Two frontlines against migraine: PACAP and Kynurenine system

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MIGRAINE

- Primary headache disorder
- High prevalence
- 3:1 female/male
- Acute treatment with drugs and intervallum therapy
- Pathophysiology?
TRIGEMINOVASCULAR SYSTEM

- meninges cortex
- peripheral
- meninges cortex
- central
- TRG
- TNC
- secondary sensory neurons

- 5-HT
- SP
- CGRP
- PACAP

Glutamate mechanism:
- Kynurenine system

- vascular theory
- neurogene inflammation
- receptor sensitization

Moskowitz et al. – Pathol Biol. (1992)
Tajti et al. – J Neural Transm (2011)
PITUITARY ADENYLATED CYCLASE ACTIVATING POLYPEPTIDE

- VIP-Glucagon-GHRH family
- Adenylate cyclase activity ↑
- 1989. isolated (Myata et al.)
- PACAP-27, PACAP-38
- Pleiotropic peptide
- VPAC1, VPAC2, PAC1 receptor
PACAP38 induces migraine-like attacks in patients with migraine without aura

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

Abstract

Most of attacks (6 out of 7) occurred during the post-hospital phase [mean time 6 h (range 2-11)]. Two healthy subjects reported migraine-like attacks after PACAP38 during the hospital phase and none during the post-hospital phase. In the hospital phase, the area under the curve (AUC) for headache score was larger during PACAP38 infusion compared to placebo in healthy subjects (P = 0.005) and tended to be larger in migraineurs (P = 0.066). In the post-hospital phase, the AUC for headache was larger after PACAP38 infusion compared to placebo in both healthy subjects (P = 0.005) and migraine patients (P = 0.013). In migraine patients, PACAP38 caused a peak decrease of 16.1% in V(MCA) and a 37.5% increase in STA diameter at 20 min after start of infusion. In conclusion, PACAP38 infusion caused headache and vasodilatation in both healthy subjects and migraine patients. In migraine sufferers, PACAP38 caused delayed migraine-like attacks. The findings stimulate further investigation of the neuronal and vascular mechanisms of PACAP38.
Peripheral and central alterations of pituitary adenylate cyclase activating polypeptide-like immunoreactivity in the rat in response to activation of the trigeminovascular system

Bernadett Tuka a, b, Zsuzsanna Helyes c, g, Adrienn Markovics c, Teréz Bagoly c, József Németh f, László Márk d, Réka Brubel c, Dóra Reglődi c, Árpád Párdutz a, János Szolcsányi c, g, László Vécsei a, b, János Tajti a, *
PACAP<sub>1-38</sub>-LIKE IMMUNOREACTIVITY in PLASMA

ELECTRICAL

NTG

Tuka et al. – Peptides (2012)
PACAP-LI in TNC, C3-C4, TRG

**ELECTRICAL**

**NTG**

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Tuka et al. – Peptides (2012)
Pituitary adenylate cyclase-activating polypeptide plays a key role in nitroglycerol-induced trigeminovascular activation in mice

Adrienn Markovics a, Viktoria Kormos a,b, Balazs Gaszner b, Arvin Lashgarara a, Eva Szoke a, Katalin Sandor a, Krisztina Szahadi c, Bernadett Tuka d, Janos Tajti d, Janos Szolcsanyi a,h, Erika Pinter a,h, Hitoshi Hashimoto e,f,g, Jozsef Kun a, Dora Reglodi b,i, Zsuzsanna Helyes a,h,i,1

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<table>
<thead>
<tr>
<th>PACAP -38 +/+</th>
<th>PACAP -38 -/-</th>
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<tr>
<td>photophobia</td>
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<tr>
<td>✓</td>
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1 Corresponding author.
Plasma samples were collected from the cubital veins in ice-cold tubes containing anticoagulant-protease inhibitor aprotinin. Separated (2000 rpm for 10 min at 4°C) and stored at −80°C. Specific and sensitive PACAP-38 and CGRP-radioimmunoassay measurements (RIA). There were no restrictions as regards food and drink intake. Migraine patients were asked not to start their usual attack treatment until cupping had been taken.
Differences in plasma PACAP-38-LI between migraineurs and healthy controls

Fig. 1/A control (n=40) vs. migraineurs during interictal period (n=80) Student’s unpaired t-test (p<0.01)
Fig. 1/B interictal (n=59) vs. migraineurs during ictal period (n=28) Student’s unpaired t-test (p<0.009)
Fig. 1/C control (n=40) vs. migraineurs during ictal period (n=28) Student’s unpaired t-test (p<0.447)

Tuka et al. - Cephalalgia (2013)
Role of VIP/PACAP in primary headaches

Lars Edvinsson

RESEARCH HIGHLIGHTS

IN BRIEF

MIGRAINE

Migraine phases linked to plasma levels of PACAP-38

A new study, published in Cephalalgia, has revealed that PACAP-38-like immunoreactivity (PACAP-38-LI) is linked to phases of migraine. PACAP-38 is involved in activation of cerebral blood vessels. Tuka et al. assessed PACAP-38-LI in plasma during both ictal and interictal phases in 87 patients with migraine. Compared with healthy controls, patients had significantly lower PACAP-38-LI in the interictal phase. Migraineurs had elevated levels of PACAP peptide during the ictal phase relative to the interictal phase. Furthermore, a negative correlation between levels of interictal PACAP-38-LI and disease duration was identified.

Elevation of pituitary adenylate cyclase activating peptide (PACAP) during headache and its reduction after treatment with sumatriptan 6 mg s.c. (postsumatriptan, *P < 0.05 compared to headache). Interattack levels of PACAP (headache free) are less than attack levels (#P < 0.05). PACAP, pituitary adenylate cyclase activating polypeptide.
Goadsby et al.
Headache and prolonged dilatation of the middle meningeal artery by PACAP38 in healthy volunteers

Faisal Mohammad Amin¹, Mohammad Sohail Asghar¹, Song Guo¹, Anders Hougaard¹, Adam Espe Hansen¹, Henrik Winther Schytz¹, Rob J van der Geest², Patrick JH de Koning², Henrik BW Larsson¹, Jes Olesen¹ and Messoud Ashina¹

Abstract

Aim: To explore a possible relationship between vasodilatation and delayed headache we examined the effect of pituitary adenylate cyclase-activating polypeptide-38 (PACAP38) on the middle meningeal artery (MMA) and middle cerebral artery (MCA) using high resolution magnetic resonance angiography (MRA).

Methods: In a double-blind, randomized, placebo-controlled study 14 healthy volunteers were scanned repeatedly after infusion (20 min) of 10 pmol/kg/min PACAP38 or placebo. In addition, four participants were scanned following subcutaneous sumatriptan (6 mg).

Results: We found significant dilatation of the MMA (p = 0.00001), but not of the MCA (p = 0.50) after PACAP38. There was no change after placebo (p > 0.40). Vasodilatation (range 16–23%) lasted more than 5 h. Sumatriptan selectively contracted the MMA by 12.3% (p = 0.043).

Conclusion: PACAP38-induced headache is associated with prolonged dilatation of the MMA but not of the MCA. Sumatriptan relieves headache in parallel with contraction of the MMA but not of the MCA.
Investigation of the pathophysiological role of intestinal adenylate cyclase-activating polypeptide-38. 

Amin FM, Hougaard A, Schytz HW, Aspelin P

Author information

Abstract

Pituitary adenylate cyclase-activating polypeptide (PACAP) receptors are expressed in various brain areas but show differences in migraine-inducing capacity. Using a controlled, double-blind, randomized, placebo-controlled design, we present a head-to-head comparison of PACAP38 and a placebo without aura were randomly allocated to PACAP38 (10 pmol/kg/min) over 20 min. We recorded incidence of migraine attacks, blood samples (plasma PACAP level), respiratory frequency, and end-tidal pCO2 (range 19-36) completed the study. There were no significant differences in the incidence of intestinal polypeptide (18%) infusion compared to placebo. PACAP38-induced attacks after PACAP38. Both groups experienced similar MRA before (A) and 2 h after (B) the start of PACAP38 infusion. PACAP38 (10 pmol/kg) was infused intravenously over 20 min.

Figure 5

MRA before (A) and 2 h after (B) the start of PACAP38 infusion. PACAP38 (10 pmol/kg) was infused intravenously over 20 min.
Change in brain network connectivity during PACAP38-induced migraine attacks: A resting-state functional MRI study.

Amin FM, Hougaard A, Magon S, Asghar MS, Ahmad NN, Rostrup E, Sprenger T, Ashina M.

Abstract

OBJECTIVE:
To investigate resting-state functional connectivity in the salience network (SN), the sensorimotor network (SMN), and the default mode network (DMN) during migraine attacks induced by pituitary adenylate cyclase-activating polypeptide-38 (PACAP38).

METHODS:
In a double-blind, randomized study, 24 female migraine patients without aura received IV PACAP38 or vasoactive intestinal polypeptide (VIP) over 20 minutes. Both peptides are closely related and cause vasodilation, but only PACAP38 induces migraine attacks. VIP was therefore used as active placebo. Resting-state functional MRI was recorded before and during PACAP38-induced migraine attacks and before and after VIP infusion. We analyzed data by Statistical Parametric Mapping 8 and the Resting-State fMRI Data Analysis Toolkit for Matlab in a seed-based fashion.

RESULTS:
PACAP38 (n = 16) induced migraine attacks and increased connectivity with the bilateral opercular part of the inferior frontal gyrus in the SN. In SMN, there was increased connectivity with the right premotor cortex and decreased connectivity with the left visual cortex. Several areas showed increased (left primary auditory, secondary somatosensory, premotor, and visual cortices) and decreased (right cerebellum and left frontal lobe) connectivity with DMN. We found no resting-state network changes after VIP (n = 15).

CONCLUSIONS:
PACAP38-induced migraine attack is associated with altered connectivity of several large-scale functional networks of the brain.
Role of PACAP in migraine headaches

László Vécsei, Bernadett Tuka, János Tajti

*Brain*, Volume 137, Issue 3, 1 March 2014, Pages 650–651,
https://doi.org/10.1093/brain/awu014

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**Editorial**
TRIGEMINOVASCULAR SYSTEM

**meninges cortex**

- **peripheral**
  - CGRP ~ 40%
  - PACAP ~ 20%
  - SP ~ 18%
  - NO ~ 15%

**TRG**

- 5-HT
- SP
- CGRP
- PACAP

**Glutamate mechanism:**
- Kynurenine system

**central**

**TNC**

**secondary sensory neurons**

- vascular theory
- neurogene inflammation
- receptor sensitization

Moskowitz et al. – Pathol Biol. (1992)
Tajti et al. – J Neural Transm (2011)
Possible sites of intervention in the glutamatergic model of migraine pathogenesis
KYNURENINE PATHWAY

Vécsei et al. – Nat. Rev. Drug (2013)
Kynurenine aminotransferase in the supratentorial dura mater of the rat: effect of stimulation of the trigeminal ganglion

Elizabeth Knyihár-Csillik, Zoltán Chadaide, Etsuo Okuno, Beata Krisztin-Péva, József Toldi, Csaba Varga, Andor Molnár, Bert Csillik, László Vécsei

https://doi.org/10.1016/j.expneurol.2003.12.001
a.) Densitometric analysis of the intensity of KAT-IR of \textbf{Schwann cells}. As compared to controls, decrease is significant after 60 min stimulation of the trigeminal ganglion.

b.) Densitometric analysis of the intensity of KAT-IR of the granules of \textbf{mast cells}. The difference between control and stimulated mast cells is significant.
I. Prodrug concept  
II. Kynurenic acid analogue concept  
III. Metabolic shift concept
Prevention of electrical stimulation-induced increase of c-fos immunoreaction in the caudal trigeminal nucleus by kynurenine combined with probenecid

Elizabeth Knyihar-Csillik a, József Toldi b, Beata Krisztin-Péva c, Zoltán Chadaide a, Hajnalka Németh b, Robert Fenyö c, László Vécsei a, d

Kynurenic acid, an endogenous NMDA receptor antagonist. Kynurenic acid, however, does not cross the blood-brain barrier, and its use as a neuroprotective agent is therefore not feasible. In contrast, kynurenine, from which kynurenic acid is formed on the action of kynurenine aminotransferase, passes the blood-brain barrier without difficulty. After the i.p. injection of kynurenine combined with probenecid it was found that the stimulation-induced increase in the c-fos immunoreactivity of the secondary sensory neurons does not occur.
Column a: saline (NP) treatment resulted in a 15% increase in the number of c-fos-immunoreactive neurons. Column c: NitroPOHL (NP) resulted in a 67% increase in the number of c-fos-immunoreactive neurons, as compared to the absolute control.

Column d: effect of nitroglycerol (NitroPOHL, NP) preceded by a 2-h SZR-72 pretreatment. The increase in the number of c-fos-immunoreactive neurons was significantly less than in the kynurenic acid-pretreated samples.

Laminae I and II of the cerebral cortex were analyzed. Column a: saline (NP) treatment resulted in a 15% increase in the number of c-fos-immunoreactive neurons. Column c: NitroPOHL (NP) resulted in a 67% increase in the number of c-fos-immunoreactive neurons, as compared to the absolute control.
Kynurenate Derivative Attenuates the Nitroglycerin-Induced CamKIIα and CGRP Expression Changes

Enikő Vámos, MSc; Annamária Fejes, MSc; Júlia Koch, MD; János Tajti, MD, PhD; Ferenc Fülöp, PhD, DSc; József Toldi, PhD, DSc; Árpád Párdutz, MD, PhD; László Vécsei, MD, PhD, DSc

Fig 4.—Histogram showing the area in μm² covered by CGRP-IR fibers in superficial laminae I and II of the TNC in segments C1 and C2 in the 3 animal groups after vehicle (light bars) or NTG (dark bars) (means ± SEM, n = 6 per group). The combined treatment and 2-(2-N, N-dimethylaminoethylamino-1-carbonyl)-1H-quinolin-4-one hydrochloride (a novel KYNA derivative) significantly attenuated the NTG-induced changes. CGRP = calcitonin gene-related peptide; IR = immunoreactive; KYNA = kynurenic acid; NTG = nitroglycerin; TNC = caudal trigeminal nucleus. *P ≤ .05; **P ≤ .01.
Altered kynurenine metabolism in chronic migraine

Authors
Martina Curci
Maurizio Sisci

Open Access
First Online
PACAP

KYNA

Kynurenine metabolites abnormality

Vasodilatation
Inflammation
Senzitization
Experimental protocol

1. Intact sampling groups:
   - ES-TRG
   - treatment

2. 0.9% NaCl (i.v.)
3. KYNA (1 mmol, i.v.)
4. KYNA derivate (1 mmol, i.v.)
5. MK-801 (4 mg/bwkg, i.v.)

- RIA (PACAP1-38 IR)
- Western blot (preproPACAP rel. opt. density)
- RT-PCR (PACAP1-38 rel. mRNA expression)

Körtési et al. – Frontiers in Neurology (2018)
Radioimmunoassay
PACAP<sub>1-38</sub> immunoreactivity

+p < 0.001 vs. Control group
**p < 0.005 vs. Vehicle-treated ES-TRG group

Körtési et al. – Frontiers in Neurology (2018)
Western blot
preproPACAP relative optical density

+++ p < 0.001 vs. Control group
*** p < 0.001 vs. Vehicle-treated ES-TRG group
## p < 0.01 vs. KYNA-a-treated ES-TRG group
Körtési et al. – Frontiers in Neurology (2018)
Real-Time PCR

PACAP_{1-38} relative gene expression

*p < 0.05 vs. Control group

**p < 0.01 vs. Vehicle-treated ES-TRG group

***p < 0.001 vs. Vehicle-treated ES-TRG group

Körtési et al. – Frontiers in Neurology (2018)
CONCLUSION

- PACAP is a potential biomarker of migraine
- KYNA and its derivates is a new future therapy in migraine
- Beside NMDAR other receptors?
Thank you for your attention!