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Title: Calcitonin-gene related peptide and disease activity in cluster headache

Agneta Snoer, MD [1]*; Anne Luise H. Vollesen, MD [1]*; Rasmus P. Beske, MD [1]; Song Guo, MD PhD [1]; Jan Hoffmann, MD PhD [2]; Jan Fahrenkrug, DMSc Prof [3]; Niklas Rye Jørgensen, DMSc Prof [4,5]; Torben Martinussen, Prof [6]; Rigmor H. Jensen, DMSc Prof [1]; Messoud Ashina, DMSc Prof [1]

[1] *Danish Headache Center and Department of Neurology, Rigshospitalet Glostrup, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark*
[2] *Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK*
[3] *Department of Clinical Biochemistry, Bispebjerg Hospital, University of Copenhagen, Denmark*
[4] *Department of Clinical Biochemistry, Rigshospitalet Glostrup, Denmark*
[5] *OPEN, Odense Patient Data Explorative Network, Odense University Hospital/Institute of Clinical Research, University of Southern Denmark, Odense, Denmark*
[6] *Section of Biostatistics, University of Copenhagen, Denmark*

* The first two authors contributed equally to the study

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Corresponding author:

Prof. Messoud Ashina, M.D., Ph.D., DMSc.
Danish Headache Center, Department of Neurology, Rigshospitalet Glostrup
Faculty of Health and Medical Sciences, University of Copenhagen
Valdemar Hansens Vej 5, DK-2600 Glostrup, Denmark
Tel: +45 38 63 33 85
E-mail: ashina@dadlnet.dk

Author contributions

Agneta Snoer: Study concept and design, acquisition of data, analysis (including statistical analyses) and interpretation, drafting the manuscript.

Anne Luise Haulund Vollesen: Study concept and design, acquisition of data, analysis and interpretation, drafting the manuscript.

Rasmus Beske: Acquisition of data and critical revision of the manuscript for important intellectual content

Song Guo: Study concept and design, critical revision of the manuscript for important intellectual content.

Jan Hoffmann: Study concept and design, critical revision of the manuscript for important intellectual content.

Jan Fahrenkrug: Analyses of biochemical data, interpretation and critical revision of the manuscript for important intellectual content.

Niklas Rye Jørgensen: Analyses of biochemical data, interpretation and critical revision of the manuscript for important intellectual content.

Torben Martinussen: **Statistical analyses** and critical revision of the manuscript for important intellectual content.

Rigmor Jensen: Study concept and design, interpretation of study result, supervision

Messoud Ashina: Study concept and design, interpretation of study result, critical revision of the manuscript for important intellectual content and supervision

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Abstract

Objective: To investigate the role of calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide-38 (PACAP38) and vasoactive intestinal polypeptide (VIP) in cluster headache (CH), we measured these vasoactive peptides interictally and during experimentally induced CH attacks.

Methods: We included patients with episodic CH in an active phase (n=9), episodic CH patients in remission (n=9) and in patients with chronic CH (n=13). Cluster headache attacks were induced by infusion of CGRP (1.5µg/min) in a randomized, double-blind, placebo controlled, two-way cross-over study. At baseline we collected interictal blood samples from all patients and during 11 CGRP-induced CH attacks.

Results: At baseline, episodic CH patients in remission had higher plasma levels of CGRP, 100.6 ± 36.3 pmol/l, compared to chronic CH patients, 65.9 ± 30.5 pmol/l, (p=0.011). Episodic CH patients in active phase had higher PACAP38 levels, 4.0 ± 0.8 pmol/l, compared to chronic CH patients, 3.3 ± 0.7 pmol/l, (p=0.033). Baseline levels of VIP did not differ between CH groups. We found no attack-related increase in CGRP, PACAP38 or VIP levels during CGRP-induced CH attacks.

Conclusions: This study suggests that CH disease activity is associated with alterations of CGRP expression. Future studies should investigate the potential of using CGRP measurements in monitoring of disease state and predicting response to preventive treatments including response to anti-CGRP monoclonal antibodies.

Introduction

The hallmark of cluster headache (CH) is periodicity and prominent cranial autonomic symptoms (CAS) [1]. Most patients report periodicity by experiencing episodic CH with month long attack periods separated by remission periods [2]. To what extent mechanisms underlying CAS contribute to initiation of CH attacks is still not fully elucidated. The trigemino-autonomic reflex activation is associated with release of sensory and parasympathetic neuropeptides [3] such as calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) and vasoactive intestinal polypeptide (VIP). Studies investigating plasma levels of these neuropeptides in CH remain, however, scarce and conflicting [4–8]. Elevated plasma CGRP, VIP and PACAP38 have been reported during spontaneous and glyceryl trinitrate (GTN) provoked CH attacks [5–7,9]. The diverging methodologies of these studies, however, make it difficult to compare findings across studies. To date, no studies have investigated the role of these vasoactive peptides in chronic CH patients. In addition, it is unknown whether plasma levels correlate to disease periodicity. As CH is unpredictable and short lasting, investigation of patients during spontaneous attacks can be immensely difficult. This challenge can be overcome by studying provoked CH attacks in a controlled setting [5]. Recently, we demonstrated that CGRP infusion could provoke CH attacks in patients in an active disease state (episodic CH in active phase and chronic CH patients), but not in patients during remission [10].

We hypothesized that baseline levels of CGRP, VIP and PACAP38 would be elevated in episodic and chronic CH patients in an active disease state compared to patients in remission. Furthermore, we hypothesized that CGRP-induced CH attacks would cause a further increase in plasma levels of neuropeptides. To test these hypotheses, we investigated plasma CGRP, VIP and PACAP38 at baseline and during CGRP induced CH attacks. In addition, we compared the baseline

concentration of neuropeptides in CH patients with historical data on migraine patients and healthy controls.

Materials and Methods

Patients were eligible for inclusion if they were aged 18 – 65 years and had a verified diagnosis of episodic or chronic cluster headache as defined by the International Headache Society classification (Headache Classification Committee of the International Headache Society (ICHD-3 beta), 2013) [11]. We recruited participants from the outpatient clinic at the Danish Headache Center (Rigshospitalet-Glostrup) in the period from December 2015 to April 2017. The present study is a predefined part of a larger parent protocol (protocol H-15006836, clinicaltrials.gov identifier NCT02466334). The first part of the study investigated the ability of CGRP to induce cluster headache like attacks and has previously been described in detail [10]. Patients were eligible for inclusion in the study if they were in active disease phase, defined as occurrence of typical CH attacks within the last 30 days; or in remission, defined as attack-free for at least 30 days. Episodic patients could participate in remission and in active disease phase. According to the ICHD-3 beta criteria, chronic patients did not have attack-free periods exceeding 30 days in the last 12 months. Exclusion criteria included any other type of headache (apart from episodic tension-type headache ≤ 5 days per month), any previous serious somatic or psychiatric condition, pregnant or nursing women, drug misuse or daily intake of medication other than preventive treatment for CH. All patients underwent a full medical examination and in women of childbearing age pregnancy testing was conducted prior to participation.

The study was approved by the Regional Committee on Health Research Ethics of the Capital Region (H-15006836) and was conducted in accordance with Helsinki II Declaration of 1964, with later revisions. The study was registered at clinicaltrials.gov (identifier NCT02466334) and

148 approved by the Danish Data Protection Agency. All patients received oral and written information
149 about the study and were given time for consideration before giving their written consent to
150 participate.
151 In the present study we conducted post hoc analyses which included previously published data on
152 migraine patients and healthy volunteers [12] (ClinicalTrials.gov identifier NCT01841827). All
153 samples from previous data were collected in an inter-ictal state, defined as the participant being
154 completely headache- and analgesic free for a minimum of 48-hours prior to sampling. Samples
155 were analyzed by the same assays in the same laboratory as the current study [12].

156 *Design and experimental protocol*
157 The study was conducted as a randomized, double-blind, placebo controlled, two-way cross-over
158 study. All patients were randomly allocated to receive a continuous infusion (Braun Perfusor,
159 Melsungen, Germany) with either 30 µg CGRP (1.5µg/min) (Calbiochem® and PolyPeptide group)
160 or placebo (saline) over 20 min on two separate days. CGRP and placebo were prepared in identical
161 vials and randomized by the regional central pharmacy. Allocation was balanced to ensure
162 approximately even numbers of participants receiving CGRP first and placebo last or vice versa.
163 The randomization code remained in the hospital during the study and was unavailable to
164 investigators until study completion.

165 On both experimental days, patients with episodic (active phase) and chronic cluster headache
166 reported themselves to the clinic when they were headache/attack free for at least 3 and 8 hours,
167 respectively. An 8-hour headache-free-interval prior to provocation was initially set for both episodic
168 patients in cluster and chronic cluster patients, but due to feasibility concerns, a revised 3-hour
169 headache-free-interval was set in order to include episodic patients with a high mid-cluster attack
170 burden.

All participants were asked to retrospectively estimate their attack frequency in the preceding 30 days. Patients were placed in a supine position and a venous catheter (Venflon®) inserted in the cubital vein on the right or left arm for CGRP infusion and drawing of blood samples. Patients were at rest for 15 min before obtaining baseline status. Blood for analysis of CGRP, PACAP38 and VIP was drawn at fixed time points: At baseline (T0), post infusion (T20), 10 min (T30) and 70 min post infusion (T90). If the patient developed a CH-like attack during the observations period, blood was drawn at the onset phase of attack (Ta0), after 15 min (Ta15) and at 30 min after the start of the attack (Ta30).

Blood collection and processing

Blood was drawn through the venous catheter and connector using two 20 ml syringes. For blood sampling the first 5 ml were discarded and after the procedure the catheter was flushed with saline. The blood was thereafter transferred into different tubes: precooled lithium heparin tubes containing aprotinin (Trasylol®) for VIP; precooled EDTA tubes with aprotinin for PACAP38 and standard EDTA tubes for CGRP. All tubes were inverted several times. The precooled tubes were stored in a cooling box (5˚C) and the rest stored at room temperature for 20 min until centrifugation. The tubes were centrifuged together at 4˚C at 1851g for 10 min. Plasma was thereafter transferred to polypropylene tubes (Greiner Cryo.s ™) and stored at -25˚C until analysis.

Radioimmunoassay

Plasma CGRP concentrations were measured with a fully evaluated radioimmunoassay for human CGRP, as described previously (Schifter, 1991) [12,13]. The tracer was prepared by the method of Iodogeneral (Pierce, Rockford, IL, USA)[14] by iodination of [Tyr0] α-CGRP (25-37) amide and purification by high liquid chromatography (HPLC). Samples, antibody and calibrators were incubated at 4°C for about 90 hours before addition of tracer and subsequent incubation for 48 hours. Free and antibody-bound tracer was separated by Sac-Cel separation.

Plasma concentration of PACAP38

The concentration of PACAP38 in plasma was measured radioimmunochemically using antiserum 733C-5 directed against the sequence PACAP28–38 [15]. The antiserum which was used at a final titer of 1.2×10^5 in a total volume of 0.8 ml/tube does not cross-react with PACAP27, VIP or other structurally related peptides. Synthetic PACAP28–38 labeled to a specific radioactivity of 30 Bq/mol with ^{125}I by the iodogen method was used as tracer and synthetic human PACAP38 was used as standard. The IC_{50} value (the concentration of PACAP38 giving 50% displacement of the tracer) was 17 pmol/l and the intra-assay and inter-assay coefficient of variation values were 3.1 and 10.1%, respectively.

Since PACAP38 in human plasma is bound to the protein ceruloplasmin [16] the peptide was freed from ceruloplasmin before measurement by the following procedure: 1.2 ml of 1% trifluoroacetic acid was added to an equal volume of plasma from each subject and mixed thoroughly for 60 s. After incubation for 10 min in an ice-bath, the mixture was neutralized by addition of 15 µl of 5 M NaOH. Subsequently 2.5 ml of absolute ethanol was added. After thorough mixing, followed by centrifugation at 1500 g for 20 min at 4 °C, the supernatant was decanted and dried under vacuum. The dried product was reconstituted to its original volume with assay buffer for assay.

Plasma concentration of VIP

The concentration of VIP in plasma, after extraction with absolute ethanol, was measured by the VIP radioimmunoassay using antiserum 5603–6 at a final titer of 1.2×10^6 in a total volume of 0.8 ml/tube [17,18]. This antiserum recognizes the mid- and C-terminal regions of the VIP molecule (sequence 11–24) and displays no cross-reactivity with other known gastrointestinal peptides or neuropeptides. The label has a specific radioactivity of 0.92 nCi/fmol (~34 Bq/fmol). The IC_{50} value (the concentration of VIP giving 50% displacement of label) was 24 pmol/l and the intra-assay and the inter-assay coefficient of variation values were 8.7 and 12.6%, respectively.

Headache characteristics and vital signs

From baseline (T-10 and T0) and throughout the entire experiment the following variables were recorded every 10 min: Headache intensity on a verbal rating scale (VRS) from 0 to 10 (0; no headache, 1; very mild headache, 10; worst imaginable headache); quality of pain (stabbing, throbbing, pulsating or resembling usual CH attack); headache localization and accompanying symptoms, these including CAS. In addition, we recorded blood pressure and heart rate. Symptoms experienced outside recording intervals were documented separately.

Statistical analysis

All absolute values are presented as mean ± standard deviation. The primary endpoints were: 1) Differences of biochemical variables (CGRP, PACAP38 and VIP) in between groups (episodic CH patients in active phase, episodic CH patients in remission and chronic CH patients) at baseline; 2) Differences over time in plasma concentrations of biochemical variables between patients developing an attack and those who did not. 3) Differences in plasma concentrations over time of biochemical variables between active and placebo days.

Distribution of demographical data was tested using the D'Agostino and Pearson normality test and group comparisons of demographical data were subsequently analyzed using parametric statistics. Evaluation of baseline variables was done using a generalized linear model with repeated measurements.

To analyze for an effect of CGRP infusion on biochemical variables we used repeated measurements analysis with random effect of subjects, attacks and further of subjects' times day. In this way we allow for correlation between measurements on the same individual, and additional correlation between measurements in the same individual on the same day. The measurement taken at time zero was used as baseline variable in the repeated measurements model. For each of the

responses VIP, PACAP and CGRP we checked model assumptions and transformed the response variables as appropriate to meet model requirements. Correlations between baseline levels and time since last attack, were calculated using Pearson correlation test.

We used GraphPad Prism 7.02, SAS Enterprise and R 3.4.3 for statistical analyses. All p-values were two-sided and considered significant if <0.05.

Data availability

The data supporting the findings of this study are not publicly available, but will be shared, in an anonymized form, by request from any qualified investigator.

Results

In total 31 patients (26 men and 5 women) completed the study (Fig. 1). The mean age was 37 years, (range 19 – 59). Nine patients reported episodic CH in active phase (6 men, 3 women; mean age 32, range 19 – 56 years), 9 episodic in remission (all men; mean age 32, range 22 – 43 years), and 13 chronic CH (10 men, 3 women; mean age 42, range 26 – 59 years). Clinical data on patients are shown in table 1. Episodic patients in remission reported remission on average for 6.6 (range 1.3–18.0) months prior to participation in the study. At baseline, blood samples were collected in all 31 patients for VIP and CGRP, but samples from one patient were lost for PACAP38. CGRP infusion induced a CH-like attack in 16 out of 31 patients, of these non in episodic patients in remission. We collected blood samples during 11 out of 16 CH attacks and all attack samples were collected prior to abortive treatment. In the remaining 5 attacks, symptoms subsided before we had the chance to engage attack sampling protocol. All provoked attacks were unilateral, located in the periorbital region and were accompanied by CAS and/or restlessness. The median severity of provoked attacks was 10 (IQR 4 – 10, range 1 – 10) and median number of accompanying symptoms was 4 (IQR 1.5 – 5, range 1 – 8). Characteristics of 11 provoked attacks are listed in

table 2. The concentration of CGRP, PACAP38 and VIP were above the detection limit in all blood samples.

CGRP

We found significantly higher baseline plasma CGRP in episodic patients in remission, 100.6 ± 36.3 pmol/l, compared to chronic patients, 65.9 ± 30.5 pmol/l, (p=0.011) (Fig. 2, table 3). The repeated measurements analysis showed no independent increase of plasma CGRP in patients who reported a CH-like attack after provocation with CGRP (p=0.36). After CGRP infusion plasma levels of CGRP increased significantly, 574.3 ± 296.4 pmol/l, compared to baseline, 81.7 ± 33.4 pmol/l, (p<0.0001). We found no changes in CGRP levels after placebo infusion compared to baseline (p=0.43).

PACAP38

We found significantly higher baseline PACAP38 levels in episodic patients in active phase, 4.0 ± 0.8 pmol/l, compared to chronic patients, 3.3 ± 0.7 pmol/l, (p=0.033) (table 3). CGRP induced CH attacks were not associated with changes in plasma PACAP38 (p=0.29). Compared to baseline, plasma levels of PACAP38 remained unchanged after CGRP (p=0.66) and placebo (p=0.57) infusion.

VIP

We found no differences in baseline plasma levels of VIP between CH groups (p > 0.05) (table 3). The repeated measurements analyses revealed a significant decrease in patients who reported a provoked attack (p=0.013). Infusion of CGRP caused a significant increase in plasma VIP compared to baseline (p<0.001), but not after placebo (p=0.53).

Post hoc analysis

We compared five groups: Episodic patients in active phase; episodic patients in remission; chronic patients; migraine without aura patients and healthy controls (Fig. 2). The analysis revealed that episodic patients in bout ($p<0.001$), patients in remission ($p<0.001$) and chronic patients ($p=0.020$) had higher baseline levels of CGRP compared to healthy controls. Furthermore, patients in active phase ($p<0.001$) and in remission ($p<0.001$) had higher CGRP levels compared to migraine patients. We found no differences in PACAP38 and VIP levels between CH patients, migraine patients and healthy controls.

In patients in an active disease state we found no difference in baseline CGRP, PACAP38 nor VIP levels in patients on prophylactic treatment vs. those without ($p>0.05$). There was no correlation between hours since last attack, nor 30-day attack burden and baseline level of CGRP, PACAP38 nor VIP ($p>0.05$).

Discussion

The main finding of the present study was that CH patients in remission had higher baseline levels of CGRP, but not VIP or PACAP38, compared to chronic CH patients. Furthermore, CGRP-induced CH attacks were not associated with elevated levels of CGRP, VIP or PACAP38. In addition, CH-patients irrespective of disease state had higher plasma levels of CGRP compared to migraine patients and healthy controls.

A novel finding of the present study was that chronic CH patients had lower plasma CGRP compared to CH patients in remission. This suggests that plasma levels of CGRP may fluctuate with disease activity. The results are very interesting when viewed in the light of recent press releases on the preventive effect of the anti-CGRP monoclonal antibodies in episodic patients in active period, but not in patients with chronic CH. Interestingly, poor treatment response in chronic CH is reported across different treatment modalities indicating basic pathophysiological differences

311 in between phenotypes [19,20]. The question is why chronic CH patients had lower CGRP levels than
312 CH patients in remission and episodic CH patients in active phase? Furthermore, how the regulation
313 of CGRP expression and release aligns with our data is difficult to reconcile but CGRP expression
314 is regulated in many ways. Animal experiments reported local elevated CGRP levels after nerve
315 damage, nerve regeneration and inflammatory response [21]. CGRP is expressed and released from C
316 fibers and its receptors are present in A delta fibers [22]. In man, application of capsaicin to the nasal
317 mucosa led to immediate release of CGRP in saliva and plasma [8]. It is possible that secreted CGRP
318 may act in an autocrine fashion to further increase CGRP release in a positive feedback loop, a
319 mechanism possibly implicated in peripheral sensitization [23]. In rats, a single subcutaneous
320 capsaicin injection in the hind-paw depleted CGRP levels in the skin and sciatic nerve after 8 and
321 10 days [24,25]. Interestingly, repeated capsaicin applications to the nasal mucosa resulted in
322 desensitization and time-dependent recovery of responses [26]. In addition, intradermal capsaicin
323 injections produced a steady increase of CGRP levels in the first sampling period but failed to reach
324 significance in the second session [27]. Thus, capsaicin-induced desensitization of sensory afferents
325 might lead to depletion of neuropeptide release from afferents or decreased activity of transient
326 receptor potential vanilloid 1 channels [28,29]. Taken together these data suggest that chronic CH
327 patients may exhibit low plasma CGRP due to depletion of CGRP from trigeminal afferents.
328 Whether a CH attack represents a comparable stimulus to capsaicin is unknown, but it is possible
329 that endogenous processes influence CGRP expression. Several factors might have influenced our
330 data. We tested for possible influence of a recent attack and attack burden (frequency) and found
331 that CGRP levels were not associated to the most recent attack or to attack burden in the 30 days
332 preceding baseline sampling. Exogenous factors such use of preventive and abortive treatments may
333 theoretically influence CGRP expression. One study reported that treatment with corticosteroids can
334 reduce CGRP levels in episodic CH patients [4]. In the present study 61.5% of chronic CH patients

335 took preventive treatments compared to 44.4 % of episodic CH patients, but an exploratory analysis
336 revealed no difference in baseline levels. Preclinical studies reported that application of 5-HT_1
337 receptor agonist decreased the synthesis of CGRP in trigeminal ganglion [30] and that 7 day infusion
338 of sumatriptan infusion upregulated CGRP expression in trigeminal dural afferents [31]. In the present
339 study, we did not record the patients use of triptans prior to baseline sampling, but in future studies
340 investigating CGRP in CH, this should be taken into consideration.

341 In the post hoc analyses we found that episodic patients in active phase and in remission had
342 higher baseline levels of CGRP than episodic migraine patients and healthy controls. Although data
343 from migraine patients and healthy controls derived is historical and thus should be interpreted with
344 caution, it is an interesting observation. One study reported that chronic migraine patients had
345 higher CGRP levels than episodic migraine patients, healthy controls or episodic CH patients in
346 remission [32]. This study also reported no difference between patients with episodic migraine and
347 CH patients [32]. As different assays have been used across studies in migraine and CH it is
348 impossible to compare results directly and further hypothesis-based studies are necessary to address
349 possible difference in CGRP levels between cluster headache and migraine.

350 Collectively, our data suggest that CGRP may be altered in CH, but the findings should be
351 reproduced in a larger cohort of CH patients, ideally in prospective studies investigating changes in
352 CGRP over time and disease state.

353 In the present study chronic CH patients had lower baseline levels of PACAP38 compared to
354 episodic patients in bout, but similar levels compared to CH patients in remission. In line with our
355 CGRP findings in chronic CH patients, this suggests possible pathophysiological differences
356 between disease states. Another important finding was that CGRP-induced CH attacks were not
357 associated with alterations in plasma PACAP38 or VIP. These data are in contrast to previous
358 studies, where VIP [7] and PACAP38 [9] were reported elevated during spontaneous attacks. In the

current study chronic patients had an average longer disease duration compared to episodic patients, which might be an influencing factor. A migraine study reported that baseline levels of PACAP38 were negatively correlated with disease duration [33], suggesting that longer disease duration or transition to the chronic phase alters the regulation of this peptide. In support, repeated chemical stimulation of dura surrounding the superior sagittal sinus decreased PACAP38 levels in the TG in rats [34]. Both PACAP38 and VIP may be considered as markers of parasympathetic activation. Higher PACAP38 levels in episodic CH patients in active phase compared to chronic patients could theoretically reflect marked parasympathetic activation, but as VIP was unchanged, this interpretation remains speculative. To notice, we found no correlation between hours since last attack and baseline VIP or PACAP38 levels, as would be expected given the short half-lives (min) of VIP and PACAP38 [35]. Infusion of CGRP induced an increase in VIP, but not PACAP38, and development of an attack was associated with a decrease in VIP. As VIP levels were highest immediately following infusion and attacks occurred on average 34 min after onset of infusion, the observed attack-associated decrease in VIP was likely to coincide with the natural fall in VIP. Interestingly, our previous study using the same assay also found elevated VIP after CGRP infusion in migraine patients [12]. These findings suggest that CGRP infusion is associated with transient elevation of plasma VIP, but it does not seem to be associated with attack development. Collectively, CH disease activity was not associated with elevation of PACAP38 and VIP. Furthermore, CGRP induced CH attack did not increase plasma VIP and PACAP38, which is in contrast to previous studies [7,9]. The inconsistency of results across studies is likely attributed to use of different assays [35].

We collected blood from the antecubital vein, and not from the external jugular vein. One might argue that plasma levels should be collected in the cranial outflow. In migraine patients, elevated plasma CGRP was reported elevated in peripheral blood ictally [36] and interictally [37]. One

study in migraine patients reported no changes in plasma CGRP during attacks. To date, no studies have compared plasma PACAP38 and VIP between the two sampling sites, but elevated levels of these neuropeptides were reported in peripheral blood during and outside of migraine attacks [9,33]. We acknowledge relatively small sample size in the present study. However, we obtained two sets of samples for baseline-comparisons (in total 62 samples) and performed a robust statistical analysis. We collected "attack samples" in 11 patients. Previous studies reported plasma alterations during and spontaneous attacks on average of 14 patients [5–7,9]. Thus, the present sample size should be sufficient to detect possible attack related changes in neuropeptides. As blood samples were not drawn in all provoked attack and element of selection bias might affect attack results. However, as these results were negative, the concern for this bias seems less important. With regards to sensitivity and specificity of CGRP assay used in the current study, we measured a robust elevation of CGRP after CGRP infusion and importantly no concomitant increase in PACAP38, which is structurally similar.

The present study demonstrated that CH disease activity might be associated with alterations of CGRP expression, possibly PACAP38 but not VIP expression. Future studies should investigate the potential of using CGRP measurements in monitoring of disease state and predicting response to preventive treatments including response to anti-CGRP monoclonal antibodies.

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Article highlights:

- The present study demonstrated that cluster headache disease activity is associated with alterations of CGRP expression, possibly PACAP38 but not VIP expression
- Our results indicate that there are basic pathophysiological differences between episodic and chronic cluster headache patients
- The observed lower CGRP levels in chronic cluster headache patients at baseline might offer an explanation to why anti-CGRP monoclonal antibodies have proven effective in episodic, but not in chronic cluster headache patients

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522 **Figure 1.** Flow chart of recruitment and inclusion of patients.

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Figure 2. **Baseline levels of CGRP, VIP and PACAP38 presented as means and SD**.

Dashed lines divide present data from historical data. Significant findings according to primary endpoints marked. Significant finings in post hoc analyses comparing CH patients to data from historical data: CGRP, eCHr and eCHa > HC, $p<0.001$; eCHr > MO, $p<0.001$; eCHa>MO, $p<0.001$; cCH > HC, $p=0.020$. No differences regarding VIP and PACAP38 levels between CH patients and MO or HC patients were found.

cCH = chronic cluster headache; eCHa = episodic cluster headache patient in active phase; eCHr = episodic cluster headache in remission; MO = Migraine without aura; HC = Healthy controls; CGRP = Calcitonin gene-related peptide; PACAP38 = pituitary adenylate cyclase-activating polypeptide-38; VIP = Vasoactive intestinal peptide.

Table 1. Clinical data on patients with episodic cluster headache in active phase (eCHa), remission (eCHr) and chronic cluster headache (cCH).

eCHa = episodic cluster headache patient in active phase; eCHr = episodic cluster headache patients in remission; cCH = chronic cluster headache patients; SD = standard deviation, W/M = Women/Men. *Treatments: Verapamil 400mg; Verapamil 560mg; Verapamil 400 mg; Recent blockade greater occipital nerve. ** Treatments: Verapamil 400mg; Verapamil 800mg. *** Treatments: Verapamil 440mg; Verapamil 480mg; Verapamil 100mg; Verapamil 400mg and Melatonin 8mg, Verapamil 240mg, Verapamil 600mg; Verapamil 240mg and Lithium 200mg; Sphenopalatine ganglion neurostimulation and melatonin 4mg.

557 **Table 2.** Clinical characteristics of provoked attacks in 11 patients.

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559 Headache intensity: 0 – 10 Verbal response scale. Ta0: Attack onset, prior to acute therapy. Ta15
560 and Ta30: 15 and 30 min after attack onset respectively. Acute therapy: Suma = sumatriptan 6mg
561 sc; Oxy = oxygen 15L/min Optimask; SPG = The Pulsante SPG Microstimulator System; Dic =
562 Diclofenac 25mg sc. Accompanying symptoms: lac = lacrimation; pto = ptosis; mio = miosis; con =
563 nasal congestion; inj = conjunctival injection; swe = forehead and facial sweating; res =
564 restlessness; rhi = rhinorrhea; ede = eyelid edema.

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575 **Table 3.** Levels of CGRP, PACAP38 and VIP at baseline, presented in means ± SD.

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577 eCH = episodic cluster headache; cCH = chronic cluster headache; CGRP = calcitonin gene-related
578 peptide; PACAP38 = pituitary adenylate cyclase-activating polypeptide-38; VIP = vasoactive
579 intestinal polypeptide

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