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# Investigation of sumatriptan and ketorolac trometamol in the human experimental model of headache

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## Abstract

**Background:** Pituitary adenylate cyclase-activating polypeptide-38 (PACAP38) induces headache in healthy volunteers but the precise mechanisms by which PACAP38 leads to headache are unclear. We investigated the headache preventive effect of sumatriptan and ketorolac on PACAP38-induced headache in healthy volunteers. In addition, we explored contribution of vascular mechanisms to PACAP38-induced headache using high resolution magnetic resonance angiography.

**Methods:** Thirty-four healthy volunteers were divided in two groups (A and B) and received infusion of PACAP38 (10 picomol/kg/min) over 20 min. Group A was pretreated with intravenous sumatriptan (4 mg) or ketorolac (30 mg) 20 min before infusion of PACAP38. Group B received infusion of sumatriptan or ketorolac as post-treatment 90 min after infusion of PACAP38. In both experiments, we used a randomized, double-blind, cross-over design. We recorded headache characteristics and circumference of extra-intracerebral arteries.

**Results:** We found no difference in AUC<sub>(0–6 h)</sub> of PACAP38-induced headache in group A, pretreated with sumatriptan or ketorolac ( $p = 0.297$ ). There was no difference between sumatriptan and ketorolac in PACAP38-induced circumference change (AUC<sub>Baseline–110 min</sub>) of MMA ( $p = 0.227$ ), STA ( $p = 0.795$ ) and MCA ( $p = 0.356$ ). In group B, post-treatment with ketorolac reduced PACAP38-headache compared to sumatriptan ( $p < 0.001$ ). Post-treatment with sumatriptan significantly reduced the circumference of STA ( $p = 0.039$ ) and MMA ( $p = 0.015$ ) but not of MCA ( $p = 0.981$ ) compared to ketorolac. In an explorative analysis, we found that pre-treatment with sumatriptan reduced PACAP38-induced headache compared to no treatment (AUC<sub>0–90min</sub>).

**Conclusions:** Post-treatment with ketorolac was more effective in attenuating PACAP38-induced headache compared to sumatriptan. Ketorolac exerted its effect without affecting PACAP38-induced arterial dilation, whereas sumatriptan post-treatment attenuated PACAP38-induced dilation of MMA and STA. Pre-treatment with sumatriptan attenuated PACAP38-induced headache without affecting PACAP38-induced arterial dilation. Our findings suggest that ketorolac and sumatriptan attenuated PACAP38-induced headache in healthy volunteers without vascular effects.

**Trial registration:** [Clinicaltrials.gov \(NCT03585894\)](https://clinicaltrials.gov/ct2/show/study/NCT03585894). Registered 13 July 2018,

**Keywords:** Headache, PACAP38, Mast cell degranulation, Plasma protein extravasation, Arterial dilation, Neuroinflammation, Pain, NSAIDs, MRA

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## Background

Pituitary adenylate cyclase-activating polypeptide 38 (PACAP38) is a pleiotropic signaling neuropeptide [33, 34] that induces headache in healthy volunteers and migraine attacks in migraine patients [4, 42]. PACAP38 is located in both sensory and parasympathetic perivascular nerve fibers [35, 52] and its infusion causes prolonged extracerebral dilation [5, 7, 13, 20, 49] and dural mast cell degranulation [12]. Recent data showed that PACAP38 activated mast cell specific receptor Mas-related G-protein-coupled receptors-b2 (Mrgprb2) [22, 38] which mediates neurogenic inflammation and pain [22]. Activation of mast cells leads to recruitment of dural immune cells involving neutrophils, monocytes and macrophages [25, 40, 47]. Dural neurogenic inflammation and mast cell mediated activation of the trigeminal pain pathway have been suggested to play a key role in migraine pathogenesis [31, 36]. The precise mechanisms by which PACAP38 leads to headache and migraine are unclear.

The anti-migraine-specific drug sumatriptan, a 5-HT<sub>1B</sub> /<sub>1D</sub> agonist [18], is a vasoconstrictor [6, 27] with anti-inflammatory properties [16] that potently blocks neurogenic plasma extravasation from dural blood vessels [17]. Ketorolac is cyclooxygenase (COX-1 and COX-2) inhibitor non-steroidal anti-inflammatory drug [41, 44, 48] that reduces mast cell degranulation [50] and blocks dural macrophage activation [37]. The neurovascular effects of ketorolac have not been studied in humans. Sumatriptan and ketorolac are used as abortive medication for migraine treatment but the site and mode of action of these drugs are not fully clarified.

In the present study, we used PACAP38 as a biomarker of headache with inflammatory and vascular components. To further elucidate the mechanisms underlying the action of sumatriptan and ketorolac, we investigated the effect of both drugs on PACAP38-induced headache in healthy volunteers. We hypothesized that both sumatriptan and ketorolac would attenuate PACAP38-induced headache but only sumatriptan infusion would abolish PACAP38-induced arterial dilation. To test this hypothesis we conducted a randomized, double-blind, crossover study and used magnetic resonance angiography (MRA) to record vascular responses.

## Methods

### Participants

We recruited thirty-four healthy volunteers. All participants were pre-screened over telephone and all potential study candidates were invited to the hospital for thorough screening. The eligibility criteria for inclusion in the study were as follows; adults  $\geq 18$  to  $\leq 50$  years of age of both sexes with body weight of 50 to 100 kg. Exclusion criteria included: daily intake of any medication

except contraceptives, magnetic resonance imaging contraindications, serious somatic disease (including any pain condition), history of migraine or any other type of headache expect episodic tension-type headache less than once a month. All participants provided detailed oral and written information about the study and written informed consent was obtained in accordance with the Helsinki declarations. The study was approved by the Ethics Committee of the Capital Region of Denmark (H-18008313) and registered at [Clinicaltrials.gov](https://clinicaltrials.gov) (ID: NCT03585894).

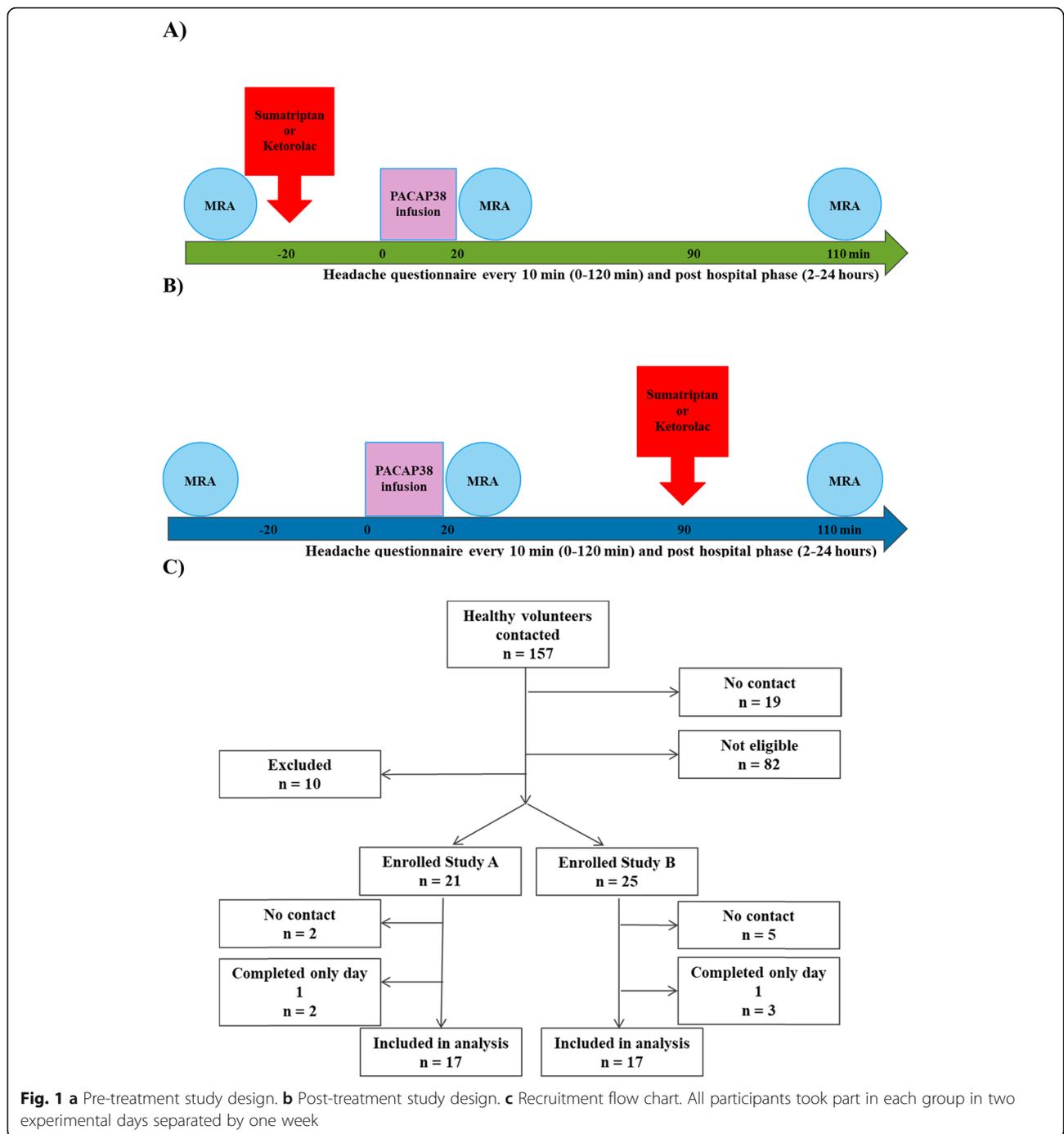
### Experimental design

We divided participants into two groups: group A and group B. In group A, participants were randomly assigned to intravenous infusion of sumatriptan 4 mg (GlaxoSmithKline Pharma A/S, Denmark) or ketorolac trometamol 30 mg (Atnahs Pharma, UK Limited) over 10 min. At 20 min after start of infusion of sumatriptan and ketorolac participants received infusion of PACAP38 (10 picomole/kg/min) [42] over 20 min (Fig. 1 a). In group B, participants first received PACAP38 infusion over 20 min and at 90 min after start of infusion randomly assigned to receive infusion of sumatriptan or ketorolac (Fig. 1 b). In each group, experiments were conducted on two separate days with a washout period of one week. The PACAP38 (Bachem) solution was prepared for the study by the Capital Region Hospital Pharmacy.

All participants were non-fasting and arrived at the hospital 24 h headache free and were instructed not to consume coffee, tea, cola, caffeine, alcohol, smoking and avoid exhausting physical activities for 12 h prior to the study day. On arrival, female participants were tested for pregnancy on both days and a venous catheter (Venflon, Becton Dickinson Infusion Therapy AB, Sweden) was inserted into the antecubital vein for infusion. After 15 min of rest in the supine position, headache intensity and characteristics, vital signs, mean arterial blood pressure (MAP), and heart rate (HR) were recorded at baseline and at fixed intervals every 10 min (0–120 min). All participants underwent three MRI sessions: baseline, 20 min and 110 min (Fig. 1). All experiments were conducted in the afternoon due to limited availability of the MRI scanner. We were therefore only able to record headache data from all participants from 0 to 6 h since most participants went to sleep thereafter.

### Headache

We used a validated headache questionnaire to record headache intensity using numerical rating scale 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) and associated symptoms. Headache characteristics (localization, quality, aggravation by



physical activity, associated symptoms (nausea, photo- and phonophobia) and prodromes (unusual fatigue, yawning, thirst, craving, mood swings, flushing and difficulty concentrating) were also recorded.

**Data acquisition and image analysis**

All scans were performed on a Philips 3 T Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) using a 32-channel phase array head coil. A 3D time-of-

flight MRA of extra-intracerebral arteries was acquired as described in previous studies [5, 6]. All acquired MRA data were saved in DICOM format and transferred to a separate workstation and analyzed by LAVA-MRA vessel wall analysis software program. LAVA-MRA was previously used in several studies [4, 8] and the method demonstrated a low < 5% inter- and intra-observer variation [3]. The software automatically detects the vessel contours and calculate the circumference every 0.2 mm perpendicular to the

center line. For each vessel an average of 26 slides (5 mm) was obtained and the measurement was repeated for all participant at the same vessel segment.

Two bilateral branches of superficial temporal artery (STA), middle meningeal artery (MMA) and middle cerebral artery (MCA) were analyzed by an investigator who was blinded to the experimental day and scan session.

### Statistical analysis

All absolute values are presented as mean with 95% confidence interval (CI). We calculated the study sample size based on headache and vascular responses to PACAP38 taking the previous findings into consideration [5]. The risk of type 1 error at 5% and a defined power at 80% and type 2 error was fixed at 20%. We calculated that thirty-four participants would be adequate for a crossover study design.

The primary endpoints of the study were a difference in the area under the curve (AUC) for headache (0–6 h) and arterial circumference change of STA, MMA and MCA from baseline to 110 min between treatment with sumatriptan and ketorolac. The secondary endpoints were difference in AUC for HR (0–120 min), MAP (0–120 min) and associate symptoms (0–24 h). Baseline values were subtracted before calculating AUC to reduce within-participant variation between sessions. The non-parametric Wilcoxon signed rank test was used to calculate the side to side difference in arterial circumference. We used Mann-Whitney test to compare the differences between the groups.

All statistical analyses were performed with SPSS version 23.0 (Chicago, IL, USA). We did not adjust for multiple comparisons, as our primary endpoints, hypotheses and statistical tests were all predefined and clearly stated in the study protocol. Five percent ( $p < 0.05$ ) was accepted as the level of significance.

### Results

Thirty-four healthy volunteers (18 females and 16 males, mean age 25 years, (range 19–39 years) and mean weight of 70 kg, (range 54–88 kg)) completed the study.

PACAP38 infusion induced headache in sixteen out of seventeen (94%) subjects pre-treated (10 females and 7 males) with ketorolac and thirteen out of seventeen (77%) pre-treated with sumatriptan. In post-treatment group (8 females and 9 males), PACAP38 infusion induced headache in sixteen out of seventeen (94%) treated with ketorolac and seventeen out of seventeen (100%) treated with sumatriptan. (Table 1).

We found no difference in arterial circumferences and vital variables at baseline between sumatriptan and ketorolac day in both study groups (A and B). There was no carry-over or period effect for baseline values between the study days. We found no difference between the right and left sided arteries ( $p > 0.05$ ) and therefore, an average of both arteries was used.

### Effect of pre-treatment of sumatriptan or ketorolac: group A

We found no difference in AUC<sub>(0–6 h)</sub> for PACAP38-induced headache between sumatriptan and ketorolac ( $p = 0.297$ ) (Fig. 2, Table 1). There was no difference in PACAP38-induced circumference change (AUC<sub>Baseline–110 min</sub>) of MMA ( $p = 0.227$ ), STA ( $p = 0.795$ ) and MCA ( $p = 0.356$ ) after sumatriptan compared to ketorolac (Fig. 3).

### Effect of post-treatment of sumatriptan or ketorolac: group B

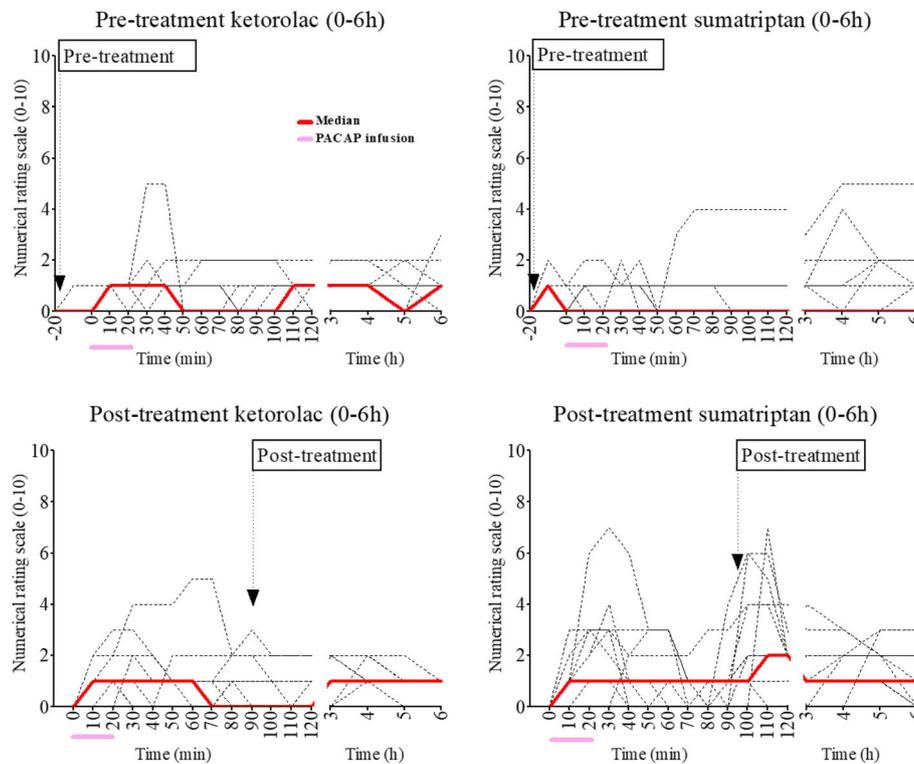
We found that ketorolac reduced the AUC<sub>(1.5–6 h)</sub> for PACAP38-induced headache compared to sumatriptan ( $p < 0.001$ ) (Fig. 2, Table 1). Sumatriptan significantly reduced the circumference of STA ( $p = 0.039$ ) and MMA ( $p = 0.015$ ) compared to ketorolac. No difference was found in circumference change of MCA ( $p = 0.981$ ) (Fig. 3).

### Explorative analyses on headache data

To explore whether both drugs prevent PACAP38-induced headache we conducted an explorative analysis on headache data between group A and B from 0 to 90 min: pretreatment with sumatriptan or ketorolac

**Table 1** Headache incidence and characteristics after PACAP38 infusion and treatment with ketorolac and sumatriptan from 0 to 2 and 2–6 h. Headache inductions rate: number of participants who developed headache; Median peak headache: peak headache intensity recorded on a numerical rating scale from 0 to 10

PACAP38 infusion/treatment	0–2 h Headache induction rate	2–6 h Headache induction rate	0–2 h Median Peak headache (range)	2–6 h Median Peak headache (range)	0–2 h Median Duration of headache (hours)	2–6 h Median Duration of headache (hours)
Pre-treatment Ketorolac	16 of 17	13 of 17	1 (0–5)	0 (0–3)	1	2
Pre-treatment Sumatriptan	13 of 17	8 of 17	1 (0–4)	0 (0–5)	0.17	1
Post-treatment Ketorolac	16 of 17	12 of 17	1 (0–5)	1 (0–2)	1.17	4
Post-treatment Sumatriptan	17 of 17	12 of 17	2 (0–7)	1 (0–4)	1.67	2



**Fig. 2** Headache intensity of individual volunteers (black lines) and the median headache intensity (red line) after PACAP38 and pre-treatment with ketorolac /sumatriptan ( $n = 17$ ) and post-treatment with ketorolac /sumatriptan ( $n = 17$ )

followed by PACAP38 infusion compared to group B where participants received only PACAP38 infusion up to 90 mins. The AUC<sub>(0–90 min)</sub> for headache score was significantly larger after PACAP38 infusion compared to PACAP38-induced headache pre-treated with sumatriptan ( $p = 0.005$ ). A trend of attenuation of PACAP38-induced headache was recorded in those who were pre-treated with ketorolac compared to only PACAP38 infusion ( $p = 0.076$ ) (Fig. 2).

#### Vital variables and adverse events

In pre-treatment group A, we found no difference in AUC<sub>(Baseline–120 min)</sub> for MAP changes between ketorolac and sumatriptan ( $p = 0.523$ ). In post-treatment group B, the AUC<sub>(Baseline–120 min)</sub> for MAP was significantly larger after sumatriptan compared to ketorolac ( $p = 0.028$ ). We found no change in AUC<sub>(Baseline–120 min)</sub> for heart rate in pre-treatment group between sumatriptan and ketorolac ( $p = 0.492$ ) and post-treatment group between sumatriptan and ketorolac ( $p = 0.356$ ) (Fig. 4).

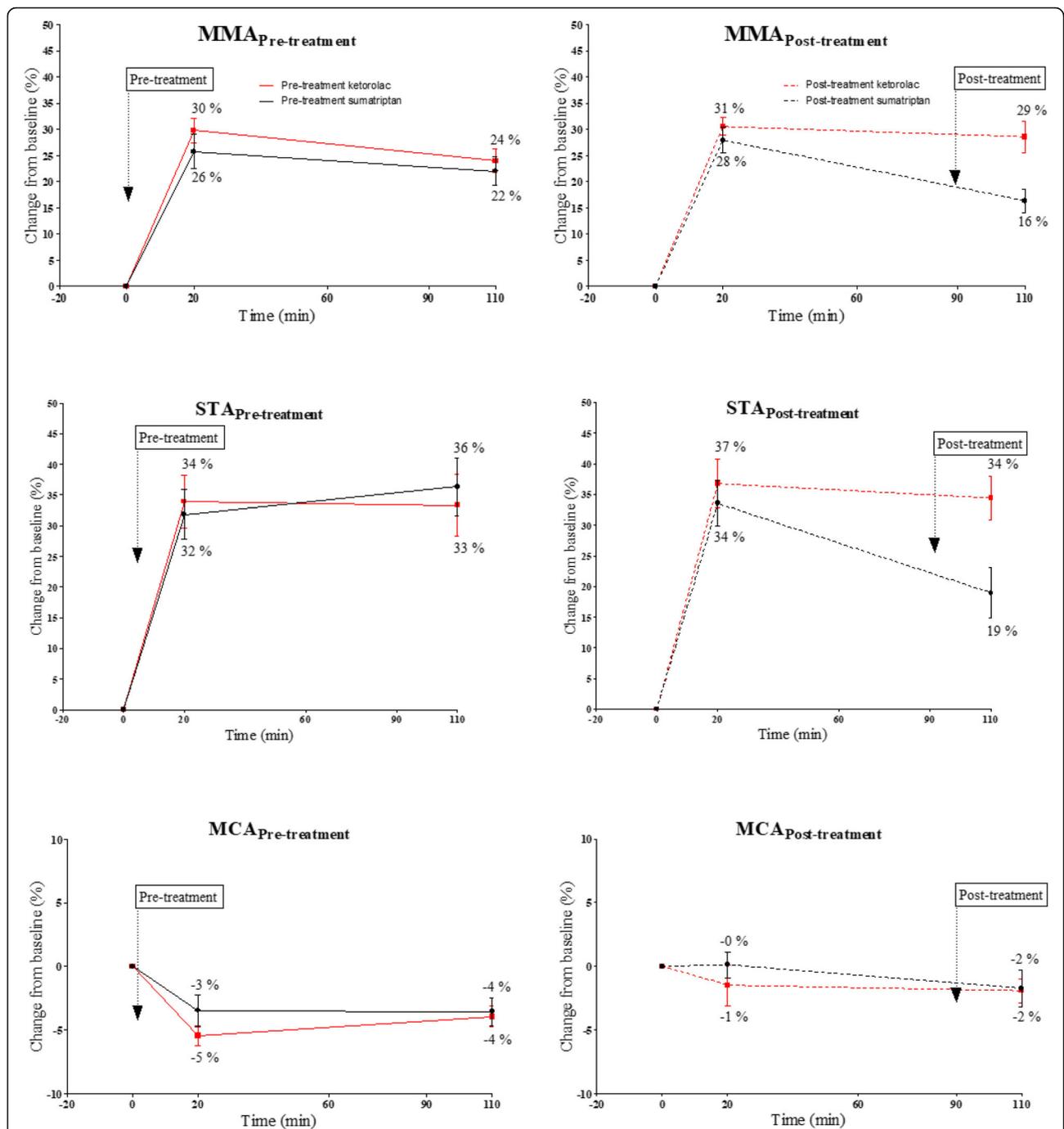
There was no difference in incidence of nausea, difficulty concentrating, neck stiffness, fatigue, thirst and facial puffiness (Table 2).

#### Discussion

The main findings of the present study were: 1) Participants who were pre-treated with sumatriptan or ketorolac reported no difference in PACAP38-induced headache; 2) Post-treatment with ketorolac was more effective in attenuating PACAP38-induced headache compared to sumatriptan. Interestingly, the ketorolac exerted its effect without affecting PACAP38-induced arterial dilation, whereas sumatriptan post-treatment attenuated PACAP38-induced dilation of MMA and STA. Explorative analysis showed that pre-treatment with sumatriptan attenuated PACAP38-induced headache without affecting PACAP38-induced arterial dilation. In the current study both drugs given as pre- or post-treatment had no effect on PACAP38-induced autonomic symptoms such as palpitation, facial flushing and heat sensation. Most participants reported facial flushing and puffiness persisting over 24 h post PACAP38 infusion. PACAP is found in human skin [53] and PACAP infusion causes intense facial flushing [21].

#### Experimentally induced headache and anti-migraine medication

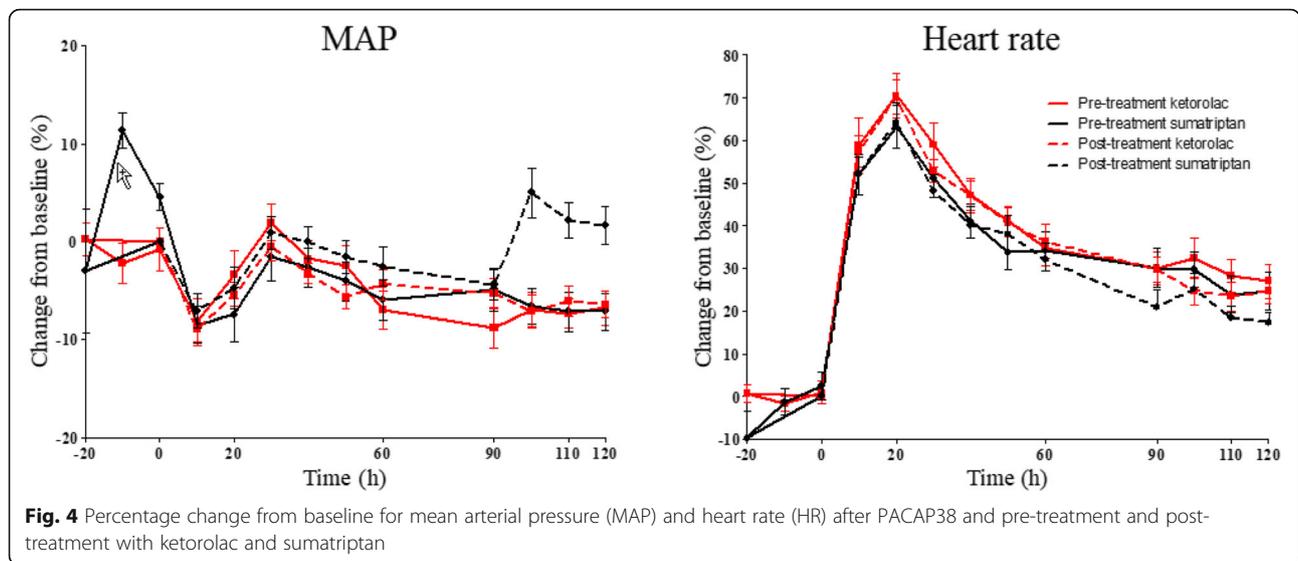
Similar to previous studies [5, 42], we found that PACAP38 infusion caused prolonged mild to moderate



**Fig. 3** Effect of pre-treatment (solid line) and post-treatment (dotted line) on extra-intracerebral arteries dilated with PACAP38. Middle meningeal artery (MMA), superficial temporal artery (STA) and middle cerebral artery (MCA)

headache in 90% of healthy volunteers. The headache preventive effect of sumatriptan when given before PACAP38 infusion is consistent with previous studies demonstrating that pre-treatment with sumatriptan reduced glycerol trinitrate (GTN) [28] and cilostazol [19] induced headache in healthy volunteers. GTN is a pro-drug for nitric oxide (NO) and cilostazol is a

phosphodiesterase 3 inhibitor which works downstream in the cascade of events associated to headache [19, 28]. It has been suggested that sumatriptan prevents headache in these models by inhibiting accumulation of cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP). Interestingly, post-treatment with triptans failed to antagonize GTN and



cilostazol induced headache in healthy volunteers [23, 46]. In line with this, we also show that post-treatment with intravenous administration of sumatriptan failed to prevent PACAP38-induced headache in healthy volunteers. The failure of sumatriptan to abort PACAP38-induced headache might be due to establishment of central sensitization and disruption of presynaptic 5-HT<sub>1B/1D</sub> receptors in the dorsal horn [32].

The lower AUC for headache score (2–6 h) after ketorolac compared to sumatriptan post-treatment suggests that ketorolac was more effective than sumatriptan when administered as post-treatment during the established PACAP38-induced headache phase in healthy volunteers. Our findings are consistent with previous studies demonstrating that ketorolac infusion is able to terminate established peripheral and central sensitization [29]. However, our results should be interpreted with caution because sumatriptan infusion caused immediate but short exacerbation of PACAP38-induced headache. The sumatriptan-induced headache was previously observed after infusion of calcitonin gene-related peptide

(CGRP), levromakalim, isosorbide-5-mononitrate (NO donor) and cilostazol in healthy volunteers [2, 9, 23, 24]. It is also a well-known side effect of sumatriptan treatment in migraine patients [15, 39]. This may explain the difference between the two drugs administered as post-treatment. However, the similar incidence of headache (Table 1) and unchanged median headache score (Fig. 2) for both drugs in post-hospital phase indicates that both drugs are not effective in aborting PACAP38-induced headache during this phase.

**Cranial artery dilation and anti-migraine medication**

Similar to previous studies [4, 5], we found that PACAP38 infusion caused sustained dilation of extracerebral arteries but not intracerebral arteries. Though earlier studies have shown that sumatriptan constricts extracerebral arteries [5, 6], sumatriptan pre-treatment was unable to counteract PACAP38-induced dilation of MMA and STA. This result shows that PACAP38 bypass the vasoconstrictive effect of sumatriptan in these arteries in healthy volunteers. The headache preventive effect

**Table 2** Adverse events after PACAP38 infusion and treatment with ketorolac and sumatriptan

Variables	Pre-treatment (Ketorolac)	Pre-treatment (Sumatriptan)	Post-treatment (Ketorolac)	Post-treatment (Sumatriptan)
Palpitation	100%	94.1%	100%	100%
Flushing	100%	100%	100%	100%
Heat sensation	100%	100%	100%	100%
Nausea	0%	0%	0%	0%
Neck stiffness	11.8%	11.7%	17.7%	17.7%
Difficulty concentrating	5.9%	5.9%	23.5%	17.8%
Fatigue	23.5%	17.7%	23.5%	23.5%
Thirst	17.7%	11.8%	11.8%	23.5%
Facial puffiness	41.2%	35.3%	41.2%	17.7%

of sumatriptan given before PACAP38 infusion without affecting the vasodilatory response to PACAP38 suggest that the headache inducing effect of PACAP38 is independent of its vasoactive property in healthy volunteers.

In the present study, we found that post-treatment with sumatriptan but not ketorolac reduced MMA and STA circumference, but the dilation of MMA and STA did not return to baseline during the observation period. In humans, sumatriptan constricts normal and pre-dilated extracerebral arteries [5, 6, 10]. In healthy volunteers, subcutaneous injection of sumatriptan totally abolished a modest CGRP induced dilation of MMA [11]. Taken together, it seems that PACAP38-induced vasodilation mechanistically differs from CGRP and its prolonged dilation of extracerebral arteries might be caused via activation of dural mast cells [12]. Identification of a putative new PACAP-receptor on mast cells provides important insight on PACAP38-induced prolonged dilation of extracerebral arteries [38]. Dural mast cells are found in close proximity to meningeal nociceptors, whereupon activation releases vasoactive neuropeptides which mediates activation of pain pathways [22].

To the best of our knowledge, no study investigated possible vasoactive properties of ketorolac using advanced MRA method. Our data demonstrated that ketorolac had no vascular effect on extra-intracerebral arteries and neither pre nor post-treatment alters the vascular effects of PACAP38. It is possible that pro-inflammatory prostanoids are released by PACAP38 induced mast cell degranulation [12, 30, 45] and the headache attenuating effect of ketorolac treatment might be via inhibition of pro-inflammatory prostanoids activated by PACAP38 infusion.

#### **Mechanisms behind the anti-nociceptive effect of sumatriptan and ketorolac**

It has been reported that PACAP38 induces sensitization of trigeminal neurons via activation of neuronal PAC<sub>1</sub> receptor [1]. In animals, pre-treatment with sumatriptan effectively blocked the development of all aspects of central sensitization by blocking the peripheral signal transmission from the meningeal nociceptors [14]. Moreover, it has been shown that sumatriptan can inhibit trigeminal activation without its vasoconstrictive effects [26]. It has been postulated that sumatriptan exerts its antinociceptive effect by disrupting communication between peripheral and central trigeminovascular neurons [14]. In the current study, we showed that pre-treatment with sumatriptan was more effective in attenuating PACAP38-induced headache. Ketorolac may exert its effect by suppression of central sensitization by directly silencing the peripheral and central trigeminovascular neurons [29]. Ketorolac treatment was more effective in terminating headache and allodynia in migraine patients who had

established central sensitization [29]. Interestingly, we found that pre-treatment with ketorolac failed to prevent PACAP38-induced headache, though as mentioned a trend was observed. We suggest that the antinociceptive effect of ketorolac may partly depend on a prior activation of pro-inflammatory prostanoids.

PACAP38 degranulates dural mast cells and this mechanism may mediate PACAP38-induced prolonged arterial dilation [12]. Recent studies reported that PACAP38 caused degranulation of mast cells and histamine release via a specific receptor Mrgprb2 [22] which leads to prolonged activation of the trigeminal pain pathway [31]. In animals, sumatriptan inhibits mast cell degranulation [31], potently blocks neurogenic plasma protein extravasation from dural blood vessels [17] and prevents release of neuropeptides from perivascular neurons [16, 17]. As a COX inhibitor ketorolac exerts its analgesic and anti-inflammatory effects via depression of prostanoid biosynthesis [43]. Prostaglandins and their receptors are widely distributed in the extra-intracerebral arteries, trigeminal ganglion and trigeminal nucleus caudalis [43]. In addition to histamine dural mast cells can release various inflammatory mediators including prostaglandin I<sub>2</sub> that can activate and sensitize meningeal sensory afferents [51].

Taken together, the current study revealed that sumatriptan pre-treatment was more effective in preventing PACAP38-induced headache. This suggests that sumatriptan may have time dependent preventative properties which require further investigation.

#### **Conclusion**

The major finding of the present study was that no difference reported in PACAP38-induced headache after pre-treatment with sumatriptan or ketorolac. We found that post-treatment with ketorolac was more effective in attenuating PACAP38-induced headache compared to sumatriptan. Ketorolac exerted its effect without affecting PACAP38-induced arterial dilation, whereas sumatriptan post-treatment attenuated PACAP38-induced dilation of MMA and STA. Explorative analysis showed that pre-treatment with sumatriptan attenuated PACAP38-induced headache without affecting PACAP38-induced arterial dilation.

#### **Abbreviations**

AUC: Under the curve; cAMP: Cyclic adenosine monophosphate; cGMP: Cyclic guanosine monophosphate; CGRP: Calcitonin gene-related peptide; CI: Confidence interval; COX: Cyclooxygenase; GTN: Glycerol trinitrate; HR: Heart rate; MAP: Mean arterial blood pressure; MCA: Middle cerebral artery; MMA: Middle meningeal artery; MRA: Magnetic resonance angiography; Mrgprb2: Mas-related G-protein-coupled receptors-b2; NO: Nitric oxide; PACAP38: Pituitary adenylate cyclase-activating polypeptide-38; STA: Superficial temporal artery

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**Authors' contributions**

HG: study concept and design, acquisition, analysis and interpretation of data, and drafting and revision of manuscript. MAA: acquisition, analysis and interpretation of data, and drafting and revision of manuscript. NA: study concept and design, critical revision of manuscript for intellectual content. MMR and MMC: acquisition and analysis of data. HBWL and FMA: study concept and design, critical revision of manuscript for intellectual content. MA: study concept and design, interpretation of data, critical revision of manuscript, and study initiation and supervision. All authors read and approved the final manuscript.

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**Availability of data and materials**

Anonymized data can be shared, until one year after publication, upon request to the corresponding author from qualified investigators for purposes of replicating procedures and results.

**Ethics approval and consent to participate**

All participants provided written consent to participate after receiving written and oral information in accordance with the Declaration of Helsinki of 1964, with subsequent revisions. The study was approved by the Ethics Committee of the Capital Region of Denmark (H-18008313). The study was registered retrospectively at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03585894) (NCT03585894).

**Consent for publication**

Not applicable.

**Competing interests**

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Messoud Ashina is a consultant, speaker or scientific advisor for Allergan, Amgen, Alder, ATI, Eli Lilly, Novartis, and Teva, primary investigator for Alder, Amgen, ElectroCore, Novartis and Teva trials. MA has no ownership interest and does not own stocks of any pharmaceutical company. MA serves as associated editor of Cephalalgia, Headache, and co-editor of the Journal of Headache and Pain. MA is the President of the International Headache Society. Mohammad Al-Mahdi Al-Karagholi has acted as an invited speaker for Novartis and received travel grant from ElectroCore. Hashmat Ghanizada, Nanna Arnggrim, Faisal Mohammad Amin, Mette Mørch-Rasmussen, Matias Metcalf-Clausen and Henrik Bo Wiberg Larsson declare they have no conflicts of interest.

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**References**

- Akerman S, Goadsby PJ (2015) Neuronal PAC1 receptors mediate delayed activation and sensitization of trigeminocervical neurons: Relevance to migraine. *Sci Transl Med* 7:308ra157. <https://doi.org/10.1126/scitranslmed.aaa7557>
- Al-Karagholi MA-M, Ghanizada H, Hansen JM, Skovgaard LT, Olesen J, Larsson HBW, Ashina M (2019) Levromakalim, an adenosine triphosphate-sensitive Potassium Channel opener, dilates Extracerebral but not arteries, cerebral. *Headache*:1468–1480
- Amin F, Lundholm E, Hougaard A, Arnggrim N, Wiinberg L, de Koning PJ, Larsson HBW, Ashina M (2014) Measurement precision and biological variation of cranial arteries using automated analysis of 3 T magnetic resonance angiography. *J Headache Pain* 15:25. <https://doi.org/10.1186/1129-2377-15-25>
- Amin FM, Hougaard A, Schytz HW, Asghar MS, Lundholm E, Parvaiz AI, De Koning PJH, Andersen MR, Larsson HBW, Fahrenkrug J, Olesen J, Ashina M (2014) Investigation of the pathophysiological mechanisms of migraine attacks induced by pituitary adenylate cyclase-activating polypeptide-38. *Brain* 137:779–794
- Amin FM, Asghar MS, Guo S, Hougaard A, Hansen AE, Schytz HW, van der Geest RJ, de Koning PJ, Larsson HB, Olesen J, Ashina M (2012) Headache and prolonged dilatation of the middle meningeal artery by PACAP38 in healthy volunteers. *Cephalalgia* 32:140–149. <https://doi.org/10.1177/0333102411431333>
- Amin FM, Asghar MS, Ravneberg JW, De Koning PJ, Larsson HB, Olesen J, Ashina M (2013) The effect of sumatriptan on cephalic arteries: a 3T MR-angiography study in healthy volunteers. *Cephalalgia* 33:1009–1016
- Anzai M, Suzuki Y, Takayasu M, Kajita Y, Mori Y, Seki Y, Saito K, Shibuya M (1995) Vasorelaxant effect of PACAP-27 on canine cerebral arteries and rat intracerebral arterioles. *Eur J Pharmacol* 285:173–179
- Arnggrim N, Schytz HW, Britze J, Amin FM, Vestergaards MB, Hougaard A, Wolfram F, de Koning PJH, Olsen KS, Secher NH, Larsson HBW, Olesen J, Ashina M (2015) Migraine induced by hypoxia: a MRI spectroscopy and angiography study. *Brain*:723–737
- Asghar MS, Becerra L, Larsson HBW, Borsook D. Calcitonin Gene-Related Peptide Modulates Heat Nociception in the Human Brain - An fMRI Study in Healthy Volunteers 2016:1–20
- Asghar MS, Hansen AE, Kapijimpanga T, Van Der Geest RJ, Van Der Koning P, Larsson HBW, Olesen J, Ashina M (2010) Dilation by CGRP of middle meningeal artery and reversal by sumatriptan in normal volunteers. *Neurology* 75:1520–1526
- Asghar MS, Hansen AE, Amin FM, van der Geest RJ, Van Der Koning P, Larsson HBW, Olesen J, Ashina M (2011) Evidence for a vascular factor in migraine. *Ann Neurol* 69:635–645
- Baun M, Pedersen MHF, Olesen J, Jansen-Olesen I (2012) Dural mast cell degranulation is a putative mechanism for headache induced by PACAP-38. *Cephalalgia* 32:337–345. <https://doi.org/10.1177/0333102412439354>
- Birk S, Sitarz JT, Petersen KA, Oturai PS, Kruse C, Fahrenkrug J, Olesen J (2007) The effect of intravenous PACAP38 on cerebral hemodynamics in healthy volunteers. *Regul Pept* 140:185–191
- Burstein R, Jakubowski M (2004) Analgesic Triptan action in an animal model of intracranial pain: a race against the development of central sensitization. *Ann Neurol* 55:27–36
- Burstein R, Jakubowski M, Levy D (2005) Anti-migraine action of triptans is preceded by transient aggravation of headache caused by activation of meningeal nociceptors. *Pain* 115:21–28
- Buzzi MG, Dimitriadou V, Theoharides TC, Moskowitz MA (1992) 5-Hydroxytryptamine receptor agonists for the abortive treatment of vascular headaches block mast cell, endothelial and platelet activation within the rat dura mater after trigeminal stimulation. *Brain Res* 583:137–149. [https://doi.org/10.1016/S0006-8993\(10\)80017-4](https://doi.org/10.1016/S0006-8993(10)80017-4)
- Buzzi MG, Moskowitz MA (1990) The antimigraine drug, sumatriptan (GR43175), selectively blocks neurogenic plasma extravasation from blood vessels in dura mater. *Br J Pharmacol* 99:202–206
- Doenicke A, Brand J, Perrin VL (1988) Possible benefit of GR43175, a novel 5-HT<sub>1</sub>-like receptor agonist, for the acute treatment of severe migraine. *Lancet* (London, England) 1:1309–1311
- Falkenberg K, Olesen J (2018) Pre-treatment with sumatriptan for cilostazol induced headache in healthy volunteers. *J Headache Pain* 19(1):71. <https://doi.org/10.1186/s10194-018-0890-y>
- Ghanizada H, Al-Mahdi Al-Karagholi M, Arnggrim N, Ghanizada M, Wiberg Larsson HB, Amin FM, Ashina M (2019) Effect of pituitary adenylate cyclase-activating polypeptide-27 on cerebral hemodynamics in healthy volunteers: a 3T MRI study. *Peptides* 121:170134. <https://doi.org/10.1016/j.peptides.2019.170134>
- Ghanizada H, Al-Mahdi Al-Karagholi M, Arnggrim N, Olesen J, Ashina M. PACAP27 induces migraine-like attacks in migraine patients. *Cephalalgia* 2019:033310241986450. doi:<https://doi.org/10.1177/0333102419864507>
- Green DP, Limjunyawong N, Gour N, Pundir P, Dong X (2019) A Mast-Cell-Specific Receptor Mediates Neurogenic Inflammation and Pain. *Neuron* 101:412–420.e3. <https://doi.org/10.1016/j.neuron.2019.01.012>
- Hansen EK, Guo S, Ashina M, Olesen J (2016) Toward a pragmatic migraine model for drug testing: 1. Cilostazol in healthy volunteers. *Cephalalgia* 36:172–178
- Hansen EK, Olesen J (2017) Towards a pragmatic human migraine model for drug testing: 2. Isosorbide-5-mononitrate in healthy individuals. *Cephalalgia* 37:11–19

25. Héron A, Dubayle D (2013) A focus on mast cells and pain. *J Neuroimmunol* 264:1–7. <https://doi.org/10.1016/j.jneuroim.2013.09.018>
26. Hoskin KL, Kaube H, Goadsby PJ (1996) Sumatriptan can inhibit trigeminal afferents by an exclusively neural mechanism. *Brain* 119:1419–1428
27. Humphrey PP, Feniuk W, Marriott AS, Tanner RJ, Jackson MR, Tucker ML (1991) Preclinical studies on the anti-migraine drug, sumatriptan. *Eur Neurol* 31:282–290
28. Iversen H, Olesen J (1996) Headache induced by a nitric oxide donor (nitroglycerin) responds to Sumatriptan. A human model for development of migraine drugs. *Cephalalgia* 16:412–418. <https://doi.org/10.1046/j.1468-2982.1996.1606412.x>
29. Jakubowski M, Levy D, Goor-Aryeh I, Collins B, Bajwa Z, Burstein R (2005) Terminating migraine with allodynia and ongoing central sensitization using parenteral administration of COX1/COX2 inhibitors. *Headache* 45:850–861
30. Jansen-Olesen I, Hougaard Pedersen S. PACAP and its receptors in cranial arteries and mast cells. *J Headache Pain* 2018;19
31. Levy D, Burstein R, Kainz V, Jakubowski M, Strassman AM (2007) Mast cell degranulation activates a pain pathway underlying migraine headache. *Pain* 130:166–176
32. Levy D, Jakubowski M, Burstein R (2004) Disruption of communication between peripheral and central trigeminovascular neurons mediates the antimigraine action of 5HT<sub>1B/1D</sub> receptor agonists. *Proc Natl Acad Sci U S A* 101:4274–4279
33. Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD, Coy DH (1989) Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun* 164:567–574
34. Miyata A, Jiang L, Dahl RD, Kitada C, Kubo K, Fujino M, Minamino N, Arimura A (1990) Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). *Biochem Biophys Res Commun* 170:643–648
35. Moller K, Zhang YZ, Hakanson R, Luts A, Sjolund B, Uddman R, Sundler F (1993) Pituitary adenylate cyclase activating peptide is a sensory neuropeptide: immunocytochemical and immunochemical evidence. *Neuroscience* 57:725–732
36. Moskowitz MA (1993) Neurogenic inflammation in the pathophysiology and treatment of migraine. *Neurology* 43:516–520
37. Pardutz A, Schoenen J (2010) NSAIDs in the acute treatment of migraine: a review of clinical and experimental data. *Pharmaceuticals* 3:1966–1987
38. Pedersen SH, la Cour SH, Calloe K, Hauser F, Olesen J, Klaerke DA, Jansen-Olesen I (2019) PACAP-38 and PACAP (6–38) Degranulate rat meningeal mast cells via the orphan MrgB3-receptor. *Front Cell Neurosci* 13:1–11. <https://doi.org/10.3389/fncel.2019.00114>
39. Peroutka SJ, McCarthy BG (1989) Sumatriptan (GR 43175) interacts selectively with 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> binding sites. *Eur J Pharmacol* 163:133–136
40. Reuter U (2001) Delayed inflammation in rat meninges: implications for migraine pathophysiology. *Brain* 124:2490–2502
41. Rice ASC, Lloyd J, Bullingham RES, O'Sullivan G (1993) Ketorolac penetration into the cerebrospinal fluid of humans. *J Clin Anesth* 5:459–462
42. Schytz HW, Birk S, Wienecke T, Kruuse C, Olesen J, Ashina M (2009) PACAP38 induces migraine-like attacks in patients with migraine without aura. *Brain* 132:16–25
43. Silberstein SD, Stirpe JC (2014) COX inhibitors for the treatment of migraine. *Expert Opin Pharmacother* 15:1863–1874. <https://doi.org/10.1517/14656566.2014.937704>
44. Sinha V, Kumar R, Singh G (2009) Ketorolac tromethamine formulations: an overview. *Expert Opin Drug Deliv* 6:961–975. <https://doi.org/10.1517/17425240903116006>
45. Theoharides TC, Donelan J, Kandere-Grzybowska K, Konstantinidou A (2005) The role of mast cells in migraine pathophysiology. *Brain Res Rev* 49:65–76
46. Tvedskov JF, Iversen HK, Olesen J, Tfelt-Hansen P (2010) Nitroglycerin provocation in normal subjects is not a useful human migraine model? *Cephalalgia* 30:928–932
47. Valledor AF, Comalada M, Santamaría-Babi LF, Lloberas J, Celada A (2010) Macrophage Proinflammatory activation and deactivation. *Quest Balance Adv Immunol* 108:1–20
48. Vane JR, Botting RM (1998) Mechanism of action of nonsteroidal anti-inflammatory drugs. *Am J Med* 104:2S–8S discussion 21S–22S
49. Vaudry D, Falluel-morel A, Bourgault S, Basille M, Burel D, Wurtz O, Fournier A, Chow BKC, Hashimoto H, Galas L, Vaudry H, National I, Sante D (2009) Pituitary Adenylate Cyclase-activating polypeptide and its receptors : 20 years after the discovery. *Pept Res* 61:283–357. <https://doi.org/10.1124/pr.109.001370.283>
50. Zhang XC, Kainz V, Burstein R, Levy D (2011) Tumor necrosis factor- $\alpha$  induces sensitization of meningeal nociceptors mediated via local COX and p38 MAP kinase actions. *Pain* 152:140–149. <https://doi.org/10.1016/j.pain.2010.10.002>
51. Zhang XC, Strassman AM, Burstein R, Levy D (2007) Sensitization and activation of intracranial meningeal nociceptors by mast cell mediators. *J Pharmacol Exp Ther* 322:806–812
52. Zhang YZ, Sjo B, Moller K, Hakanson R, Sundler F (1993) Pituitary adenylate cyclase activating peptide produces a marked and long-lasting depression of a C-fibre-evoked flexion reflex. *Neuroscience* 57:733–737
53. Ødum L, Petersen LJ, Skov PS, Ebskov LB (1998) Pituitary adenylate cyclase activating polypeptide (PACAP) is localized in human dermal neurons and causes histamine release from skin mast cells. *Inflamm Res* 47:488–492

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