nonheadache symptoms reported by patients who did and did not develop migraine attacks after CGRP or PACAP38 infusion (0-12 hours).

Nonheadache symptoms after CGRP (0-12 h)	Patients reported migraine attacks (n = 25)	Median time to onset of nonheadache symptoms (range), h	Patients reported no migraine attacks (n = 15)	Median time to onset of nonheadache symptoms (range), h	*P**
Any of the nonheadache symptoms	96 (24)	2 (0.17-7)	80 (12)	2.5 (0.17-9)	0.139
Fatigue	84 (21)	3 (0.17-9)	67 (10)	4.5 (0.33-10)	0.256
Photophobia	72 (18)	0.5 (0.33-9)	20 (3)	0.33 (0.33-8)	0.003
Nausea	68 (17)	3 (0.33-10)	20 (3)	1.33 (0.33-4)	0.008
Phonophobia	48 (12)	0.75 (0.17-8)	0	NA	0.001
Yawning	44 (11)	2.33 (0.67-9)	33 (5)	3 (0.33-9)	0.740
Stiff neck	40 (10)	3 (0.67-8)	40 (6)	3 (0.33-7)	1.000
Mood swings	20 (5)	4 (3-9)	7 (1)	9	0.381
Hunger (craving)	12 (3)	1 (0.5-1)	0	NA	0.279
Poor concentration	4 (1)	4	0	NA	1.000
Nonheadache symptoms after PACAP38 (0-12 h)	**Patients reported migraine attacks (n = 23)**	**Median time to onset of nonheadache symptoms (range), h**	**Patients reported no migraine attacks (n = 9)**	**Median time to onset of nonheadache symptoms (range), h**	***P****
Any of the nonheadache symptoms	96 (22)	0.33 (0.17-2)	89 (8)	0.33 (0.17-3)	0.490
Fatigue	91 (21)	0.5 (0.16-5)	78 (7)	0.33 (0.16-4)	0.557
Nausea	74 (17)	3 (0.17-11)	44 (4)	0.33 (0.33-4)	0.213
Yawning	65 (15)	1.17 (0.33-5)	56 (5)	0.67 (0.33-3)	0.696
Stiff neck	61 (14)	0.5 (0.16-6)	44 (4)	1.83 (0.16-10)	0.453
Photophobia	61 (14)	2 (0.33-6)	33 (3)	0.33 (0.13-0.5)	0.243
Thirst	52 (12)	3 (0.16-6)	33 (3)	2 (0.33-4)	0.444
Poor concentration	52 (12)	1.75 (0.5-3)	22 (2)	1.75 (0.16-5)	0.235
Hunger (craving)	43 (10)	1.75 (0.33-8)	56 (5)	2 (0.16-3)	0.699
Phonophobia	43 (10)	5 (0.17-10)	11 (1)	11	0.115

Median time to onset of CGRP- and PACAP38-induced migraine attacks was 3 hours (range 0.17–12 hours) and 5 hours (range 2–10 hours), respectively. Median time to onset of *head pain* after both CGRP and PACAP38 was 0.33 hours (range 0.17–7 hours). Migraine-associated symptoms are marked in gray.

Data are presented as % (n): number of patients.

* *P* value: Fisher exact test.

NA, not applicable.

4. Discussion

The major outcome of this study was that CGRP did not induce PS, whereas PACAP38 did induce PS in 48% of patients. However, CGRP and PACAP38 did not induce more PS in patients who developed an attack compared with those who did *not* develop an attack. Furthermore, we found no difference of nonheadache symptoms in the headache and postdrome phase or for the entire recording period (0-12 hours). Only 2 patients fulfilled the strict IHS definition for PS after PACAP38 administration, and no patients fulfilled these criteria after CGRP. We found that patients with a familial predisposition of migraine were not more susceptible to PS, and they did not report more nonheadache symptoms induced by CGRP or PACAP38.

We acknowledge the fact that we did not have a control group of healthy volunteers or placebo-treated patients, which would make it possible to distinguish between migraine-related symptoms from substance-related symptoms. Another limitation is that patients who experienced PS before their spontaneous attacks were not asked whether the induced PS mimicked their usual symptoms. Nonetheless, the strength of our study is the large sample size of pharmacologically triggered patients (attack vs *no* attack group was compared) and the use of a detailed questionnaire for recording the PS.

Our findings, in particular the lack of PS symptoms after CGRP infusion, suggest that peripheral mechanisms of CGRP induced migraine attacks. To support this, CGRP has been shown to pass the blood–brain barrier (BBB) very poorly[36] and CGRP antibodies, which also do not cross the BBB,[42] have shown efficacy as preventive treatment for migraine.[15,16] In addition, a recent study has shown that CGRP binds to receptors localized on human vascular smooth muscle cells of dural meningeal arteries and neurons in the trigeminal ganglion reinforcing its role in trigeminal sensitization.[34]

Conversely, PACAP38 showed a tendency to induce PS in at least some patients, although it also poorly passes the BBB.[17] This could be because PACAP38 is able to cross the BBB modestly by a specific saturable transporter[13] and thus exerting some central effects.[43] A plausible site of action for PACAP38 in migraine induction is the PAC_1 receptor possibly activating both trigeminal and central neurons.[40] A study in rats showed that centrally located PAC_1 receptors were able to mediate dural-nociceptive trigeminocervical neurons,[4] which suggests that migraine is likely to be triggered through PACAP38 within the brain. This difference of effect between CGRP and PACAP38 is also supported by a recent study showing that the blood–brain barrier remains intact during triggered migraine attacks.[37] However, we cannot rule out that both peripheral and central mechanisms are involved in the induction of PACAP38-triggered attacks. Nonetheless, PACAP38 is widely distributed in the central nervous system and has been shown to possess other neurological functions acting both as a neurohormone, a neurotransmitter, and a trophic factor.[45] The most abundant population of PACAP38-containing neurons is found in the hypothalamus,[10] indicating that PACAP38 is a potent modulator of hypothalamic activity. Studies have also suggested a role of PACAP38 on the pineal gland[41] and the pituitary gland.[35] This role is supported by

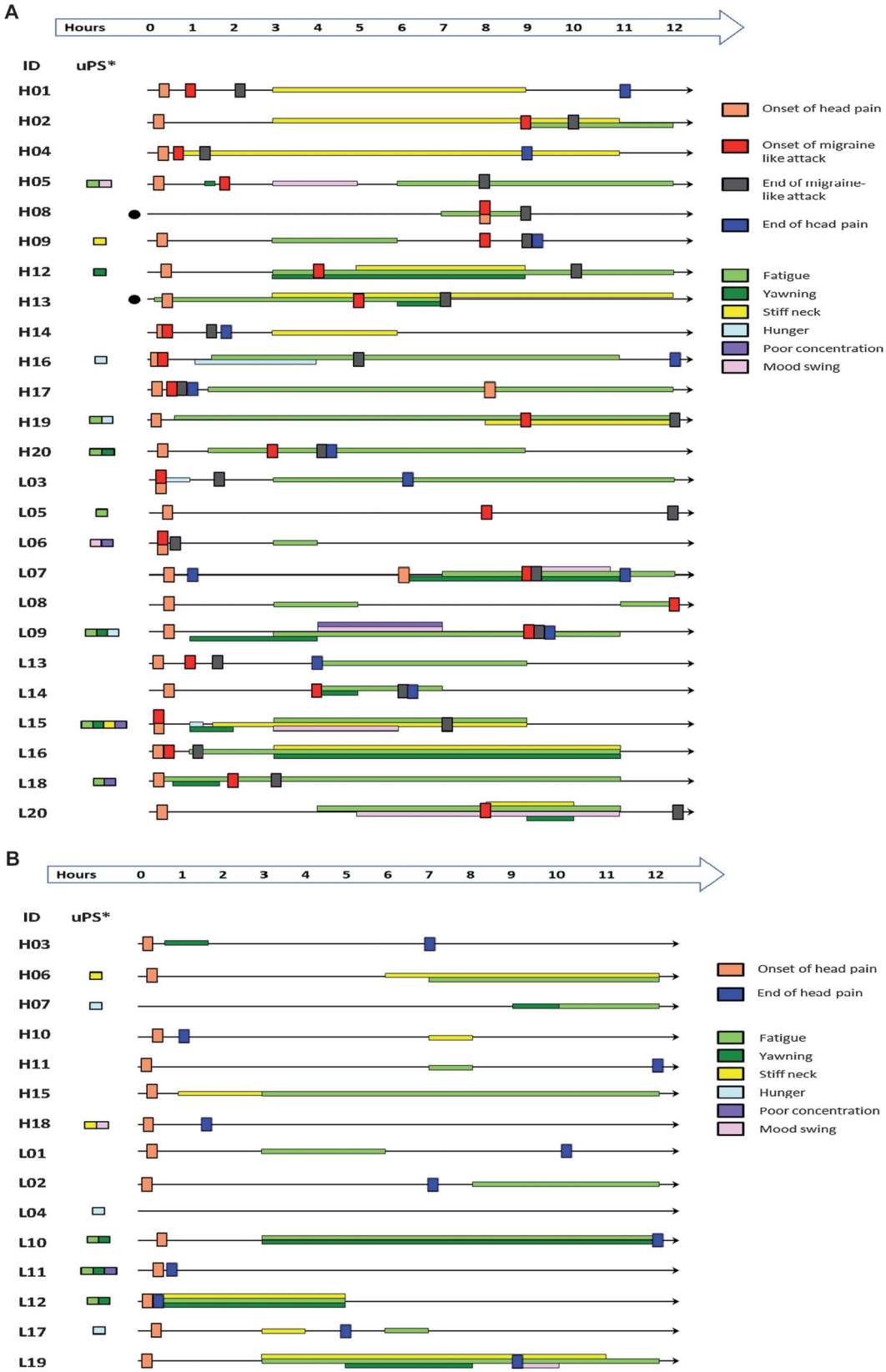

Figure 4. Nonheadache symptoms of patients who did (A) and did not (B) develop CGRP-induced migraine-like attacks. *uPS = patient's usual premonitory symptoms before their spontaneous migraine attacks. One patient fulfilled our definition of premonitory symptoms and is marked with (●).

a recent study showing that PACAP38 increases the plasma levels of thyroid-stimulating hormone and prolactin in patients with migraine.[23] Thus, PACAP38 is a multifunctional peptide involved in various physiological responses likely to cause nonheadache symptoms, which makes it difficult to distinguish between genuine induced PS and side effects of PACAP38.

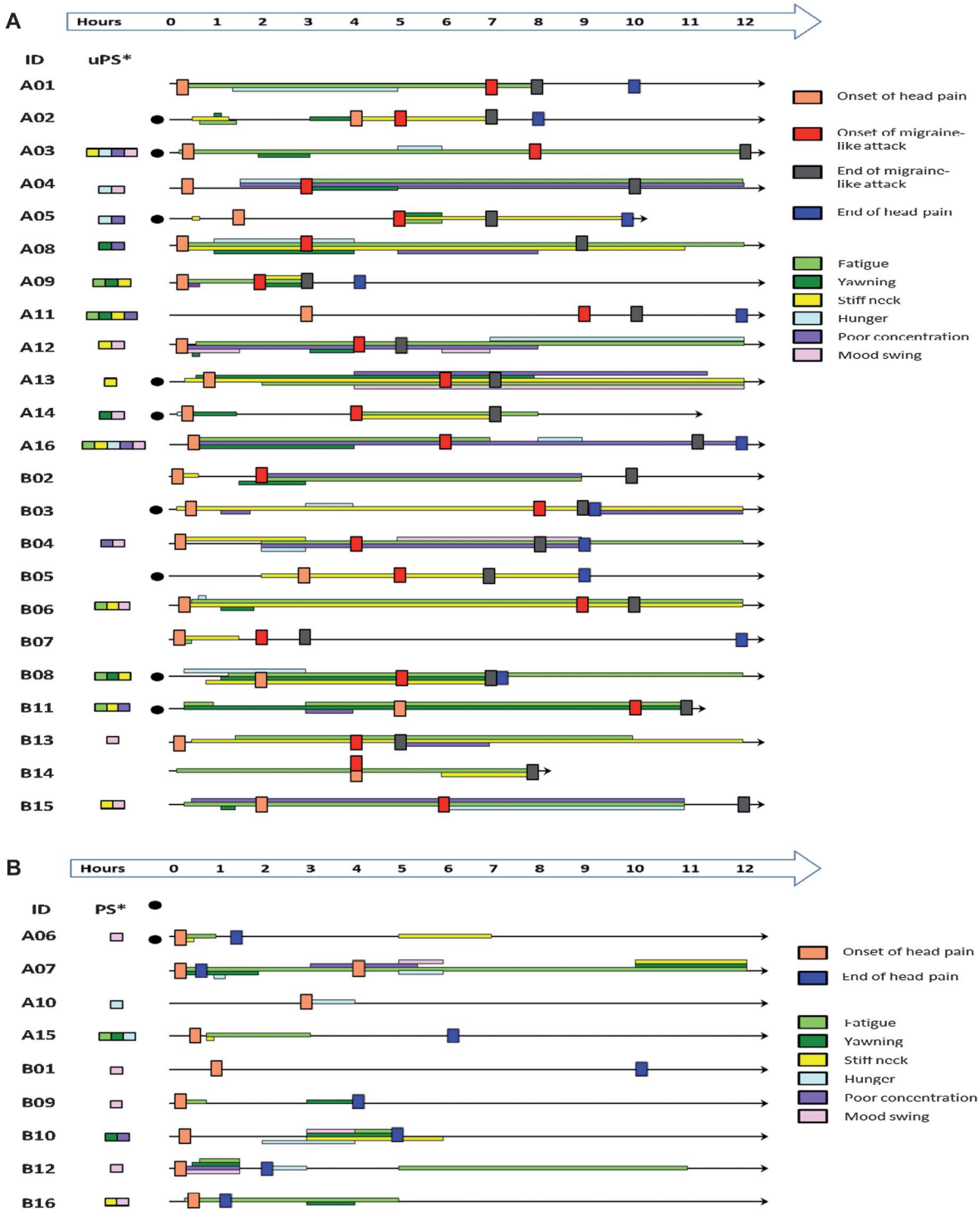

Figure 5. Premonitory and nonheadache symptoms of patients who did (A) and did not (B) develop PACAP38-induced migraine-like attacks. *uPS = patient's usual premonitory symptoms before their spontaneous migraine attacks. Eleven patients fulfilled our definition of PS and are marked with (●).

Accordingly, it is important to compare between patients who did and did not report attacks or to compare patients with healthy volunteers. After all, everyone may experience nonheadache symptoms such as yawning, craving for food, or mood swings without having headache. A control group of healthy participants is therefore crucial for studying genuine PS. It also might be interesting to investigate how often patients with PS experience PS but no migraine develops, or experience migraine attacks with no PS. As far as we know, this has not yet been systematically investigated.

None of the previous provocation studies with CGRP and PACAP38 in healthy volunteers reported nonheadache symptoms except for palpitation, heat sensation, nausea, and flushing.[7,25,39] Notably, however, the participants were not specifically asked about such nonheadache symptoms in these studies.

Another explanation for our findings could be that the CGRP- and PACAP38-induced migraine-like attacks are different from spontaneous attacks. However, we believe that this is unlikely because patients reported that their induced attack mimicked their usual spontaneous attacks and that the induced attack responded effectively to their usual migraine medication.

A provocation study of GTN in an unselected group of patients with migraine reported PS in 36% (12 of 33) before the induced migraine-like attacks[1] and showed that PS were highly reproducible with a mean latency of around 1 hour. In addition, the same research group reported PS after GTN provocation in a selective and screened group of patients with migraine with PS showing hypothalamic activation during the premonitory phase using positron emission tomography scans.[31] In this study, however, no control group was included, and it can therefore not be ruled out that the observed activation changes relate to GTN administration rather than to migraine. Moreover, none of these studies compared the PS in patients who reported and did *not* report attacks. In contrast to CGRP and PACAP38, GTN is a lipophilic compound that easily crosses the BBB and hence may activate brain structures.[2,31] GTN studies defined PS as symptoms before the onset of the triggered migraine headache,[1,31] which is different from the definition of the IHS.[44] In the present study, we used the same definition for PS as in GTN studies because pharmacologically induced migraine-like attacks usually develop within hours (range 0.33-11 hours) after start of infusion,[8,11,29,39] and the induced PS may therefore develop only shortly before or simultaneously with the onset of headache. Prospective data on PS showed no 2-hour gap between the end of PS and the beginning of pain.[21] We have no clinical experience suggesting a gap of this duration either. Thus, the strict and arbitrary IHS definition for a spontaneous PS, stating that the symptom must begin 2 to 48 hours before the headache or aura in patients with migraine (The International Classification of Headache Disorders, third edition [beta version], 2013), may *not* be applicable in migraine attacks induced by pharmacological triggers. In addition, no terminology exists for the phase between minus 2 hours and the onset of head pain. Therefore, we support the suggestion[32] that the IHS definition of PS should be regarded as "symptoms preceding and forewarning the migraine attack before the onset of head pain," and encourage the International Headache Society Classification Committee to consider this definition before the completion of the beta round.

Most of the previous studies on PS in migraine are retrospective,[6,18,38,46] and the underlying mechanisms of the PS are still unclear. In this study, most patients reported nonheadache symptoms simultaneously with headache or migraine, which may suggest that these in fact are migraine-accompanying symptoms. Interestingly, a recent study showed that neck pain is more likely to be an integral part of the migraine attack rather than a PS.[28] The nonheadache symptoms also could be side effects of CGRP and PACAP38. In particular, PACAP38 is known to exert many indirect systemic effects,[45] which likely could cause these symptoms.

Recently, a large study (n = 2223) demonstrated a prevalence of PS in 77% of selected patients with familial predisposition to migraine and higher prevalence among patients with hemiplegic migraine (95%) and migraine with aura (79%) compared with MO (75%).[30] In addition, severity and female gender were positively correlated to a higher number of PS. These findings could indicate an association between familial predisposition and PS, which we investigated in the present study. However, we found no association between PS and high family load.

In conclusion, intravenous administration of CGRP did not induce PS, suggesting a peripheral site of action, and therefore CGRP is unsuitable to use as a provocation model for PS. PACAP38 showed a tendency to induce PS in some patients, possibly because it is able to pass the BBB to a modest degree. However, we found no statistical difference in PS between the 2 groups of patients who did and did not develop a migraine-like attack after administration of CGRP or PACAP38. In addition, we found no association between PS and familial predisposition. It is important that future studies of PS assess each nonheadache symptom prospectively by a specific questionnaire or by direct interview. Other methods are unreliable in the study of such complex symptoms.

Conflict of interest statement

J. Olesen has received grants and/or research support from, has been a consultant and/or scientific adviser for, and has been in the speakers' bureau of Allergan Inc, AstraZeneca Pharmaceuticals LP, Boehringer Ingelheim, Eli Lilly, GlaxoSmithKline, Janssen Pharmaceutical Products, Lundbeck, Merck, Amgen, Alder, and Pfizer. M. Ashina is a consultant and/or scientific adviser/speaker for the ATI, Allergan, Amgen, Alder, and Eli Lilly. The remaining authors have no conflicts of interest to declare.

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Appendix A. Supplemental Digital Content

Supplemental Digital Content associated with this article can be found online at http://links.lww.com/PAIN/A337.

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