Investigation of migraine-related molecules in the activated trigeminovascular system

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Ph.D. Thesis summary

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INTRODUCTION

Migraine is a common, paroxysmal primary headache disorder, which has high socio-economic and personal impacts on the quality of life. This pain syndrome is typically characterized by recurrent attacks of unilateral, throbbing headache of moderate or severe intensity. Most frequenty migraine-associated phenomena include nausea, vomiting, photophobia, phonophobia and allodynia. Epidemiological studies have revealed that the prevalence of migraine is 12% and it is most common in young adult woman. Two main subtypes of this neurological disease are the migraine with and without aura. Medications used to combat migraines fall into two broad categories: acute and preventive medications. Main reason for the limited therapeutic potential is the pathophysiology of migraine is stilll not fully known. However, the activation of trigeminovascular system (TS) is crucial in these processes. The TS consists of pseudounipolar neurons within the trigeminal ganglion (TRIG). The peripheral branches of these neurons innervate the cranial vessels and meningeal tissues, while their central afferents project to the nociceptive second-order neurons in the trigeminal nucleus caudalis (TNC) located in the brainstem and more caudally in the upper region of the spinal cord.

TS activation produces a significant release of vasoactive molecules and various neuropeptides from the terminals of the trigeminal branches, such as calcitonin gene-related peptide (CGRP) and pituitary adenylate cyclase-activating polypeptide (PACAP). These peptides have important role

in several migraine-related processes, therefore the current migraine researches have focused on these peptides, as new potential therapeutic targets.

Besides the TS activated neuropeptide release the importance of glutamatergic system is also pivotal in the mechanism of hyperexcitability manifested in migraine. Interesting and promising endogenous regulators of the glutamatergic neurotransmission include certain metabolites of the kynurenine pathway formed during the catabolism of tryptophan. Some of these metabolites are neuroactive for example kynurenic acid (KYNA), which is an endogenous NMDA receptor antagonist. Since the NMDA receptor mediated excitotoxicity play crucial role in the pathomechanism of migraine, the modulation of glutamatergic system may be another therapeutic strategy.

AIMS

Our goals were to investigate:

1.) the alterations of CGRP and PACAP in the experimental models of activated TS.

2.) the potential link between PACAP and glutamate system.

3.) the possible therapeutic effects of KYNA and its synthetic analogue (SZR72).

Hypotheses of this study were that:

1.) the CFA-induced sensitization may evoke elevated PACAP and CGRP expression level in the TS, which can be manifested in the alteration of mechanical hyperalgesia.

2.) the KYNA and SZR72 may influence, presumably decrease the concentration of PACAP.

3.) the different treatments may cause diverse effects due to the receptor specificity of drugs.

MATERIALS AND METHODS

106 young adult male SPRD rats were used for the experiments and two different, TS activated animal models were applied. These methods are well-described, widely used and generally certified.

1.) Orofacial hyperalgesia with Complete Freund's Adjuvant (CFA)

The right whisker pad of rats was injected with 50 µl CFA or saline. A mechanical allodynia test was performed with von Frey filaments before and after the treatment. Animals were transcardially perfused at 24, 48, 72 and 120 hours after the injection and it was followed by dissection of the TNC. The relative optical density of CGRP and preproPACAP was analyzed by western blot.

2.) Electrical stimulation of TRIG

Rats were pretreated with KYNA, SZR72, MK-801 or saline (vehicle). Next, the right TRIG was electrically stimulated, the animals were transcardially perfused following 180 min and the TNC was removed. In the TNC samples, the $PACAP_{1-38}$ immunoreactivity was measured by radioimmunoassay, the relative optical density of preproPACAP was assessed by western blot analysis and the $PACAP_{1-38}$ mRNA was detected by real-time polymerase chain reaction.

RESULTS

1.) Results of orofacial hyperalgesia model

A.) Orofacial CFA treatment resulted significant preproPACAP increase in the TNC

Orofacial CFA injection caused significant ($p<0.01$, $p<0.001$) preproPACAP elevation 24 (0.58), 48 (0.69), 72 (1.01) and 120 hours (0.85) after the treatment in the TNC. The highest preproPACAP concentration was measured 72 hours after the CFA injection.

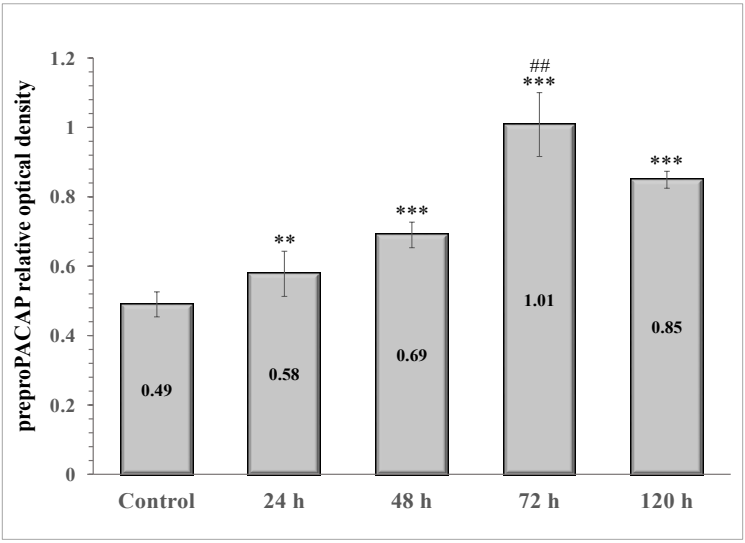

Figure 1. Relative optical density of the preproPACAP protein in the TNC following orofacial CFA treatment. *** $p < 0.001$ vs. control group, ** $p < 0.01$ vs. control group, ## $p < 0.01$ vs. 120 h group. Mean ± SD, $n = 6$.

B.) Orofacial CFA treatment significantly elevated the CGRP relative optical density in the TNC

Significant ($p<0.01$, $p<0.001$) CGRP expression increase was observed 24 (0.99), 48 (1.26), 72 (2.36) and 120 hours (2.1) after the CFA treatment in the TNC. The highest CGRP concentration was detected 72 hours after the CFA injection.

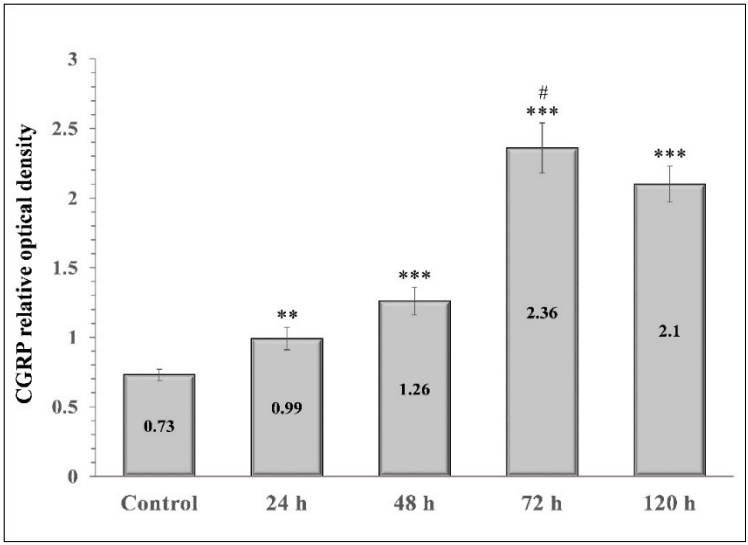

Figure 2. Relative optical density of the CGRP protein in the TNC following orofacial CFA treatment. *** $p < 0.001$ vs. control group, ** $p < 0.01$ vs. control group, # $p < 0.05$ vs. 120 h group. Mean ± SD, n = 6.

C.) Orofacial CFA treatment decreased the mechanonociceptive threshold

Significant ($p<0.01$, $p<0.001$) decrease was observed at 24 (6.64), 48 (4.10), 72 (2.10) and 120 hours (2.77) after the CFA treatment in the mechanonociceptive threshold compared to the control measurement (7.77). The lowest mechanonociceptive threshold was detected 72 hours after the CFA injection.

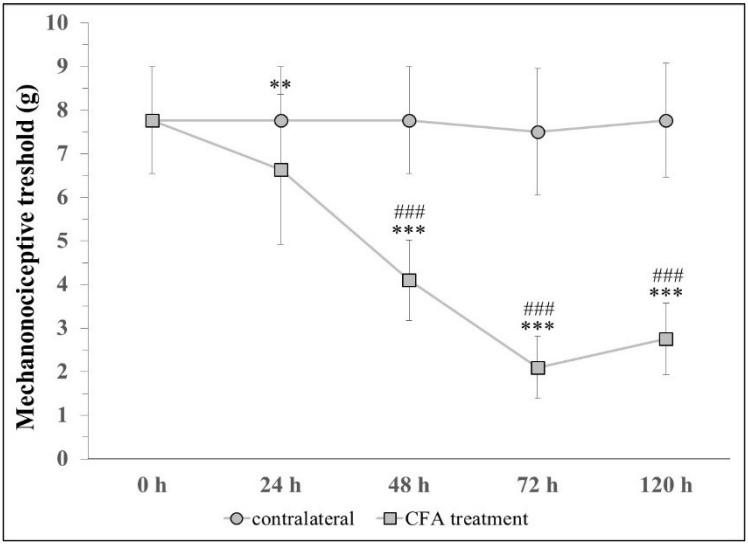

Figure 3. Changes in mechanical theshold before (0) and 24, 48, 72, 120 hours after CFA treatment. *** $p < 0.001$ vs. control measurement (0 h) in CFA treatment group, ** $p < 0.01$ vs. control measurement (0 h) in CFA treatment group, ### $p < 0.001$ vs. contralateral side. Mean ± SD, n = 6 - 24.

D.) Correlation between the expression of neuropeptides and the mechanical hyperalgesia

Reverse relationship was observed between the concentrations of neuropeptides and the value of the evoked mechanical threshold (CFA treated whisker pad) depending on the time ($p_{CGRP}<0.001$, $R_{CGRP}=-0.846$; n= 30, $p_{PACAP}<0.001$, $R_{PACAP}=-0.792$).

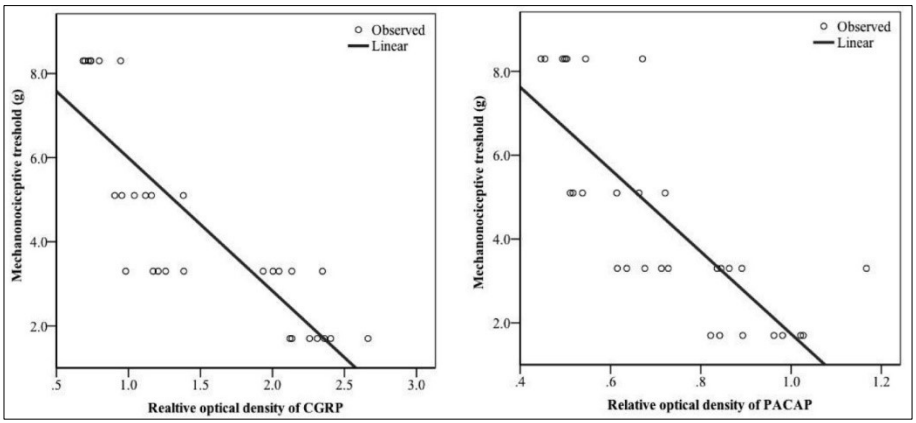

Figure 4. Negative, linear correlation between the expression levels of neuropeptides and the mechanonociceptive threshold.

2.) Results of ES-TRIG model

A.) Effects of SZR72 treatment on the ES-TRIG induced $PACAP_{1-38}$ mRNS overexpression

ES-TRIG resulted significant ($p<0.05$) $PACAP_{1-38}$ elevation in the TNC (27.49) compared to the control animals (19.31). SZR72 pretreatment prevented this ES-TRIG-evoked elevation (14.68).

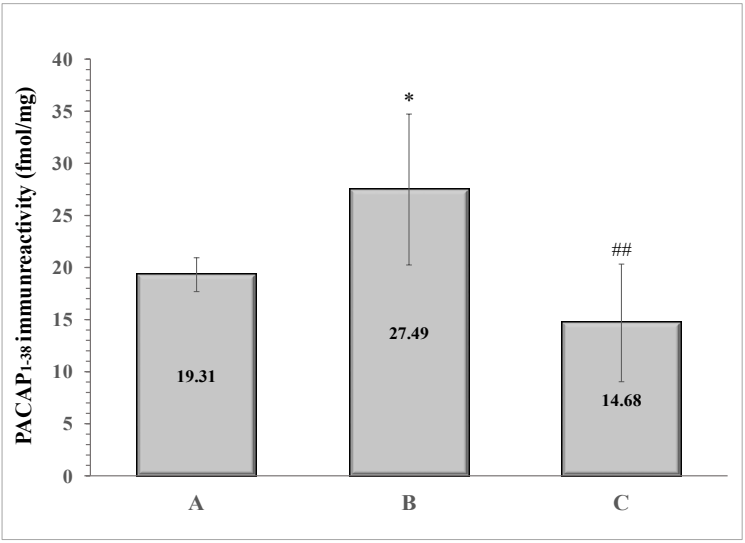

Figure 5. Immunreactivity of $PACAP_{1-38}$ in the TNC following electrical stimulation of the TRIG. A: control group B: vehicle-treated ES-TRIG group C: SZR72-treated ES-TRIG group. $^{*}p < 0.05$ vs. control group, $^{\#\#}p < 0.005$ vs. vehicle-treated ES-TRIG group. Mean ± SD, n = 6.

B.) Effects of KYNA, SZR72 and MK-801 treatment on the ES-TRIG induced preproPACAP protein overexpression

ES-TRIG induced preproPACAP overexpression (1.78) was significantly ($p<0.001$) reduced by the KYNA (0.74), SZR72 (0.51) and MK-801 (1.04) treatments. Difference of the preproPACAP level between the MK-801-, and the SZR72-treated groups was significant ($p<0.01$).

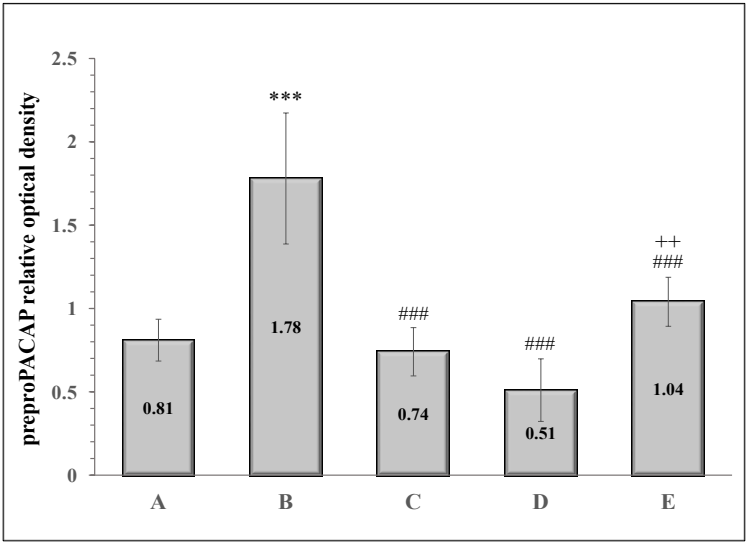

Figure 6. Relative optical density of the preproPACAP protein in the TNC following ES-TRIG. A: control group B: vehicle-treated ES-TRIG group C: KYNA-treated ES-TRIG group D: SZR72-treated ES-TRIG group E: MK-801-treated ES-TRIG group. $^{***}p < 0.001$ vs. control group. $^{###}p < 0.001$ vs. vehicle-treated ES-TRIG group. $^{++}p < 0.01$ vs. SZR72-treated ES-TRIG group. Mean ± SD, n = 6.

C.) Effects of KYNA, SZR72 and MK-801 treatment on the ES-TRIG induced $PACAP_{1-38}$ mRNS overexpression

ES-TRIG caused significant ($p<0.05$) $PACAP_{1-38}$ mRNA release in the TNC (1.16) compared to the control group. It was significantly ($p<0.01$, $p<0.001$) reduced by the KYNA (0.80), SZR72 (0.66) and MK-801 (0.72) treatments. Comparison of the different treatments did not reveal any significant difference.

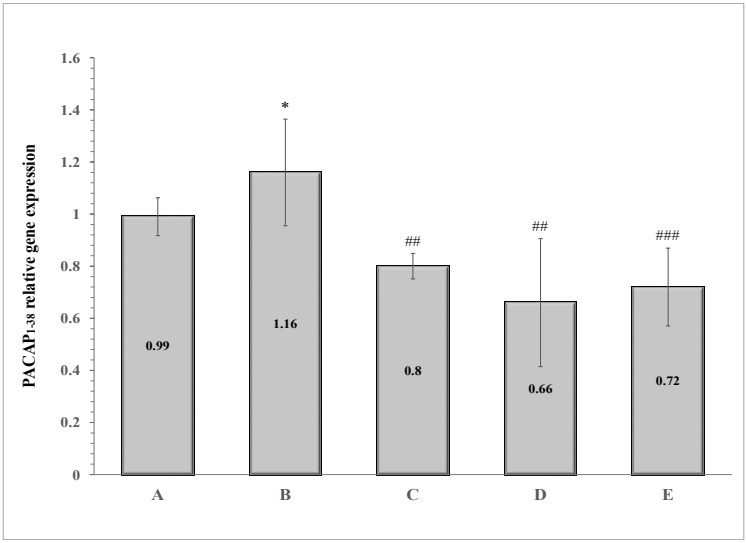

Figure 7. Relative mRNA expression of $PACAP_{1-38}$ in the TNC ES-TRIG. A: control group B: vehicle-treated ES-TRIG group C: KYNA-treated ES-TRIG group D: SZR72-treated ES-TRIG group E: MK-801-treated ES-TRIG group. $^{*}p < 0.05$ vs. control group. $^{\#\#}p < 0.01$ vs. vehicle-treated ES-TRIG group. $^{\#\#\#}p < 0.001$ vs. vehicle-treated ES-TRIG. Mean ± SD, n = 5.

DISCUSSION

Recent years the migraine research has focused on the neuropeptides as potential pathogenic factors and possible therapeutic alternatives. Nevertheless, alterations of the kynurenine metabolism have remarkable role in several neurological diseases. KYNA is a neuroprotective endogenous NMDA receptor antagonist, which may be a new agent in the therapy of migraine.

Our results proved that the activation of TS cause significant and simultaneous preproPACAP and CGRP release in the central migraine-related area in the brainstem, thereby these data support the neuropeptides theory of migraine pathomechanism.

Orofacial CFA treatment evoked significant CGRP and preproPACAP increase in the TNC. The neuropeptide levels reached theirs maximum at 72 hours after the CFA injection, correspondingly to the peak of facial allodynia. Another important result of present study is that the alterations of CGRP and preproPACAP expression show correlation with the change of mechanonociceptive threshold.

ES-TRIG caused significantly elevated preproPACAP/$PACAP_{1-38}$ expression both at the levels of proteom and transcriptome in the TNC. KYNA, SZR72 and MK-801 inhibited the ES-TRIG induced PACAP overexpression. Remarkable finding of this investigation that the expression levels of preproPACAP were significantly different between MK-801-, and SZR72-treated groups raises the possibility of the involvement of

additional targets of KYNA besides the NMDA (AMPA, kainate, aryl hydrocarbon, GPR35, opiod, etc.).

CONCLUSION

Our results provided the first direct evidence that the expression levels of CGRP and PACAP simultaneously increase after CFA induced trigeminal activation in the central region of the TS. Correlations, which were found between the alterations of CGRP/preproPACAP expression and mechanonociceptive threshold have proved the influence of neuropeptides in the mechanism of hyperalgesia.

Another important observation of the present study, that the NMDA receptor inhibition prevented the overexpression of PACAP in an experimental model of migraine. Remarkable difference was detected between the MK-801 and SZR72 treatments, which supports that targets at the non-NMDA glutamatergic systems may have therapeutic value in migraine.

These data contribute to the better understanding of migraine pathogenesis and thereby to the development of more efficient therapeutic approaches.

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Original publications directly related to the Ph.D. thesis:

I. **Körtési Tamás**, Tuka Bernadett, Tajti János, Bagoly Teréz, Fülöp Ferenc, Helyes Zsuzsanna, Vécsei László. Kynurenic Acid Inhibits the Electrical Stimulation Induced Elevated Pituitary Adenylate Cyclase-Activating Polypeptide Expression in the TNC. *Frontiers in Neurology. 2018 Jan 16;8:745. Impact Factor: 2.635, Citation:6*

II. **Körtési Tamás**, Tuka Bernadett, Nyári Alíz, Vécsei László. Tajti János. The effect of orofacial complete Freund's adjuvant treatment on the expression of migraine-related molecules. *Journal of Headache and Pain. 2019 Apr 29;20(1):43. Impact Factor: 3.918, Citation: 0*

Cumulative impact factor of the publications directly to the thesis: **6.553**

Publications not directly related to the Ph.D. thesis:

I. Tuka Bernadett, Szabó Nikolett, Tóth Eszter, Kincses Zsigmond Tamás, Párdutz Árpád, Szok Délia, **Körtési Tamás**, Bagoly Teréz, Helyes Zsuzsanna, Edvinsson Lars, Vécsei László, Tajti János. Release of PACAP-38 in episodic cluster headache patients - an exploratory study. *Journal of Headache and Pain. 2016 Dec;17(1):69. Impact factor: 3.580 Citation: 35*

II. Cseh Edina Katalin,Veres Gábor, **Körtési Tamás**, Polyák Helga, Nánási Nikolett, Tajti János, Párdutz Árpád, Klivényi Péter, Vécsei László, Zádori Dénes. Neurotransmitter and tryptophan metabolite concentration changes in the CFA model of orofacial pain. *Journal of Headache and Pain. Publishing under process. Impact factor: 3.580 Citation: 0*

Cumulative impact factor of publications not directly related to the thesis: **7.160**

Total impact factor: **13.713**