Effect of Adrenomedullin on Migraine-like Attacks in Patients With Migraine: A Randomized Crossover Study

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Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.
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Search terms: 1: Headache, 2: arterial dilation, 3: CGRP, 4: CLR/RAMP, 5: migraine pathophysiology

Figures: 3
Tables: 4

Acknowledgement: The investigators of the study are deeply thankful to all migraine patients for participating in the study.

Study Funding: The study received financial support from Lundbeck Foundation (R155-2014-171).

Disclosure
The following authors, Hashmat Ghanizada, Mette Mørch-Rasmussen and Nanna Arngrim reports no disclosure. Mohammad Al-Mahdi Al-Karagholi received personal fee from Novartis and travel grant from ElectroCore. Debbie L. Hay has received research funding from Alder, Living Cell Technologies, and is a consultant for Merck and Intarcia Therapeutics. Christopher S. Walker has obtained funding for research from Alder, Allergan and Living Cell Technologies. Messoud Ashina (MA) is a consultant/speaker/scientific advisor for AbbVie, Allergan, Amgen, Eli Lilly, Lundbeck,
Novartis and Teva. MA is also the primary investigator for ongoing, Allergan, Amgen, Eli Lilly, Lundbeck and Novartis clinical trials. MA own no stocks or ownership interest in any pharmaceutical companies. MA serves as associate editor of Headache, Journal of Headache and Pain and Cephalalgia. MA is also President of the International Headache Society. MA reports research grants from Lundbeck Foundation, Novo Nordisk Foundation, and research grant from Novartis.

Objective

To determine whether the intravenous infusion of adrenomedullin, a potent vasodilator belonging to calcitonin family of peptides, provokes attacks of migraine in patients.

Methods

Twenty migraine without aura patients participated in a placebo-controlled and double-blinded clinical study. In a randomized and crossover design the patients received an intravenous infusion of human adrenomedullin (19.9 picomole/kg/min) or placebo (saline) administrated via an automated intravenous pump (20 minutes). The patients participated in two study days with washout period of minimum of seven days. The primary outcome of the study was predefined as a
difference in migraine incidence (0–12 h) and the secondary outcome were the headache intensity score’s area under curve (AUC_{0-12 h}) and the (AUC_{0-90 min}) for MAP, flushing and HR.

**Results**

Eleven migraine without aura patients (55%) fulfilled migraine attacks criteria after adrenomedullin infusion in comparison to only three patients reported attack (15%) after placebo ($P=0.039$). We found that patients reported in a period of (0-12 hours) stronger headache intensity after adrenomedullin in comparison to placebo infusion ($P=0.035$). AUC_{0-90 min} for HR and, flushing ($P<0.05$) were significant and MAP ($P=0.502$) remain unchanged. Common adverse events reported were facial flushing, heat sensation and palpitation ($P<0.001$).

**Conclusion**

Our data implicate adrenomedullin in migraine pathogenesis. This suggests that adrenomedullin and/or its receptors are novel therapeutic targets for the treatment of migraine. However, we cannot discount for the possibility that adrenomedullin may be acting through the canonical CGRP receptor.

**Introduction**

Adrenomedullin (AM) and, calcitonin gene-related peptide (CGRP) shares structural homology and, both peptides are belonging to one peptide superfamily $^1$. AM is identified as a strong vasoactive hormone peptide, consisting of 52 amino acids and having limited sequence identity with CGRP $^2$. The biological activity of AM is mediated through receptor complexes, composed of a heterodimer structure consist of a G protein-coupled receptor (GPCR) and, an independent single transmembrane protein: Receptor Activity-Modifying Protein (RAMP). The interaction of GPCR: Calcitonin Receptor-Like Receptor (CLR) with RAMP at the cell membrane creates combination of receptors which are responsive to AM and CGRP peptides $^3$. In mammals RAMPs work as pharmacological switches to modulate the function of GPCR and to date 3 RAMPs [1-3] are
identified. The interaction between CLR with RAMP2&3 generate respectively AM receptors (AM₁ & 2). Interestingly, the association of CLR/RAMP1 complex construct the canonical CGRP receptor which is responsive to AM but with lower potency compared to CGRP.

AM is mainly found in the cerebral vasculature. Multiple biological functions have been linked to AM, including the regulation of vascular tone, cerebral blood flow, facial flushing, and limited evidence also links AM to inflammation and nociception. For example, the dorsal root ganglion (DRG) is reported to express AM receptor components, and it is overexpression contributes to the development of inflammatory pain. Interestingly, AM receptor components or its receptor components have been reported in several migraine-related structures, like trigeminal ganglion (TG), cerebral vasculature, the medulla, and other locations in the central nervous system.

CGRP is a well-established key component of migraine pathogenesis. However, CGRP-infusion only induces migraine attacks in approximately 60% of patients, and anti-CGRP monoclonal antibodies only reduce monthly migraine days in approximately 60% of patients. This suggests heterogeneity in migraine induction and treatment response. Therefore, in-depth study of CGRP related peptides and their receptors is warranted to identify new therapeutics for migraine treatment. Molecular and structural similarities between CGRP and AM peptides and their receptor components suggest that AM could be involved in migraine pathophysiology, like CGRP. To further explore the possible role of AM we investigated whether AM infusion in migraine patients provoked migraine-like attacks using human models of migraine.

Methods

Participants

Twenty-three participants (three healthy volunteers for pilot study and 20 patients previously diagnosed with migraine without aura) for main study were enrolled. We recruited the participants via advertisement in Danish website (forsoegsperson.dk) for clinical research volunteers. The
inclusion criteria for participants were age 18-60 of both sexes with body weight of 50-100 kg. The exclusion criteria for healthy volunteers were serious somatic or psychiatric diseases, any pain condition including migraine, family disposition for migraine, intake of daily medication (except oral contraceptives) and only tension-type headache less than once a month were allowed.

The inclusion criteria for migraine patients were, a minimum of two migraine attacks pr. month, in accordance to the criteria of the ICHD-3 (beta) 18. Exclusion criteria were intake of daily or migraine preventative medication or suffering from any medical condition but headache of tension-type less than five days and episodic migraine without aura, only acute migraine medication, oral contraceptive and vitamins were allowed. The study involved a telephone interview to assess eligibility criteria and further thorough screening carried out by study investigator (HG) including complete neurological and physical examination at the hospital.

**Standard protocol, patient consents, and registrations**

The study protocol has been thoroughly reviewed the Ethics Committee, Capital Region, (Denmark) and, approved with the following journal ID (H-18020494). Prior to the study initiation the protocol recorded at Clinicaltrials.gov database with following identification number NCT04111484. The study participants were informed orally by the study investigator (HG) and written information provided as recommended by Declaration of Helsinki 2013. The study participants have been given adequate reflection time before obtaining the signed informed consent. The study has been completed from September 2019 and January 2020 at the Danish Headache Center, Rigshospitalet-Glostrup, Denmark. Data available from Dryad (Participants information sheet), DOI: https://doi.org/10.5061/dryad.8gtht76nn

**Pilot study**

In a randomized, single-blind, multiple-ascending-dose finding pilot study, we investigated the safety and tolerability of AM on three healthy volunteers (Fig. 1). A previous dose-finding pilot study with AM used cumulative doses of AM administrated (t=20 min) infusion with a washout
period of only 60 min in migraine patients. The first patient in the study experienced severe
tachycardia; therefore, lower doses of AM were used for the remainder of study. To avoid similar
methodological errors in the current study, all participants received an infusion of AM on three
separate days with a washout period of five days. All participants completed the pilot study without
developing any serious adverse events, and all doses were well-tolerated. Therefore, the highest
dose with a strong vasodilatory effect was selected.

Main study

Design and randomization

The Capital Region Central Pharmacy prepared the study drug and preformed a balanced block
(size 4) randomization. The study randomization code was stored at site in accordance to the Danish
data protection rules and regulation and the investigators of the study had no access until the
completion of the study.

Based on the result of pilot study, all patients for the main study were randomly allocated to two
experimental days to receive an infusion of human AM (19.9 picomole/kg*min ) or placebo
(isotonic saline) administered over a period of (20 min). AM was synthesized by Bachem Holding
(AG, Bubendorf, Switzerland). The experimental days were separated by a minimum of one 7 days
to accommodate adequate washout period.

Experimental procedures

The patients arrived for the experiment ≥ 48 hours headache and three-day free of any migraine
attack and without taking any analgesics. In accordance to the study protocol, all the patients were
informed in screening day to refrain from alcohol, tea, caffeine, coffee, cocoa, and smoking for a
period of twelve hours prior to study day. On arrival, a urine sample collected from female
participants to measure hCG levels. All patients on arrival to the hospital were asked to rest in
supine position for 20 min in a temperature and light controlled lab. A venous catheter on the
antecubital vein of right or left inserted for intravenous infusion. All baseline values were recorded at the end of resting period. We have used a previously, well-validated headache questionnaire to register any head pain and its associated symptoms (10-90 min) at the clinic and patients recorded their headache and associated symptoms every hour for 12 hours.

We constantly monitored the following parameters under experiment 1) heart rate (HR), 2) systolic/diastolic pressure and, 3) the mean arterial blood pressure (MAP). Flux were recorded by a speckle camera purchased from (moorFLPI, MOOR Instruments, Devon, UK) to record and quantify the facial vasculature dilation (facial flushing) as previously described. The experiment initiated at time (0 min) by start of infusion and the vital parameters, speckle and the questionnaire for headache and associated symptoms were repeatedly collected every 10 min. In the end of the study (90 min) all patients carefully instructed to record their headache and its associate symptoms every hour for 12 hours in post-hospital phase. To abort an attack of migraine anytime, typical migraine medication was recommended.

**Migraine-like attack criteria**

Human migraine models uses a modified but validated migraine criterium for experimentally provoked migraine attacks as the ICHD-3 criterium requires that migraine attacks are spontaneous. Furthermore, migraine typically evolves slowly and last between four and up to 72 hours. In experimentally provoked migraine the patients cannot be denied recue medication if they wanted to treat their headache. Therefore, in this study, we have used the modified migraine criteria as previously described.

**Migraine-like attack criteria**

**One of the following criteria [1] or [2] should be fulfilled as previously described:**

[1] The ICHD-3 classification requires that migraine without aura attack must fulfill the following criteria C and D. C) Headache reported by the patients must have the following two
characteristics. The headache is unilateral of pulsating quality and the head pain must be of moderate to severe intensity in the NRS ≥4. Furthermore, the headache intensity is aggravated by a coughing under the experiment or by common physical activity in the post-hospital phase. D) Following symptoms one symptom should also be present during the headache phase (Nausea, vomiting or (both), photo-phonophobia). [2] If the migraine attack reported by the patient as mirroring the patient’s typical migraine attack and the attack were terminated with patients own acute migraine medication (rescue medication) 20.

**Headache and associated symptoms**

As published previously 21, the headache intensity and its associated symptoms collected in a validated headache diagnostic questionnaire and, the headache intensity score documented by NRS, where 0 is no pain, 5 is the moderate pain and 10 the most strong pain. We have recorded the premonitory symptoms (craving, mood swings, unusual fatigue, difficulty in concentrating, thirst, and yawning), headache intensity and migraine associated symptom (Nausea, vomiting, photophobia, phonophobia) for 12 hours.

**Facial skin blood flow**

The facial flushing of the patients was quantified by laser contrast technique as a biomarker of vasodilation. The speckle measures the facial flow intensity (flux) changes via a dual camera which was positioned (30 cm) above the head of the patients. The camera automatically captures data every 5 seconds however, due to sensitivity of the camera to motion, the patients were asked to remain still with minimal facial movement for a minute to avoid motion related artifacts as previously described 22.

**Statistics and data analysis**

We planned to include 20 patients in each group in a cross-over design and expected to provide 80% power with type 1 error determined at 5% with significance level of (P< 0.05), assuming the
type 2 error at 20% in according to the study protocol. Based on our previous study, we assumed that approximately 60% of migraine patients may report an attack of migraine after AM and after placebo about 20%. This assumption was based on the migraine induction rate of known migraine triggers like CGRP\textsuperscript{23}. Furthermore, we recently found that the pooled incidence of migraine attacks after placebo was 8.1% with an upper limit of 15.5% response rate\textsuperscript{24}. Therefore, we estimated that 20 patients would be adequate in a cross-over study. In accordance to study protocol the incidence of migraine attacks after infusion of AM and placebo (12 hours post infusion) were set as the primary endpoint of the study and statistical test (McNemar’s test) were used for this analysis. The study predefined secondary endpoints were the headache intensity score’s area under curve (AUC: 0-12 h) and the (AUC: 0-90 min) for MAP, facial flushing and HR. These outcomes were collected by a blinded personnel (MMR).

The data are shown as mean of Standard Deviation (± SD) in the graphs and, percentage changes (%) from baseline shown with 95% confidence interval and only the individual headache intensity score shown as median. AUC for the study secondary endpoints were calculated in accordance to the trapezium rule, as previously defined\textsuperscript{25} to approximate and analyzed any change in the AUC between AM and placebo. In order to minimize within the patient’s variation for the baseline values which obtained on the two experimental days, we subtracted the baseline values and used Non-Parametric Wilcoxon-signed rank to test the difference in AUC for each individual data set.

The baseline measurements recorded prior to infusion of AM or placebo and the baseline defined as time (t\textsubscript{0}). Cross-over studies requires to examine for period/carry-over effect (Mann-Whitney test) for the baseline values and all the statistical work were performed using SPSS version 23. Furthermore, due to predefined primary and secondary end points in the study, no test carried out for multiple analyses.
Data availability

The senior author of the study may share all study related data in the anonymized form to any qualified research after an appropriate request.

Results

Twenty patients completed the study. The participants were of both sexes (18 women and 2 men). The mean age of the participants were 25 years old: (19-38) and a mean bodyweight of 67 kg (50-93) (Table 1). There was no difference in MAP and HR ($P > 0.05$) for the baseline values found. No of carry-over or period effect were detected. The headache intensity ($\text{AUC}_{0\text{-}12\text{h}}$) was significantly greater after AM infusion in comparison to the placebo ($P = 0.035$). Sixteen patients (80%) reported headache after AM infusion compared to eleven (55%) after placebo (Table 2).

Migraine-like attacks

AM provoked significantly more migraine-like attacks in comparison to infusion of placebo ($P = 0.039$). 55% (n=11) of the patients reported attacks of migraine after AM infusion, in comparison to 15% (n=3) after placebo (Fig. 2A). The initiation of migraine-like attacks median time was 4 h (0.83-7 h) and, for headache onset the median time was 0.25 h (0.17-2 h). The peak headache reported as 2.8 (0-8), and the median headache duration was 6 h (0.17-12 h) after AM and 1 (0-4), 1 h (0.17-12 h) after placebo.

Vital signs and facial skin blood flow

We found that the $\text{AUC}_{0\text{-}90\text{ min}}$ for HR ($P = 0.001$); and, facial flushing ($P < 0.001$); were significantly larger after AM compared to placebo (Fig. 3A, 3B-C). However, the $\text{AUC}_{0\text{-}90\text{ min}}$ for MAP ($P = 0.502$) remain unchanged (Fig. 3A).
**Adverse events**

The incidence of heat sensation, palpitation and facial flushing were also significantly ($P < 0.001$) larger in the entire period of experiment after AM compared to placebo. Prevalence of other adverse events were insignificant ($P > 0.05$) (Table 3).

**Discussion**

The novel finding of this current study is that AM provoked migraine-like attacks in 55% of patients and the attack mimicked their usual migraine. Furthermore, AM increased heart rate and induced facial flushing. Our data provide insights into how AM and/or its receptor activity may play a key part in migraine pathogenesis.

Cerebral vasculature is the main source of AM expression which mainly localized in endothelium and smooth muscle cells $^{4,5}$, and AM in a autocrine/paracrine manner involve in the maintenance of vascular tone by increasing the cellular cyclic adenosine monophosphate (cAMP) dependent or independent mechanisms $^6$. Interestingly, AM secreted by cerebral endothelial cells is thought to be vital for the preservation of vascular integrity and blood brain-barrier (BBB) function $^{26}$.

Furthermore, components of AM receptors, (CLR, RAMP2 & 3) reported in TG $^{15}$, cerebral vasculature, including the middle cerebral and middle meningeal arteries (MCA and MMA) $^{27}$.

However, pharmacological analysis of vasodilation responses and the presence of RAMP1 mRNA suggested that AM maybe, in part or full, acting through the CGRP receptor in these arteries $^{27}$.

Similar conclusions have been made using in vivo models to study AM-induced vasodilation in cerebral arterioles, dural arteries, and pial arteries $^{28}$.

Interestingly, several studies highlight the role of AM in nociception $^{11,29}$. For example, intrathecal AM infusion caused prolonged hypersensitivity to heat and inflammatory heat hyperalgesia $^{11}$. In addition, AM deficient mice display alterations in pain sensitivity $^{12}$ and endothelium dysfunction $^{30}$. In addition, AM was upregulated in (DRG neurons) during the initial phase of inflammatory process which may advance the progression of neurogenic inflammation $^{13}$. In rats, AM receptor
signaling was found to play an vital part in acute morphine-induced analgesia. Interestingly, this cascade of events was mediated by the nitric oxide-CGRP signaling pathway. AM is reportedly found in the DRG neurons of small to medium size and co-localized with CGRP in perivascular nerves of rat mesenteric artery. However, to date, there are only limited studies investigating AM expression in migraine-related structures. However, given the structural and functional similarities between the DRG and TG, it would be surprising if their patterns of AM expression were different. More detailed studies with CGRP and AM peptide and receptor components using antibodies that are properly validated to detect only their specific targets are needed to provide clear information regarding the likelihood of AM signaling in migraine-relevant structures.

A previous study with AM in migraine patients was inconclusive due to a high placebo effect (33%), which usually is between 10-15% in migraine provocation studies. In the present study, the placebo effect on migraine induction was 15%. However, patients also reported headache both after AM infusion (80%) and after placebo (55%), which is similar to previous study. In contrast to biphasic migraine-like attacks, non-migraine headaches were mild, transient, and monophasic. These headaches are most likely due to anticipation of developing a headache during experiments.

Interestingly, previous studies have shown that CGRP infusion induces migraine in 57-75% of participants. The most common adverse events reported after CGRP infusion were facial flushing, warm sensations, and palpitation. The CGRP infusion induced migraine with median time for onset of attack at 3 h (0.17-12 h), and the patients subsequently taken their rescue medication at 5 h (0.5-12 h). CGRP-induced migraine pain was localized mainly in frontal and temporal regions of the cranium (Fig. 2B). In the current study, we found that the migraine induction rate after AM was 55%, and the median peak headache intensity was milder than after previously published CGRP headache intensity (Table 4). The median time for start of migraine after AM was 4 h (0.83-7 h). Like CGRP, AM-induced headache was predominately frontal and temporal (Fig. 2B). Further, multiple administration and prolonged infusion of AM caused a repeated headache in healthy volunteers. These data suggest that migraine induction after infusion
of AM may have some phenotypical similarities with CGRP, but slight differences in headache intensity and the onset of migraine were observed. Overall, our data suggest that AM via receptors that it can activate might play a vital part in migraine pathogenesis.

Both AM and CGRP potently dilate cerebral vessels \(^8\), with and without endothelium \(^{27,38}\), possibly via increasing cAMP production \(^2\). Infusion of CGRP in healthy volunteers dilates extracerebral but not intracerebral arteries \(^39\), and in migraine patients, both intra-extracerebral arteries were dilated after infusion of CGRP \(^35\). An ultrasonography study showed that the infusion of AM in migraine patients dilates the arteria temporalis superficialis (STA) (1.11 to 1.45 mm) but had no effect on the MCA \(^9\). Likewise, CGRP induces marked (1.26 to 1.70 mm) and prolong dilation of STA \(^40\). In the current study, we quantified facial flushing as a biomarker of extracerebral vasodilation after the infusion of AM, which confirms the vasodilatory effect of the peptide in migraine patients. However, more data is required to understand the role of intracerebral vasodilation. We suggest that the vasorelaxant effect of CGRP and AM on extra-intracerebral arteries in migraine patients should be studied in a cross-over design using an advanced MRA method to increase our knowledge of both peptides. Furthermore, the blinding of our study may be compromised due to autonomic AM induced symptoms, like facial flushing, palpitations and heat sensation. However, we believe that the study design helped to mitigate against this methodological error. We found a significant increase in the HR after the infusion of AM, but no alterations found in MAP. Although decreased blood pressure was linked to an increase in migraine prevalence \(^41\), our data indicate that alterations in vital signs are unlikely to contribute to AM-induced migraine.

There are several possible mechanisms that could explain the findings of the current study in patients.

AM and CGRP have both overlapping and distinct receptor pharmacology and biological activities, which can make it challenging to determine which receptor may be involved in an activity of each peptide \(^3\). AM and CGRP potently activates canonical CGRP receptor CLR/RAMP1 \(^3\). However,
AM overall has a lower affinity than CGRP at the CGRP receptor. Interestingly, both peptides can induce facial flushing and dilate extracerebral arteries, however, in animal models CGRP was 10-30 times more potent compared to AM. Interestingly, this effect is consistent with the known pharmacology of AM and CGRP activity at the CGRP receptor. However, in rats, AM-induced vasorelaxation was reportedly mediated via both AM and CGRP receptors. This may reflect the differences in receptor pharmacology between humans and rodents or highlight the need to consider the concentrations of AM employed in a study given its potential to activate the closely related CGRP receptor. In humans, AM plasma concentrations are in the range of 3 pM and half-life of around (22 min). Thus, in present study we estimated that AM reached a steady state concentration of \(8.6 \times 10^{-9}\) mol/L (steady state concentration \(C_{ss}\) = Infusion rate \(R_{in}\) / Clearance (CL)). Given the known potencies of AM in activating CGRP and AM receptors, this concentration, is thus theoretically adequate to partially or fully activate CGRP, AM1 and AM2 receptors simultaneously. Therefore, we cannot entirely rule out the likelihood of that the CGRP receptor (CLR/RAMP1) is at least partially activated in this study. However, considering the lack of efficacy of recently approved CGRP targeting monoclonal antibodies for treatment of migraine in a substantial percentage of patients and together with the result of the present study, we speculate that both CGRP and AM or its receptors may play an a key part in the genesis of migraine. Antibodies to AM have been generated and are in clinical trials. These could be valuable for examining the effect of AM in migraine.

AM receptor activation increases cAMP, which subsequently triggers protein kinase A (PKA) activation which leads to the opening of ATP-sensitive potassium channel (\(K_{ATP}\)). A recent study showed that the \(K_{ATP}\) channel opener (levcromakalim) induces migraine. Lastly, new evidence is suggesting that the mechanosensitive receptors (Piezo ion channels) in facilitating the transmission of meningeal nociception. Recently, this channel was identified in the TG of post-mortem humans. It has been shown that the Piezo receptors activation in the TG neurons and meningeal nerve fibers induce pain mediated by the release of CGRP. Moreover, vascular endothelial Piezo...
receptor in response to fluid stress mediate release of AM from endothelial cells, which regulates vascular tone through activation of AM receptors. We hypothesize that AM-induced migraine may be mediated via activation of mechanosensitive Piezo ion channels found on the vascular endothelium and in meningeal nerve endings of the trigeminal ganglion.

Our study demonstrated that infusion of AM provokes migraine-like attacks in patients. The AM induced attacks had phenotypical similarities to CGRP induced migraine attacks, but some differences were identified. We speculate that AM induced migraine may in part be mediated via the canonical CGRP receptor CLR/RAMP1. However, further investigation is required and the participation of recently identified mechanosensitive Piezo ion channels expressed on the endothelial vascular cells and meningeal nociceptors activated by AM cannot be ruled out. Future studies should investigate the role of AM in migraine patients treated with canonical CGRP receptor monoclonal antibodies or receptor antagonists.

Appendix 1. Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hashmat Ghanizada, MD, PhD</td>
<td>Danish Headache Center, DK</td>
<td>Designed and conceptualized the study, acquired, analyzed and interpreted the data, drafted the manuscript for intellectual content</td>
</tr>
<tr>
<td>Mohammad Al-Mahdi Al-Karaghali, MD</td>
<td>Danish Headache Center, DK</td>
<td>Designed and conceptualized the study, acquired, analyzed and interpreted the data, drafted the manuscript for intellectual content</td>
</tr>
<tr>
<td>Nanna Arngrim, MD, PhD</td>
<td>Danish Headache Center, DK</td>
<td>Interpreted the data, revised the manuscript for intellectual content</td>
</tr>
<tr>
<td>Mette Mørch-Rasmussen, MD</td>
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<td>Major role in data collection</td>
</tr>
<tr>
<td>Christopher S. Walker, PhD</td>
<td>Auckland University, NZ</td>
<td>Interpreted the data, revised the manuscript for intellectual content</td>
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<td>Name</td>
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<td>Danish Headache Center, DK</td>
<td>Designed and conceptualized study, interpreted the data, revised the manuscript for intellectual content</td>
</tr>
</tbody>
</table>

Reference


25. Matthews JNS, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in


Figure 1. Workflow chart and study design.

(A) Pilot dose finding study on healthy volunteers (B) Flowchart of participant recruitment of patients with migraine without aura (C) Main study design (D) Workflow chart.
Figure 2. AM induction of headache and migraine.

(A) The black lines showing the individual headache intensity score over time and the red line shows the median headache intensity after infusion of AM compared to placebo (n=20). (B) The median-time for onset of migraine-like attacks and intake of rescue medication for adrenomedullin (red) and previously published CGRP data (35) (black) and headache localization for patients with migraine attacks.
**Figure 3.** AM effect on vital parameters and facial flux.

(A) The left graph shows the mean arterial pressure (MAP) in percentage change from baseline and the right graph shows the heart rate (HR) after adrenomedullin and placebo. (B) Facial skin blood flow (Flux) changes after infusion of adrenomedullin and placebo (0-90 min). (C) Facial skin blood flow shown as a percentage change.
Table 1. Showing the demographic and clinical characteristics of included patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Female (n=18)</th>
<th>Male (n=2)</th>
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</thead>
<tbody>
<tr>
<td>Age (range), year</td>
<td>24 (19-28)</td>
<td>32 (26-38)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24 (17-27)</td>
<td>25 (23-27)</td>
</tr>
<tr>
<td>Age at migraine onset, year</td>
<td>15 (12-19)</td>
<td>17.5 (15-20)</td>
</tr>
<tr>
<td>Migraine attacks per month</td>
<td>3.22 (2-5)</td>
<td>4</td>
</tr>
<tr>
<td>Headache (Tension-type) days per month</td>
<td>2.77 (1-4)</td>
<td>4 (3-5)</td>
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<tr>
<td>Migraine specific medication use (%)</td>
<td>78%</td>
<td>100%</td>
</tr>
<tr>
<td>Non-migraine specific medication use (%)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Previous migraine preventive medication use</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Family disposition to migraine</td>
<td>100%</td>
<td>100%</td>
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Table 2. Showing detailed headache characteristics and its associated symptoms in after infusion of adrenomedullin vs placebo (0–12 h)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Peak headache</th>
<th>Headache characteristics</th>
<th>Associated symptoms</th>
<th>Mimics usual migraine</th>
<th>Migraine-like attacks (onset)</th>
<th>Treatment (time/efficacy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 AM</td>
<td>9h (4h)</td>
<td>Left/6/throb/+</td>
<td>+/- +/-</td>
<td>Yes</td>
<td>Yes (6 h)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>70min (7h)</td>
<td>Left/2/pres/-</td>
<td>-/-/-</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>2 AM</td>
<td>4h (4h)</td>
<td>Left/3/throb/+</td>
<td>+/- +/-</td>
<td>Yes</td>
<td>Yes (4 h)</td>
<td>Sumatriptan 50 mg (4) / Yes</td>
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<td></td>
<td>Placebo</td>
<td>None</td>
<td></td>
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<tr>
<td>3 AM</td>
<td>90 min (9h)</td>
<td>Bilat/4/pres/+</td>
<td>-/+ +/-</td>
<td>Yes</td>
<td>Yes (90 min)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2 h (6 h)</td>
<td>Bilat/2/pres/+</td>
<td>-/+/-</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>4 AM</td>
<td>None</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
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<td></td>
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<tr>
<td>5 AM</td>
<td>7h (7 h)</td>
<td>Bilat/6/throb/-</td>
<td>+/- +/-</td>
<td>Yes</td>
<td>Yes (7h)</td>
<td>Paracetamol 1g (7 h) / NA</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>8 h (2 h)</td>
<td>Bilat/1/throb/-</td>
<td>-/+/-</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>6 AM</td>
<td>3h (12 h)</td>
<td>Bilat/4/throb/+</td>
<td>+/- +/-</td>
<td>Yes</td>
<td>Yes (4 h)</td>
<td>Sumatriptan 50 mg (4) / Yes</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7 AM</td>
<td>None</td>
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<td></td>
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<tr>
<td></td>
<td>Placebo</td>
<td>None</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>8 AM</td>
<td>3 h (10 h)</td>
<td>Bilat/8/pres/+</td>
<td>+/- +/-</td>
<td>Yes</td>
<td>Yes (3h)</td>
<td>Paracetamol 1g + Ibuprofen 400 mg (3 h) / Yes</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>Duration</th>
<th>Side</th>
<th>Type</th>
<th>Location</th>
<th>Intensity</th>
<th>Pain</th>
<th>Nurse</th>
<th>Pain</th>
<th>Method</th>
<th>Intensity</th>
<th>Doctor</th>
<th>Medication</th>
<th>Intensity</th>
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<tbody>
<tr>
<td>9 AM</td>
<td>Placebo</td>
<td>6h (10h)</td>
<td>Bilat</td>
<td>3/pres</td>
<td>+ / + / -</td>
<td>Yes</td>
<td>Yes (10h)</td>
<td>Paracetamol 1g + Ibuprofen 400 mg (10h) / Yes</td>
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<td></td>
</tr>
<tr>
<td>3h (8h)</td>
<td>Placebo</td>
<td>4h (8h)</td>
<td>Right</td>
<td>4/throb</td>
<td>- / + / +</td>
<td>Yes</td>
<td>Yes (80 min)</td>
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</tr>
<tr>
<td>20 min (20 min)</td>
<td>AM</td>
<td>Placebo</td>
<td>50 min (9h)</td>
<td>Right</td>
<td>4/pres</td>
<td>+ / + / -</td>
<td>Yes</td>
<td>Yes (50 min)</td>
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<td>20 min (30 min)</td>
<td>AM</td>
<td>Placebo</td>
<td>20 min (20 min)</td>
<td>Left</td>
<td>1/pres</td>
<td>- / - / -</td>
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<td>No</td>
<td>None</td>
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<tr>
<td>4h (10h)</td>
<td>AM</td>
<td>Placebo</td>
<td>40 min (4h)</td>
<td>Right</td>
<td>1/pres</td>
<td>- / - / -</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 min (8h)</td>
<td>AM</td>
<td>Placebo</td>
<td>90 min (8h)</td>
<td>Bilat</td>
<td>5/throb</td>
<td>+ / + / +</td>
<td>Yes</td>
<td>Yes (50 min)</td>
<td>None</td>
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<tr>
<td>None</td>
<td>AM</td>
<td>Placebo</td>
<td>10min (40 min)</td>
<td>Diffus</td>
<td>1/throb</td>
<td>- / - / -</td>
<td>No</td>
<td>No</td>
<td>None</td>
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</tr>
<tr>
<td>4h (9h)</td>
<td>AM</td>
<td>Placebo</td>
<td>10 min (3 h)</td>
<td>Diffus</td>
<td>1/pres</td>
<td>- / - / -</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>8h (7h)</td>
<td>AM</td>
<td>Placebo</td>
<td>8h (7h)</td>
<td>Left</td>
<td>4/pres</td>
<td>- / - / -</td>
<td>No</td>
<td>No</td>
<td>Paracetamol 1g + Ibuprofen 400 mg (9h) / Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6 h (7 h)</td>
<td>AM</td>
<td>Placebo</td>
<td>6h (7h)</td>
<td>Bilat</td>
<td>4/throb</td>
<td>-/+/-</td>
<td>Yes</td>
<td>Yes (6h)</td>
<td>*Treo 550 mg (6 h) / Yes</td>
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<td></td>
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</tr>
<tr>
<td>None</td>
<td>AM</td>
<td>Placebo</td>
<td>10 min (2 h)</td>
<td>Bilat</td>
<td>1/pres</td>
<td>- / - / -</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>None</td>
<td>AM</td>
<td>Placebo</td>
<td>40 min (2 h)</td>
<td>Bilat</td>
<td>3/throb</td>
<td>- / + / -</td>
<td>Yes</td>
<td>Yes (90 min)</td>
<td>*Treo 550 mg (90 min) / Yes</td>
<td></td>
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</tr>
<tr>
<td>None</td>
<td>AM</td>
<td>Placebo</td>
<td>10 min (2 h)</td>
<td>Bilat</td>
<td>1/pres</td>
<td>- / - / -</td>
<td>No</td>
<td>No</td>
<td>None</td>
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<td></td>
</tr>
</tbody>
</table>

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In parentheses duration of headache in hours. (a) Showing the headache localization/intensity/quality (throb = throbbing; pres = pressing)/aggravation (cough in hospital-phase and by movement during post-hospital phase). (b) Nausea / photo or phonophobia. (c) Attacks criteria the described in methods. (d) Pain freedom or pain relief (≥50% decrease of intensity) within 2 h. (e) Acetylsalicylic acid 500 mg and caffeine 50 mg.’’

Table 3. Adverse events recorded in (0-12 h) after Adrenomedullin and placebo in all patients

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Adrenomedullin</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flushing</td>
<td>17</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heat sensation</td>
<td>17</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Palpitation</td>
<td>15</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nausea</td>
<td>5</td>
<td>2</td>
<td>0.375</td>
</tr>
<tr>
<td>Neck stiffness</td>
<td>3</td>
<td>2</td>
<td>1.000</td>
</tr>
<tr>
<td>Difficulty concentrating</td>
<td>4</td>
<td>1</td>
<td>0.375</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4</td>
<td>0</td>
<td>0.125</td>
</tr>
<tr>
<td>Craving</td>
<td>4</td>
<td>1</td>
<td>0.250</td>
</tr>
</tbody>
</table>

Yawning urge (n=3); after placebo, yawning urge (n=1) reported by few patients.
Table 4. Comparison of variables between adrenomedullin and previously published CGRP studies \textsuperscript{32,34}.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adrenomedullin (n=20)</th>
<th>CGRP \textsuperscript{32} (n=9)</th>
<th>CGRP \textsuperscript{34} (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Double-blinded, placebo-control</td>
<td>Double-blinded, placebo-control</td>
<td>Open-label</td>
</tr>
<tr>
<td>Migraine \textsuperscript{a}</td>
<td>55%</td>
<td>65%</td>
<td>63%</td>
</tr>
<tr>
<td>Headache \textsuperscript{a}</td>
<td>80%</td>
<td>100%</td>
<td>94%</td>
</tr>
<tr>
<td>Flushing</td>
<td>85%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Heat sensation</td>
<td>85%</td>
<td>100%</td>
<td>95%</td>
</tr>
<tr>
<td>Palpitation</td>
<td>75%</td>
<td>NA</td>
<td>53%</td>
</tr>
<tr>
<td>Nausea</td>
<td>25%</td>
<td>88%</td>
<td>52%</td>
</tr>
<tr>
<td>Heart rate \textsuperscript{b}</td>
<td>42%</td>
<td>59%</td>
<td>NA</td>
</tr>
<tr>
<td>Systolic blood pressure \textsuperscript{c}</td>
<td>-3.7%</td>
<td>-4.0%</td>
<td>NA</td>
</tr>
<tr>
<td>Diastolic blood pressure \textsuperscript{b}</td>
<td>-9.6%</td>
<td>-18.9%</td>
<td>NA</td>
</tr>
<tr>
<td>Median peak headache intensity</td>
<td>2.8 (0-8)</td>
<td>4 (1-6)</td>
<td>5 (2-9)</td>
</tr>
<tr>
<td>Median-time to peak headache intensity (h)\textsuperscript{c}</td>
<td>4 (0-9)</td>
<td>5 (1-12)</td>
<td>3 (1-11)</td>
</tr>
</tbody>
</table>

(a) Headache and migraine incidence in percentage.

(b) Mean change from baseline

(c) Range in hours
Effect of Adrenomedullin on Migraine-like Attacks in Patients With Migraine: A Randomized Crossover Study
Hashmat Ghanizada, Mohammad Al-Mahdi Al-Karagholi, Nanna Arngrim, et al.
Neurology published online April 7, 2021
DOI 10.1212/WNL.0000000000011930

This information is current as of April 7, 2021