REVIEW ARTICLE

Migraine, Neurogenic Inflammation, Drug Development - Pharmaco-chemical Aspects

Melinda Lukács[1], János Tajti[1], Ferenc Fülöp[2], József Toldi[3], Lars Edvinsson[4,5], and László Vécsei[1,6,*]

[1]*Department of Neurology, University of Szeged, Hungary;* [2]*Institute of Pharmaceutical Chemistry and MTA-SZTE Research Group for Stereochemistry, University of Szeged, Hungary;* [3]*Department of Physiology, Anatomy and Neuroscience, University of Szeged, Hungary;* [4]*Department of Clinical Sciences, Division of Experimental Vascular Research, Lund University, Lund, Sweden;* [5]*Department of Clinical Experimental Research, Copenhagen University, Glostrup Hospital, Copenhagen, Denmark;* [6]*MTA-SZTE Neuroscience Research Group, Szeged, Hungary*

Abstract: ***Background:*** Migraine is a primary headache disorder. Despite numerous studies conducted with the aim to understand the pathophysiology of migraine, several aspects are still unclear. The trigeminovascular system plays a key role. Neurogenic inflammation is presumed to be an important factor in migraine pathophysiology, mediated by the activation of primary neurons, leading to the release of various pro-inflammatory neuropeptides and neuro-transmitters such as Calcitonin Gene-Related Peptide (CGRP), substance P (SP), and vasoactive intestinal peptide (VIP). Nitric oxide (NO), Pituitary adenylate cyclase-activating polypeptide (PACAP) and Glutamate (Glu) also play an important role in the modulation of inflammatory mechanisms.

Objective: To review the literature focusing on novel therapeutic targets in migraine, related to neurogenic inflammation.

Method: A systematic literature search in the database of PUBMED was conducted regarding therapeutic strategies in migraine, focusing on substances and cytokines released during neurogenic inflammation, published in January 2017.

Results: Ongoing phase III clinical studies with monoclonal antibodies against CGRP and CGRP receptors offer promising novel aspects for migraine treatment. Preclinical and clinical studies targeting SP and nitric oxide synthase (NOS) were all terminated with no significant result compared to placebo. New promising therapeutic goal could be PACAP and its receptor (PAC$_1$), and kynurenic acid (KYNA) analogues.

Conclusion: Current migraine treatment offers pain relief only for a small proportion of migraine patients and might not be adequate for patients with cardiovascular comorbidity due to side effects. Better understanding of migraine pathophysiology might, therefore, lead to novel therapeutic lines both in migraine attack treatment and prophylaxis.

1. INTRODUCTION

1.1. Migraine

Migraine is a painful episodic neurological disease being the third most prevalent and the seventh most disabling disease worldwide [1]. Migraine has not only a large impact on individual and public health but its socio-economic costs are extensive [2, 3]. Clinically, migraine pain is considered a unilateral, pulsating headache, aggravated by coughing or physical activity

---

*Address correspondence to this author at the Department of Neurology, University of Szeged, Hungary and MTA-SZTE Neuroscience Research Group, Szeged, Hungary; Tel: +3662545384; Fax: +3662545597; E-mail: laszlo.vecsei@med.u-szeged.hu

associated with nausea, vomiting, and photophobia [4]. The attack can be preceded by the aura phenomenon that occurs in the wake of the migraine attack and is represented in 99% of cases by visual symptoms (scotomas, flashing lights etc.). Nonvisual aura presents mainly as sensory symptoms (paresthesia); however, but rare auras like olfactory hallucinations, language difficulties (dysarthria) or temporary muscle weakness (hemiparesis in case of familial hemiplegic migraine-FHM) might also occur [4-6]. According to the latest classification of the International Headache Society migraine can be divided into episodic (migraine with or without aura) and chronic form (headache occurs on at least 15 days per month with more than 8 typical migraine attacks lasting for at least 3 months) [6].Various studies have addressed the pathophysiology of migraine but some aspects still remain unclear. Nevertheless, we can firmly postulate that the trigeminovascular system plays a crucial role in the generation and transmission of pain sensation. The system consists of the trigeminal ganglion (TG) with pseudounipolar neurons that innervate the meningeal vasculature and project centrally to the second-order neurons of the trigeminal nucleus caudalis (TNC) in the brainstem extending to the C_1-C_2 region of the spinal cord [7]. These second-order neurons transmit pain signals into the thalamus and cortical regions [8, 9]. Recent neuroimaging studies revealed other regions of the central nervous system (CNS) (ex. cerebellum, insula, pulvinar, etc.) that might play a role in the modulation of pain sensation [10, 11]. In 1938, Wolff and coworkers have set up the vascular theory of migraine headache, suggesting that the headache might be generated in the cranial arteries due to a short vasoconstriction and a reactive vasodilation occurring during a migraine attack [12]. New findings have questioned this theory. Currently the concept is that migraine is a neurovascular disorder, that originates in the CNS causing a hypersensitivity of the peripheral trigeminal nerve fibers that innervate meningeal blood vessels [13].

Regarding migraine treatment different medications are used for an acute migraine attack and for prevention. For acute migraine treatment non-steroid anti-inflammatory drugs (NSAID) like aspirin, ibuprofen, diclofenac or naproxen are recommended. However, these are not migraine specific and pain restarts after 2-4 hours in almost 80% of patients [14-17]. Triptans are small agonist molecules acting on 5-HT$_{1B/1D}$ receptors and represent level A recommendation according to the European Federation of Neurological Societies (EFNS) guideline [18, 19]. Certain aspects of their mechanism of action in migraine pain relief is, however, still un-

clear [20]. One possible site of action could be an inhibitory effect on plasma protein extravasation and neurogenic inflammation [21]. Clinical trials show pain-relief in 28-59% of the cases [22], the proportion of pain-free patients after 2 hours following oral treatment is 18-58% [23]. After application of sumatriptan severe cardio- and cerebrovascular adverse events have been reported [24], therefore triptans are contraindicated in hypertension, Raynaud syndrome, coronary artery disease, stroke and in pregnancy [19]. Their use is restricted to 9 days per month as high risk for chronification was noted following the use of 12 days per month [25, 26]. Therefore, treating chronic migraine represents a therapeutic challenge for both clinicians and researchers. Preventive daily treatment is needed when headache frequency exceeds 8-10 days per month or the use of NSAIDs or triptans for more than 8-9 days per month is needed [27]. Beta-blockers (metoprolol, propranolol), calcium channel blockers (flunarizine), antiepileptic drugs (valproic acid, topiramate) or antidepressants (amitriptyline, venlafaxine) are recommended for migraine prevention [19]. Lately Botulinum toxin A (BoNTA) has proven to be effective and it improves the quality of life in chronic migraine treatment [28-31]. An important disadvantage of BoNTA is its route of administration: intramuscular injection in the muscles of the face and neck [28, 30]. On the basis of all these, we can conclude that migraine is a highly prevalent painful neurological condition with an important socio-economic impact. In order to reach an optimal disease-control new therapeutic strategies are needed, especially in chronic migraine.

1.2. Neurogenic Inflammation

The concept of 'neurogenic inflammation' was introduced by the classic experiment of Goltz (1874) and Bayliss (1901) observing skin vasodilation following electrical stimulation of the dorsal horn, that could not be linked to the immune system [32]. Activation of sensory nerve fibers causes transmission of pain signals not only orthodromically, but also antidromically in the yet inactive afferent nerve fibers [32, 33]. At the peripheral ending of the fibers substance P (SP), calcitonin gene-related peptide (CGRP), and neurokinin A (NKA) are released which lead to the activation of various cell-types (endothelial cells, mast cells, macrophages, T cells, dendritic cells). Moreover, these cells release numerous other substances, such as prostaglandins, tumor necrosis factor alpha (TNFα, interleukins (ILs), glutamate (Glu), nerve-growth factor, vasoactive intestinal peptide (VIP) also causing plasma protein extravasation (Fig. **1**), thus creating a whole
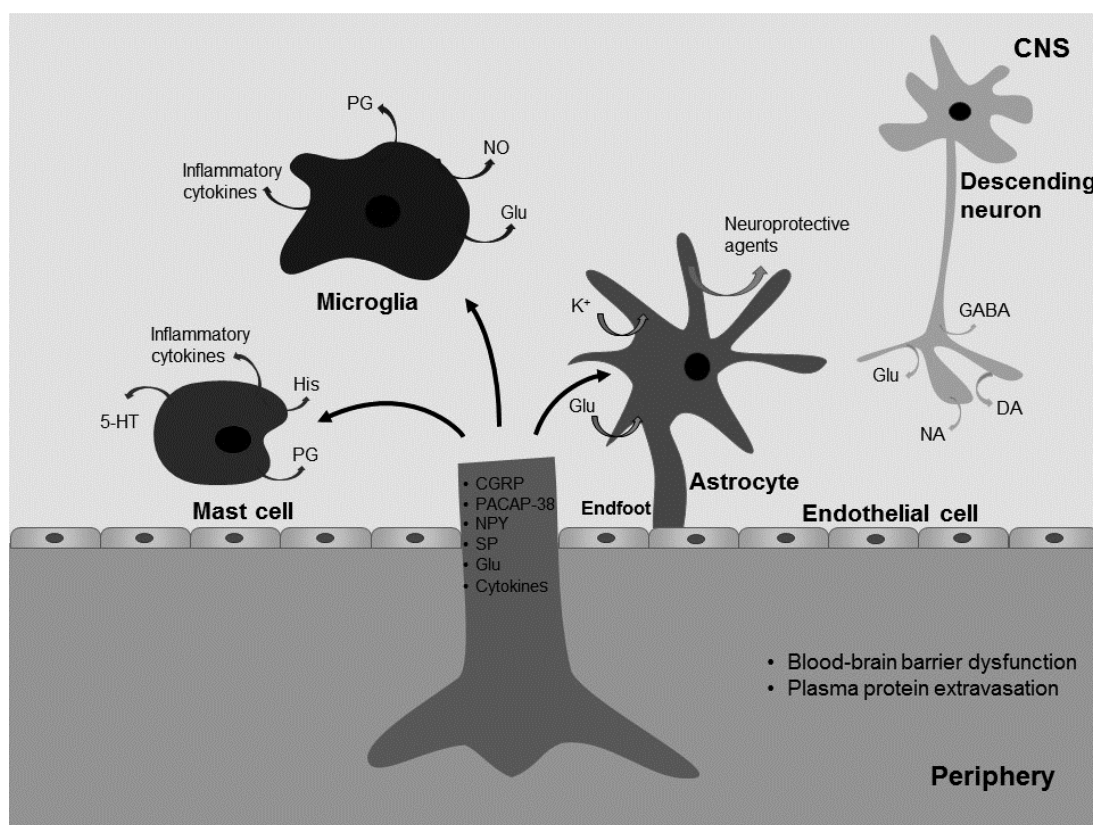
Fig. (1). Neurogenic inflammation. Enhanced activation of the peripheral nerve endings results in the release of various neuropeptides (*e.g.,* CGRP, NPY, SP, and Glu etc.), leading to neurogenic inflammation. This involves, but it is not limited to the activation of astrocytes, microglia, and mast cells, which under inflammatory conditions are able to pass the blood-brain barrier (BBB). Plasma protein extravasation and BBB dysfunction are also important consequences of neurogenic inflammation. Release of numerous pro- and anti-inflammatory agents from the cells involved are able to further modify the inflammatory process. Higher-order centers of the CNS can intensify the process, leading to a self-amplifying reaction that might cause peripheral and central sensitization.

CGRP = calcitonin gene-related peptide; CNS = central nervous system; DA = dopamine; GABA = gamma-aminobutyric acid; Glu = glutamate; 5-HT = serotonin; His = histamine; NPY= neuropeptide Y; PACAP-38 = 38-amino-acid isoform of pituitary adenylate cyclase-activating polypeptide, PG = prostaglandins; NO = nitric oxide, NA = noradrenaline (a.k.a. norepinephrine); SP = substance P.

picture of an 'inflammatory state' [33, 34]. Neurogenic inflammation is presumed to be an important factor in various neurological diseases: brain injury [35] , neuropathic pain [36], epilepsy [37, 38] and primary headache diseases [39]. A role of neurogenic inflammation in migraine pain has been suggested for decades [40]. Plasma protein extravasation, an important phenomenon that occurs in neurogenic and non-neurogenic inflammation has been described in animal models of trigeminovascular activation: following electrical stimulation of the rat TG or chemical stimulation with intravenous capsaicin, plasma protein leakage was reported in the dura mater but also within extracranial tissues (eyelids, conjunctiva, lips, gingiva) [41]. In these models plasma protein extravasation is presumed to take place through the fenestrated endothelium of the small vessels in the dura mater and is mediated *via* perivascular myelinated and unmyelinated fibers [41, 42]. Dural neurogenic inflammation is further mediated by the release of vasoactive neuropeptides (CGRP, SP, and NKA) from the perivascular nerve fibers [43]. Plasma protein extravasation has been shown to be blocked by triptans and NSAIDs, selectively in the dura mater but not in the extracranial structures or in the brain or brain vasculature [42, 44, 45]. The process of neurogenic inflammation can be self-amplifying, where Glu, the major excitatory neurotransmitter, plays a key role in sensitization of sensory nerve fibers. Furthermore, CNS centers might aggravate peripheral neurogenic inflammation, playing a crucial role in long-term potentiating processes which lead to the chronification of migraine pain [33]. The above listed proc-

esses and cytokine mechanisms might have relevance not only by offering a better understanding of migraine pathophysiology, but might also serve as site of action for novel therapeutic approaches. The importance of neurogenic inflammation is suggested by the presumption that triptans, the current gold-standard therapy in migraine act against neurogenic inflammation as they reduce plasma protein extravasation and the release of vasoactive peptides (CGRP, substance P) [46, 47].

2. THERAPEUTIC TARGETS INVOLVING NEUROGENIC INFLAMMATION

In the present paper, we aimed to review the therapeutic possibilities in migraine in relation to neurogenic inflammation. A systemic literature search was conducted in the database of PUBMED in January 2017. The search strings used were: "migraine", "neurogenic inflammation", and "migraine treatment".
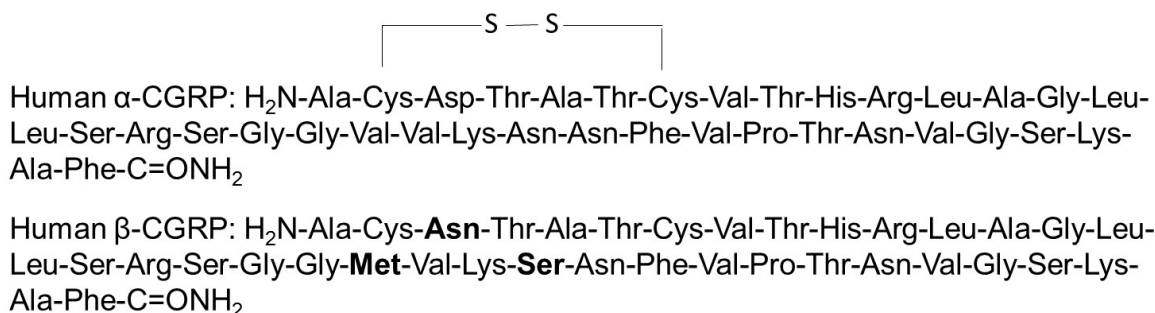
2.1. Calcitonin Gene-related Peptide (CGRP)

CGRP is a 37-amino-acid protein shown to be linked to migraine pathophysiology and neurogenic inflammation [48]. It has a vasodilatory effect and plays a role in the transmission of pain sensation [49]. CGRP exists in two active forms differing only in three amino acids in humans: α-CGRP is expressed in the peripheral and central nervous system, whereas β-CGRP is expressed predominantly in the enteric nervous system [50, 51]. The structure of human α-CGRP contains four domains: the first seven residues with the N-terminal, linked together by a disulfide bridge form the first domain. The second domain is built up by residues 8-18, deletion of this domain causes high decrease in binding affinity. The third domain contains residues 19-27 and whereas the fourth domain is made up by residues 28-37containing the C-terminus representing the binding epitope [52]. The structure of human CGRP is presented in Fig. (**2**).

In the 90's, calcitonin receptor-like receptor (CLR) was discovered, consisting of 461 amino acids with seven transmembrane domains [53, 54]. As CLR is widely expressed in different cell types, another protein called receptor activity modifying protein (RAMP) is needed for the site-specific bioactivity of CGRP. Three types of RAMPS are known: RAMP1, RAMP2 and RAMP3. These receptors become activated by the heterodimerization of one transmembrane RAMP and CLR proteins [55]. Heterodimerization of CLR and RAMP1 creates a CGRP receptor with high binding affinity for CGRP (Fig. **3**), while co-expression of CLR and RAMP2 or RAMP3 yields adrenomedullin receptors (AM1 and AM2 receptor) [56]. The mechanism of CGRP binding to the receptor is referred to as the 'two-domain model'. During the first step, the COOH terminal part of CGRP binds to the NH_2 terminal of the extracellular domain of the receptor, during the second step the NH_2 terminal of the protein binds to the juxta membrane region of the receptor, leading to the activation of intracellular pathways [57]. An additional protein called receptor component protein (RCP), a small membrane-associated protein, is needed for the activation of the cyclic adenosine monophosphate (cAMP)- and inositol diphosphate (IP_2)-generating pathways (Fig. **3**). RCP is not necessary for receptor activation but is essential for proper signal transduction [58].

Mapping of CGRP and its receptors in the trigeminovascular system allows the identification of potential sites of action of anti-CGRP therapies [59-62]. In 2000, Boehringer Ingelheim presented the first selective non-peptide CGRP antagonist, Olcegepant [63]. In phase II clinical trials, the effect of Olcegepant was comparable to the effect of triptans, having no influence on systemic hemodynamics, suggesting that Olcegepant will not have cardiovascular side effects [64]. Due to its

$$S — S$$

Human α-CGRP: H_2N-Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-C=ONH_2

Human β-CGRP: H_2N-Ala-Cys-**Asn**-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-**Met**-Val-Lys-**Ser**-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-C=ONH_2

Fig. (2). Structure of α-CGRP and β-CGRP. CGRP is a 37-amino-acid protein having two active forms: α-CGRP expressed in the CNS and β-CGRP expressed in the enteric nervous system. Their structure differs in three amino acids. CGRP = calcitonin gene-related peptide.
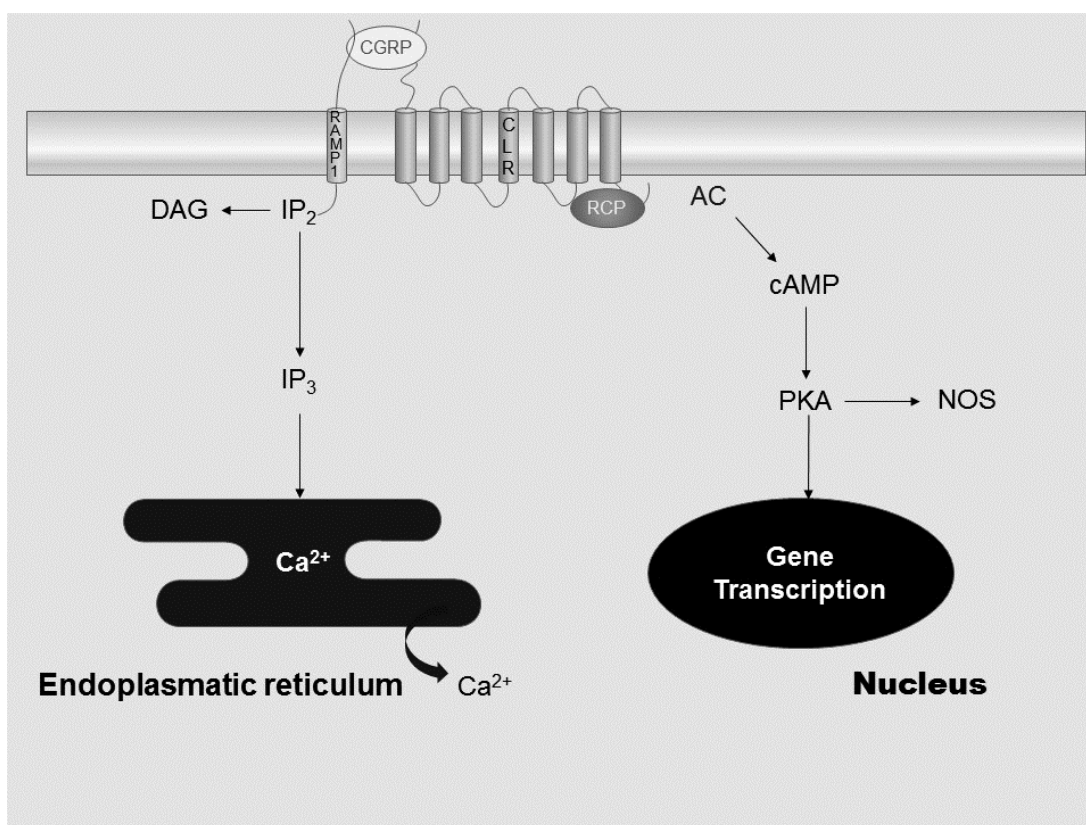
Fig. (3). Binding of CGRP to its receptors. The receptor for CGRP is considered to be formed by the CLR/RAMP1 complex, where CLR has seven whereas RAMP1 has one transmembrane domain. For the optimal function of the protein, another membrane-associated component, called RCP, is needed. Binding of CGRP to the receptor leads to the activation of intracellular signaling pathways: 1. Elevation of cAMP, leading to activation of PKA, resulting in gene transcription and protein synthesis. NO production might occur following phosphorylation of NOS. 2) Another signaling pathway mediated by IP_2 results in Ca^{2+} release from the endoplasmic reticulum.

Abbreviatons: CGRP- Calcitonin gene-related peptide, CLR- calcitonin receptor-like receptor, RAMP1- receptor activity modifying protein 1, RCP- receptor component protein. AC- adenylate cyclase, cAMP- cyclic adenosine monophosphate, PKA- protein kinase A, NOS- nitric oxide synthase, IP_2- inositol-diphosphate, DAG- diacylglycerol, IP_3- inositol triphosphate.

high molecular weight Olcegepant (and being a dipeptide) could only be administrated intravenously [65]. A great effort has been invested in the development of orally available CGRP antagonists. In 2007, Merck published details of Telcagepant with oral administration reported to be potent in acute migraine treatment [66, 67]). The studies had to be interrupted at phase III clinical trials due to elevated transaminase levels and hepatotoxicity following twice daily Telcagepant administration for 3 months [68]. Although many compounds of this class have been proven effective in migraine treatment, their clinical development had to be suspended due to side-effect associated with long-term use [69]. More recently, monoclonal antibodies targeting CGRP or its receptors have been developed. Zeller *et al.* have shown for the first time that function-blocking CGRP antibodies were able to block neurogenic vasodilation in the skin and meningeal blood ves-

sels in rats [70]. These monoclonal anti-CGRP antibodies are macromolecules that bind to CGRP and neutralize the excessively released CGRP from the trigeminal nerve fibers, or target CGRP receptors, blocking CGRP induced activation of the trigeminovascular system. These antibodies have various potential benefits compared to CGRP antagonists in the light of their biochemical properties [69]: 1. the target specificity of monoclonal antibodies prevents appearance of side-effects, such as off-target hepatotoxicity, 2. due to their pharmacokinetic profile and long-term half-life a less frequent dosing is needed [71]. Anti-CGRP antibodies are macromolecules, unable to pass the blood-brain barrier (BBB), thus the conclusion appears obvious that their effect can only be exerted through a peripheral site of action delivered through the intravenous or subcutaneous routes [69]. The development of such monoclonal antibodies represents a great challenge compared

to that of small molecules as their immunogenicity influences the pharmacokinetic properties and their toxicity. Therefore, different types of bioanalytical assays are needed for their early development [72]. Another disadvantage might be the result of systemic immunological presenting as an immune response against the therapeutic protein. Therefore, most antibodies are humanized mAbs, and anti-drug antibodies need to be screened prior treatment to prevent immunological side-effects [71]. These anti-drug antibodies are able to diminish the effect of mAbs either by uplifting the clearance of the antibodies, which leads to their reduced concentration, or by preventing the mAbs to bind to their target [70]. Three anti-CGRP and one anti-CGRP receptor mAb are under development. LYD2951742 (Arteaus Therapeutics, USA, Eli Lilly and Co., USA) has been proven to have better outcome than placebo in phase II clinical studies and three phase III studies are underway [73, 74]. ALD 403 (Alder Biopharmaceuticals, USA) has shown promising results in a phase II trial [75]. The ongoing phase III trial is planned to be completed in June 2017 [74]. LBR101/TEV-48125 (Labys Biologics, Pfizer, USA - Teva Pharmaceuticals, USA) has been proven to be efficient in migraine prevention [76] and an ongoing phase III clinical trial will be completed by October 2017 [74]. AMG 334 (Amgen, USA-Novartis) is the only mAb that targets the CGRP receptors [77]. A phase III trial was started in 2015 and will end in March 2017 [74]. Details of mAbs are summarized in Table **1**.

Due to their serious side effects during long-term use, the future therapeutic role of CGRP and CGRP receptor antagonists is questionable and their clinical use is limited. The most promising novel therapeutic line is represented by mAbs. Although their oral administration is not possible, the infrequent dosing leads to better compliance as being more acceptable for the patients. The fact that mAbs are large molecules not able to penetrate the BBB prevents CNS-related side effects, and they do not affect the liver or the kidney. Further studies providing detailed assessment of safety and tolerability aspects during long-term use are eagerly awaited.

2.2. Substance P

SP is an 11-amino-acid protein (Fig. **4**), known to be another key mediator implicated in neurogenic inflammation. Release of SP from trigeminal nerve endings causes plasma protein extravasation and vasodilation [78]. SP binds to three tachykinin receptors (NK_1, NK_2, NK_3) with highest affinity to NK_1. The NK_1 receptor is a G protein-coupled receptor or a seven-transmembrane receptor. For SP binding, the N-terminal segment and the third and seventh transmembrane domain has major importance. PRP 100893, a non-peptide NK_1 receptor antagonist, a member of the perhydroindolone family, has been suggested as a therapeutic target in migraine, having been reported to block neurogenic inflammation in animal models [79]). Unfortunately, clinical studies (double blind, placebo controlled) were not able to support the positive effects of NK_1 receptor antagonists either in acute or in the prophylactic treatment of migraine. Nevertheless, no side-effects have been noted [80, 81]. In the light of their ineffectiveness, future studies involving SP have not been undertaken. The role of SP remains a question of debate for migraine scientists, considering that SP

Table 1.   Monoclonal antibodies targeting CGRP and its receptors.

Monoclonal Antibody	Target	Type of Headache	Route of Administration	Ongoing Clinical Study
LYD2951742	CGRP	episodic/chronic migraine	s.c.	Phase 3 (June September 2017, April 2018)
ALD403	CGRP	frequent episodic/chronic migraine	i.v.	Phase 3 (June 2017)
LBR101/TEV-48125	CGRP	frequent episodic/chronic migraine	s.c.	Phase 3 (October 2017)
AMG 334	CGRP receptor	episodic/chronic migraine	s.c.	Phase 3 (March 2017, February 2018)

Substance P: H- Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met- H_2N

PACAP-27: H-His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-Ser-Arg-Tyr-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Ala-Ala-Val-Leu-H_2N

PACAP-38: H-His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-Ser-Arg-Tyr-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Ala-Ala-Val-Leu-Gly-Lys-Arg-Tyr-Lys-Gln-Arg-Val-Lys-Asn-Lys-H_2N

Fig. (4). Sequences of substance P, PACAP-27, and PACAP-38. The figure presents the amino acid sequences of substance P, built from 11 amino acids, PACAP-27, and PACAP-38 (the two active forms of PACAP), built from 27 and 38 amino acids, respectively. PACAP-27 is a polypeptide fragment of PACAP-38 being able to induce the activation of intracellular signaling pathways.
PACAP-27 = 27-amino-acid isoform of pituitary adenylate cyclase-activating polypeptide; PACAP-38 = 38-amino-acid isoform of pituitary adenylate cyclase-activating polypeptide.

levels were not elevated during spontaneous migraine attacks, in contrast with CGRP [82, 83]. Another explanation might be that dural plasma extravasation does not in fact play a crucial role in migraine pathomechanism [84]. To our knowledge, no ongoing studies have been reported regarding SP. The questionable role of SP in migraine pathophysiology and the ineffectiveness of NK-1 receptor antagonists together make any future attempt on novel therapeutic approaches regarding SP beyond reason.

2.3. Nitric Oxide

Nitric oxide (NO) is a labile gas with pleiotropic effects in different organs and especially in the brain. NO is produced by three iso-enzymes, called nitric oxide synthases (NOS): neuronal NOS (nNOS), endothelial NOS eNOS) and inducible NOS (iNOS) [85]. NOS consists of two domains that work independently. The first is a C-terminal reductase domain, which represents the binding site for NADPH and Ca-calmodulin. Binding of Ca- calmodulin triggers the activation of NOS [86]. The N-terminal region contains a binding site to tetrahydrobiopterin (BH4), heme and L-Arginine (L-Arg) [87]. From the three iso-enzymes, iNOS is the only NOS the activity of which is unrelated to the Ca-calmodulin complex and dependent on its *de novo* synthesis caused by various inflammatory cytokines (*e.g.,* interferon-gamma) [88]. In migraine, NO is supposed to be produced in the perivascular nerve endings [89], and human studies have shown increase of platelet nitrates, known as markers of endogenous NO production, suggesting a possible role of NO in migraine pathophysiology [90]. A non-selective NOS inhibitor, namely N(G)-mono-methyl-L-arginine (L-NMMA), lead to pain relief in migraine attack; however, poten-tial vascular side-effects (bradycardia and high blood pressure) due to eNOS inhibition have also been noted [91]. Therefore, a scientific demand has been raised related to selective iNOS and/or nNOS inhibitors. The role of iNOS has been suggested in inflammation; therefore, selective iNOS inhibitors have been tested in migraine (GW274150, GW273629) [92]. No superiority has been reported compared to placebo in human studies either in acute treatment or for prophylaxis [93]. A new selective iNOS inhibitor and 5-HT$_{1B/1D}$ receptor agonist has shown promising results in preclinical studies [94], but phase II clinical studies have failed to show efficacy in acute treatment [95]. With regard to NOS inhibitors it needs to be considered that NO can be harmful, especially in terms of oxidative stress. Nevertheless, it is also needed for physiological processes in the brain; therefore, any future therapeutic strategies involving modulation of NO synthesis should be handled carefully.

2.4. Vasoactive Intestinal Peptide

VIP is a 28-amino-acid peptide, a member of the secretin/glucagon superfamily, which acts on G protein-coupled receptors [96]. Despite its proposed role in protein extravasation, current studies have questioned its role in migraine pathogenesis. Intravenous infusion of VIP indeed caused vasodilation in the temporal superficial artery; however, it did not induce migraine attacks [97]. Interestingly though, a recent study has reported increased VIP levels in the serum of chronic migraine patients compared to healthy subjects [98]. In conclusion, we assume that VIP might have a strong vasodilator effect, but its role in initiating migraine attack is uncertain. To our knowledge, there are no ongoing clinical studies targeting VIP or its receptors.

## 2.5. Pituitary Adenylate Cyclase-activating Peptide

PACAP is a member of the VIP/secretin/glucagon family first isolated in 1989 from ovine hypothalamus extract and named after its ability to stimulate cAMP in rat pituitary cells [99]. Sequencing of the peptide showed that it's C-terminal is α-amidated and it consists of 38 amino acids (Fig. **4**). In the structure of PACAP1-38 an internal cleavage-amidation site is found, that might cause formation of a polypeptide fragment, containing 27 amino acids, called PACAP1-27 (Figure 4) [99]. PACAP1-27 has proven to be 68% identical to VIP with a much higher ability to stimulate cAMP (Figure 5) [99-101]. PACAP1-38 and VIP bind with the same affinity to $VPAC_1$ and $VPAC_2$ receptors, whereas PAC_1 receptor has a much higher affinity to PACAP1-38 (Fig. **5**) [102]. $VPAC_1$ and $VPAC_2$ receptors are G protein-coupled receptors class B having seven transmembrane domains. The N-terminal ectodomain of the receptor represents the binding site for the C-terminal region of VIP and the N-terminal region of VIP binds to the first transmembrane domain of the receptor, forming a so called 'Sushi' domain [103]. PACAP1-38 is a pleiotropic molecule shown to be present in various components of the trigeminal system [89, 104-106] and its role in neurogenic inflammation has been suggested by various studies [107, 108]. Elevated levels pf PACAP1-38 were found in the ictal phase compared to the interictal phase in migraine patients [109], and the intravenous administration of PACAP1-38 caused delayed migraine-like headache [110]. Although PACAP1-38 seems to play a crucial role in migraine, some aspects of the nociceptive effects of PACAP1-38 in migraine pathophysiology are still unsettled [111, 112]. PACAP1-38 causes mast cell degranulation in the dura mater leading to the activation of peripheral trigeminal nerve fibers [113, 114]. The vasodilatory effect of PACAP1-38 is less potent than that of VIP in meningeal and coronary arteries and it was not influenced by PAC_1-receptor antagonism, suggesting that PACAP1-38 does not contribute to migraine pathophysiology *via* its vasodilatory effect [115]. PACAP1-38 induced migraine pain might be generated by the activation of the trigeminal nociceptive fibers that innervate the dura *via* intracellular cAMP increase and activation of IP_2 pathway initiated by the binding of PACAP to PAC_1 receptor. While PACAP1-38 is supposed to generate migraine *via* peripheral mechanisms, the development of such PAC_1 receptor antagonists, that penetrate the blood-brain-barrier (BBB) might also act centrally on the second-order neurons and not having vascular side effects [116]. In an animal model of dural electrical stimula-

tion, intravenous administration of PAC_1 antagonist inhibited meningeal vasodilation but did not affect the neuronal responses. Only the intra-cerebro-ventricular delivery was able to modify activation of the TNC neurons [117]. Maxadilan is a 61 amino acid protein, a potent vasodilator isolated from the saliva of sand flies [118]. The maxadilan binding site was found to be the PAC_1 receptor, making maxadilan a PACAP1-38 receptor agonist [119]. Deletion of amino-acids between position 24 and 42 generated M65, a potent and selective PAC_1 antagonist [120]. No human studies have been performed to test PAC1 receptor antagonists as a novel therapeutic tool in acute migraine treatment [116, 121]. Additionally, the presence of a novel, not yet identified PACAP1-38 receptor has been suggested, as PACAP induced CGRP release from the TNC, but not from the TG or the dura mater. Strikingly, this effect was not mediated by any of the already known receptors [122] yielding a potential target for new therapeutic strategies. Taken all together, a high amount of evidence suggest that PACAP1-38 and its receptors play a pivotal role in the initiation of a migraine attack and the sensitization the pain. Regarding future perspectives PACAP1-38 might act as a biomarker for migraine attack, and therapies acting on PACAP1-38 and its receptors might represent new strategies for drug discovery. Current efforts focus on understanding the exact role of PACAP1-38 in migraine.

2.6. Kynurenic Acid

Tryptophan (TRP), an essential α-amino-acid, is the precursor of the neurotransmitter 5-HT. 5-HT is synthesized involving the action of tryptophan hydroxylase [123]. Triptans are small agonist molecules acting on $5-HT_{1B/1D}$ receptors and have level A recommendation according to EFNS [18, 19]. The major route for TRP metabolism is the kynurenine pathway (KP), resulting in NAD^+ and $NADP^+$ as end products. In this metabolic pathway, L-kynurenine (KYN) can be metabolized through two branches (Fig. **6**): one branch providing the neuroprotective kynurenic acid (KYNA) and another branch providing the neurotoxic quinolinic acid (QUIN) [124-126]. Both neuroactive molecules have been shown to play important roles in various CNS diseases [127, 128]. *N*-methyl-D-aspartate (NMDA) receptor consists of three subunits (NR1, NR2, and NR3) and is activated by Glu and glycine. Glycine is essential for NMDA receptor function with the glycine binding site being located on the NR1 subunit [129, 130]. In higher (micromolar/millimolar) doses, KYNA acts on the strychnine-insensitive glycine binding site of the NMDA receptor [130]. In low (nanomolar) con-
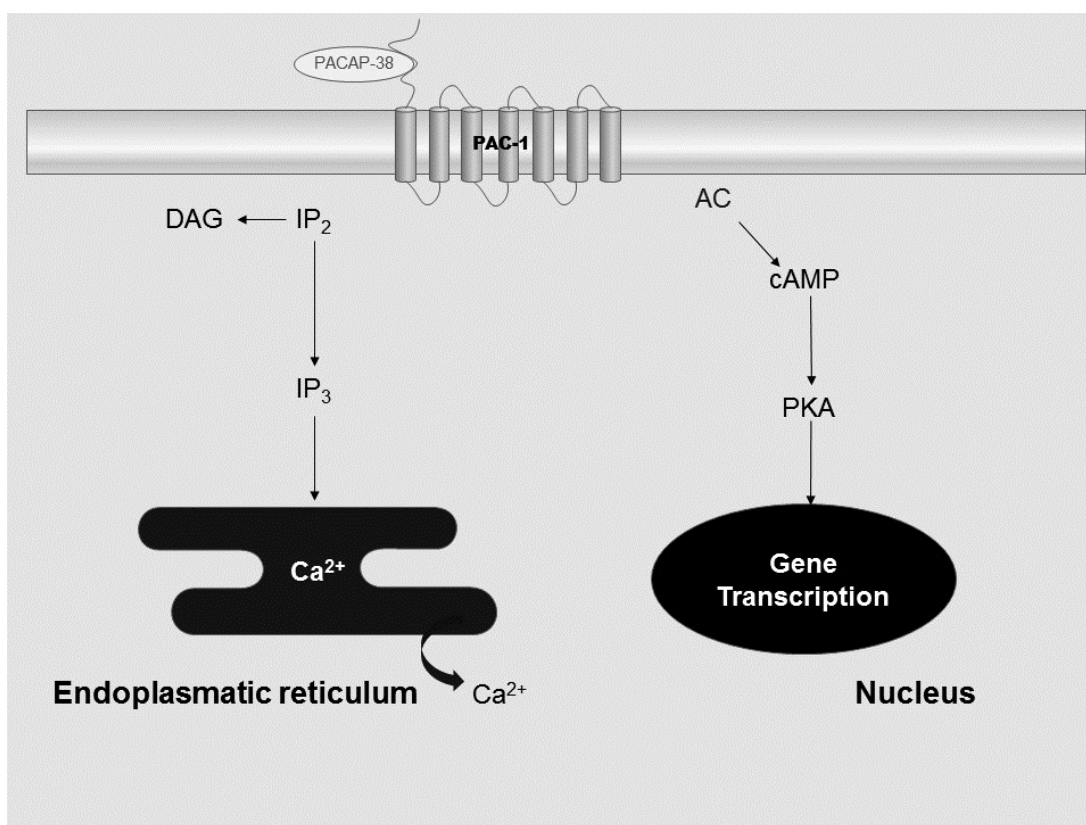
Fig. (5). Binding of PACAP to its receptor. PAC1 is a seven-transmembrane protein receptor. Binding of PACAP-38 to PAC1 causes increase in intracellular cAMP levels, leading to the activation of various signaling pathways described in Fig. **3**.
AC = adenylate cyclase; cAMP = cyclic adenosine monophosphate; DAG = diacylglycerol; IP_2 = inositol diphosphate; IP_3 = inositol triphosphate; NOS = nitric oxide synthase; PACAP = pituitary adenylate cyclase-activating polypeptide; PAC_1 = pituitary adenylate cyclase-activating polypeptide receptor 1; PKA = protein kinase A.

centrations, however, KYNA enhances α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-sensitive Glu receptors [131, 132]. A key enzyme of the KP is the indolamine 2,3-dioxygenase (IDO), which was shown to be modulated by the immune system *via* inflammatory cytokines and molecules (Fig. **6**) [133]. This may explain the low availability of 5-HT in the interictal phase of migraine [134].

In a model of electrical stimulation of the rat TG, immunoreactivity of kynurenine aminotransferase (KAT), the synthesizing enzyme of KYNA, was reported to be decreased in dural mast cells, macrophages, and Schwann cells [135]. A possible site of action of KYNA might be the TG [134, 136]. In the TNC, KYNA was not able to attenuate nitroglycerin-induced activation [137], as KYNA can poorly penetrate the BBB. In turn, L-kynurenine (L-Kyn) combined with probenecid were able to mitigate the activation of second-order neurons in the TNC both in the model of electrical stimulation of the TG [137], and in the nitroglycerin model [138]. In order to fac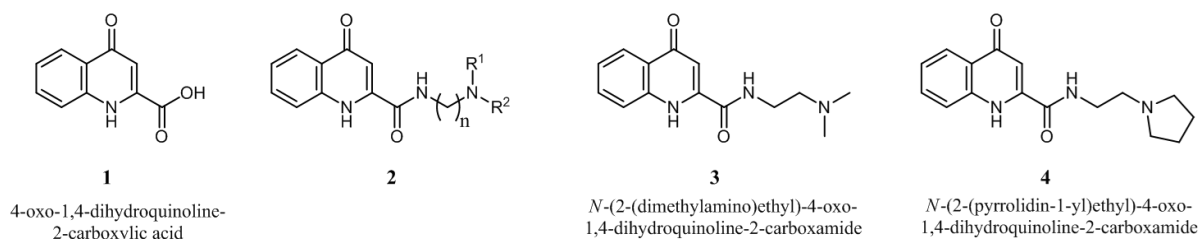ilitate BBB penetration, novel KYNA-derivates are being synthesized by our research group (Fig. **7**) [139]. The KYNA amide has been designed in the Department of Pharmaceutical Chemistry and Research Group for Stereochemistry, University of Szeged Hungary. The synthesis was performed by adding 2-dimethylaminoethylamine followed by treatment with ethanolic hydrogen chloride, yielding *N*-(2-*N*,*N*-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide hydrochloride. Structural properties of KYNA derivate are: the presence of a water-soluble side-chain, the inclusion of a new cationic center, and aside-chain substitution (Fig. **7**) in order to facilitate brain penetration [140].

Our research group has developed an animal model of migraine chronification, using complete Freund's Adjuvant (CFA) on the rat dura mater that causes pERK1/2, Il-1β and CGRP activation in the TG [141]. This effect was mitigated by a novel KYNA derivate [142]. In another experimental model, CFA was injected into the temporomandibular joint of rat, inducing inflammation in the TG, which was subsequently mitigated by KYNA and KYNA derivate [143]. Beside the
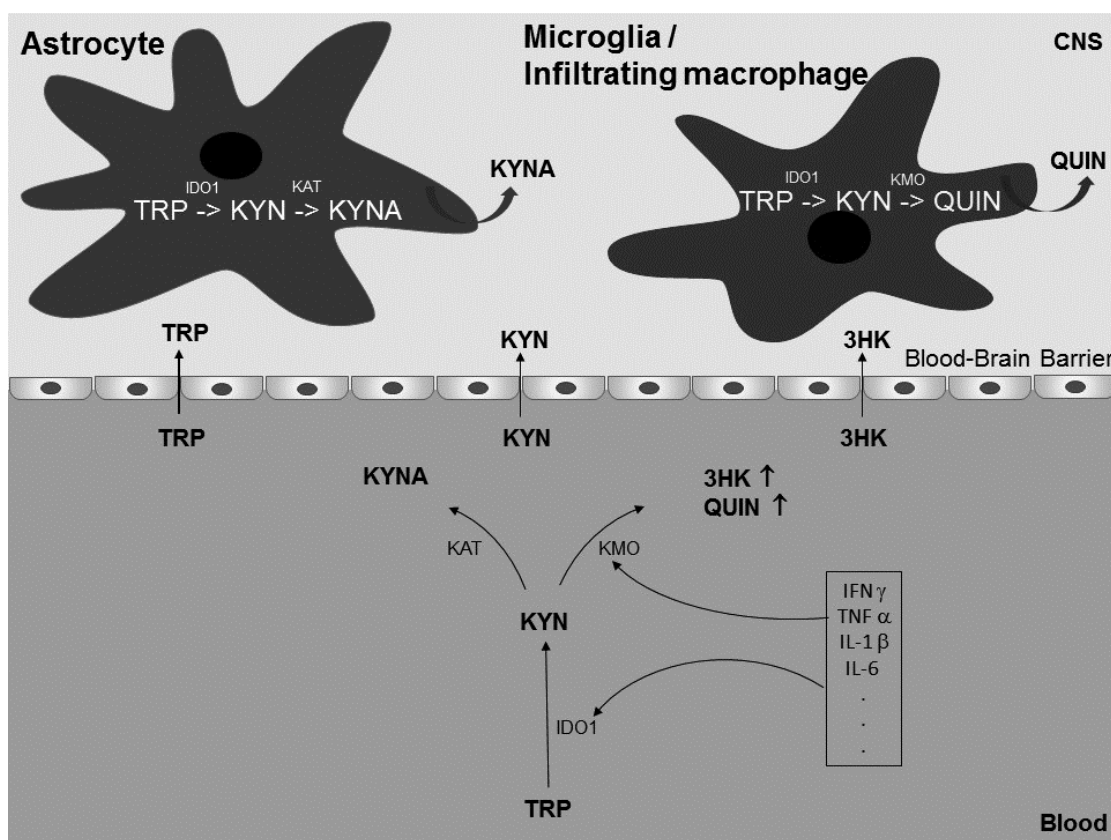
Fig. (6). Kynurenine pathway and inflammation. The major route for TRP metabolism is the kynurenine pathway (KP). The figure presents a simplified version of the KP. In the first metabolic step, TRP is converted to KYN in a process mediated by IDO1. KMO mediates the metabolism of KYN into 3HK, converted after multiple metabolic steps to QUIN, a neurotoxic metabolite. The other, neuroprotective branch of the KP is mediated by KAT, resulting in the production of KYNA. Inflammatory mediators increase the activity of IDO1 and KMO, leading to elevated levels of 3HK and QUIN. Inflammatory mediators have no effect or even decrease the activity of KAT. Excess peripheral 3HK and KYN can be transported across the blood-brain barrier (BBB) and can be used for further QUIN production in the CNS. QUIN cannot pass the BBB. Microglia and macrophages, cells that under inflammatory conditions can cross the BBB, express the KMO branch of the KP, leading to elevated levels of toxic 3HK and QUIN. On the other hand, astrocytes contain KAT, converting TRP to KYNA, and they are unable to produce QUIN, as they lack KMO.

CNS = central nervous system; 3HK = 3-hydroxykynurenine; IDO1 = indolamine 2,3-dioxygenase 1, KAT = kynurenine aminotransferase; KMO = kynurenine 3-monooxygenase; KYN = L-kynurenine; KYNA = kynurenic acid; QUIN = quinolinic acid; TRP = tryptophan.

1	2	3	4
4-oxo-1,4-dihydroquinoline-2-carboxylic acid		*N*-(2-(dimethylamino)ethyl)-4-oxo-1,4-dihydroquinoline-2-carboxamide	*N*-(2-(pyrrolidin-1-yl)ethyl)-4-oxo-1,4-dihydroquinoline-2-carboxamide

Fig. (7). Kynurenic acid (KYNA) and its derivates. 1. Chemical structure of KYNA; 2. General chemical structure of the KYNA derivates produced by our research group, aiming to facilitate blood-brain-barrier (BBB) penetration by inclusion of a new cationic side-chain; 3, 4. Chemical structure of the two most commonly used KYNA derivates in animal models of trigeminovascular activation having the following structural properties: a new cationic center, presence of a water-soluble side-chain and a side-chain substitution to help crossing through the BBB.

peripheral action, a potential central modulatory effect of KYNA analogues would be useful in preventing central sensitization [134]. The new KYNA analogues have proved their efficacy in the TNC following nitroglycerin-induced c-fos activation [138, 144]. The spectrum of action that occurs following the treatment with different KYNA analogues is still untangled. Ongoing behavioral, immunohistochemical and pharmacokinetic studies might elucidate the possible effect site of the KYNA analogues [145]. A possible interaction between KYNA and inflammatory cytokines (IFNα, IFNγ, TNFα, TGF-β, IL-1β, IL4, IL6, IL23) suggests an interaction between the kynurenine pathway and the immune system, leading to the idea that one possible site of action for KYNA derivates could be neurogenic inflammation [146, 147]. Summarizing all the above mentioned preclinical and clinical studies, we conclude that the KP is involved in the pathophysiology of migraine. KYNA analogues might be able to pass the BBB might also have a central effect beside that on the TG. Future clinical studies are needed to elucidate potential alterations in the KP in migraine. In animal experiments, KYNA analogues represent a promising innovative antimigraine therapy.

CONCLUSION

Epidemiological studies have demonstrated that migraine is an important socio-economic problem, having huge impact on individual health and wellbeing. Large neurobiological efforts have revealed partly the pathophysiology of migraine and the underlying phenomena that might generate migraine pain. Animal models aiming at the activation of primary or secondary trigeminal neurons have been developed, and various human studies and genetic investigations have been performed. In spite of all the advances in neurobiology of primary headache diseases, the role of neurogenic inflammation as an initiator of migraine headache pain (heralding for decades) remains a subject of debate among scientists. Undoubtedly, activation of dural afferents occurs with the release of CGRP and SP. This leads to plasma protein extravasation and release of pro-inflammatory cytokines causing sterile inflammation. Currently, migraine is treated either with general pain-killers (NSAID), not specific to migraine pain, or drugs with unpredictable effectiveness that might have severe side-effects. Triptans represent the gold-standard in the current treatment of migraine. However, they are not recommended for patients with high cardiovascular risk and can also lead to medication overuse headache; therefore, they are not recommended for chronic migraine. BoNTA could represent a good therapeutic

strategy for chronic migraine treatment, but its way of administration might influence patient-compliance. Further studies are needed to better understand migraine pain and promote the development of new, commercially available drugs that offer consistent efficacy and acceptable pain-relief. While a number of challenges persist in migraine treatment, its complex pathophysiology offers great opportunities for the discovery of new therapeutic strategies. First of all, migraine is a very heterogeneous disease, with many subtypes and a presumably diverse pathophysiological background. The complexity of events potentially occurring during a migraine attack leads to the conclusion that variability in treatment response to a certain therapeutic target might appear among patients. Another problem is the lack of specific biomarkers required for drug discovery in any of the primary headache diseases. In spite of the progress that has been achieved in migraine research, the diagnosis of primary headaches is still based on the clinical symptoms and subjective evaluation.

This review has focused on giving a summary of potential therapeutic targets in migraine in relation to neurogenic inflammation. The most promising therapeutic strategy seems to be related to CGRP and its receptor. Despite their efficacy, the first specific CGRP antagonists failed due to their hepatotoxic side effects but Allergan has restarted the field and ubrogepant, is now in phase III for acute therapy in migraine attacks. This gepants is likely without liver toxicity. Monoclonal antibodies against CGRP and its receptors have no such side-effect and have proven demonstrated great potency in clinical studies. Ongoing phase III clinical studies will presumably end in 2017/2018. Studies in relation to SP (NK$_1$ receptor antagonists) and NO (NOS antagonist) were terminated due to lack of efficacy, which makes the role of SP and NO questionable in migraine attacks. Nevertheless, they might be involved in the pathophysiology of some migraine subtypes (e.g. NOS antagonists in nitroglycerine induced headache); therefore potentially effective in a small headache subpopulation with more homogenous clinical features. Preclinical studies have shown effectiveness of PACAP antagonists and KYNA analogues in animal models of dural stimulation. Here we need to emphasize the limitations of predictive animal models in migraine research, therefore further preclinical studies are needed in order to understand the role of PACAP and KYNA analogues in migraine along with clinical studies that assess their effectiveness in acute or prophylactic treatment. All these medical leads pro-

vide hope for novel migraine treatments in the near future.

ABBREVIATIONS

CNS = Central nervous system

Glu = Glutamate

KYNA = Kynurenic acid

CGRP = Calcintonin gene related peptide

SP = Substance P

NO = Nitric oxid

NOS = Nitric oxid synthase

PACAP = Pituitary adenylate cyclase activating peptide

PAC$_1$ = PACAP receptor type 1

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

REFERENCES

[1]   Murray, C.J.; Vos, T.; Lozano, R.; Naghavi, M.; Flaxman, A.D.; Michaud, C.; Ezzati, M.; Shibuya, K.; Salomon, J.A.; Abdalla, S.; Aboyans, V.; Abraham, J.; Ackerman, I.; Aggarwal, R.; Ahn, S.Y.; Ali, M.K.; Alvarado, M.; Anderson, H.R.; Anderson, L.M.; Andrews, K.G.; Atkinson, C.; Baddour, L.M.; Bahalim, A.N.; Barker-Collo, S.; Barrero, L.H.; Bartels, D.H.; Basanez, M.G.; Baxter, A.; Bell, M.L.; Benjamin, E.J.; Bennett, D.; Bernabe, E.; Bhalla, K.; Bhandari, B.; Bikbov, B.; Bin Abdulhak, A.; Birbeck, G.; Black, J.A.; Blencowe, H.; Blore, J.D.; Blyth, F.; Bolliger, I.; Bonaventure, A.; Boufous, S.; Bourne, R.; Boussinesq, M.; Braithwaite, T.; Brayne, C.; Bridgett, L.; Brooker, S.; Brooks, P.; Brugha, T.S.; Bryan-Hancock, C.; Bucello, C.; Buchbinder, R.; Buckle, G.; Budke, C.M.; Burch, M.; Burney, P.; Burstein, R.; Calabria, B.; Campbell, B.; Canter, C.E.; Carabin, H.; Carapetis, J.; Carmona, L.; Cella, C.; Charlson, F.; Chen, H.; Cheng, A.T.; Chou, D.; Chugh, S.S.; Coffeng, L.E.; Colan, S.D.; Colquhoun, S.; Colson, K.E.; Condon, J.; Connor, M.D.; Cooper, L.T.; Corriere, M.; Cortinovis, M.; de Vaccaro, K.C.; Couser, W.; Cowie, B.C.; Criqui, M.H.; Cross, M.; Dabhadkar, K.C.; Dahiya, M.; Dahodwala, N.; Damsere-Derry, J.; Danaei, G.; Davis, A.; De Leo, D.; Degenhardt, L.; Dellavalle, R.; Delossantos, A.; Denenberg, J.; Derrett, S.; Des Jarlais, D.C.; Dharmaratne, S.D.; Dherani, M.; Diaz-Torne, C.; Dolk, H.; Dorsey, E.R.; Driscoll, T.; Duber, H.; Ebel, B.; Edmond, K.; Elbaz, A.; Ali, S.E.; Erskine, H.; Erwin, P.J.; Espindola, P.; Ewoigbokhan, S.E.; Farzadfar, F.; Feigin, V.; Felson, D.T.; Ferrari, A.; Ferri, C.P.; Fevre, E.M.; Finucane, M.M.; Flaxman, S.; Flood, L.; Foreman, K.; Forouzanfar, M.H.; Fowkes, F.G.; Fransen, M.; Freeman, M.K.; Gabbe, B.J.; Gabriel, S.E.; Gakidou, E.; Ganatra, H.A.; Garcia, B.; Gaspari, F.; Gillum, R.F.; Gmel, G.; Gonzalez-Medina, D.; Gosselin, R.; Grainger, R.; Grant, B.; Groeger, J.; Guillemin, F.; Gunnell, D.; Gupta, R.; Haagsma, J.; Hagan, H.; Halasa, Y.A.; Hall, W.; Haring, D.; Haro, J.M.; Harrison, J.E.; Havmoeller, R.; Hay, R.J.; Higashi, H.; Hill, C.; Hoen, B.; Hoffman, H.; Hotez, P.J.; Hoy, D.; Huang, J.J.; Ibeanusi, S.E.; Jacobsen, K.H.; James, S.L.; Jarvis, D.; Jasrasaria, R.; Jayaraman, S.; Johns, N.; Jonas, J.B.; Karthikeyan, G.; Kassebaum, N.; Kawakami, N.; Keren, A.; Khoo, J.P.; King, C.H.; Knowlton, L.M.; Kobusingye, O.; Koranteng, A.; Krishnamurthi, R.; Laden, F.; Lalloo, R.; Laslett, L.L.; Lathlean, T.; Leasher, J.L.; Lee, Y.Y.; Leigh, J.; Levinson, D.; Lim, S.S.; Limb, E.; Lin, J.K.; Lipnick, M.; Lipshultz, S.E.; Liu, W.; Loane, M.; Ohno, S.L.; Lyons, R.; Mabweijano, J.; MacIntyre, M.F.; Malekzadeh, R.; Mallinger, L.; Manivannan, S.; Marcenes, W.; March, L.; Margolis, D.J.; Marks, G.B.; Marks, R.; Matsumori, A.; Matzopoulos, R.; Mayosi, B.M.; McAnulty, J.H.; McDermott, M.M.; McGill, N.; McGrath, J.; Medina-Mora, M.E.; Meltzer, M.; Mensah, G.A.; Merriman, T.R.; Meyer, A.C.; Miglioli, V.; Miller, M.; Miller, T.R.; Mitchell, P.B.; Mock, C.; Mocumbi, A.O.; Moffitt, T.E.; Mokdad, A.A.; Monasta, L.; Montico, M.; Moradi-Lakeh, M.; Moran, A.; Morawska, L.; Mori, R.; Murdoch, M.E.; Mwaniki, M.K.; Naidoo, K.; Nair, M.N.; Naldi, L.; Narayan, K.M.; Nelson, P.K.; Nelson, R.G.; Nevitt, M.C.; Newton, C.R.; Nolte, S.; Norman, P.; Norman, R.; O'Donnell, M.; O'Hanlon, S.; Olives, C.; Omer, S.B.; Ortblad, K.; Osborne, R.; Ozgediz, D.; Page, A.; Pahari, B.; Pandian, J.D.; Rivero, A.P.; Patten, S.B.; Pearce, N.; Padilla, R.P.; Perez-Ruiz, F.; Perico, N.; Pesudovs, K.; Phillips, D.; Phillips, M.R.; Pierce, K.; Pion, S.; Polanczyk, G.V.; Polinder, S.; Pope, C.A., 3rd; Popova, S.; Porrini, E.; Pourmalek, F.; Prince, M.; Pullan, R.L.; Ramaiah, K.D.; Ranganathan, D.; Razavi, H.; Regan, M.; Rehm, J.T.; Rein, D.B.; Remuzzi, G.; Richardson, K.; Rivara, F.P.; Roberts, T.; Robinson, C.; De Leon, F.R.; Ronfani, L.; Room, R.; Rosenfeld, L.C.; Rushton, L.; Sacco, R.L.; Saha, S.; Sampson, U.; Sanchez-Riera, L.; Sanman, E.; Schwebel, D.C.; Scott, J.G.; Segui-Gomez, M.; Shahraz, S.; Shepard, D.S.; Shin, H.; Shivakoti, R.; Singh, D.; Singh, G.M.; Singh, J.A.; Singleton, J.; Sleet, D.A.; Sliwa, K.; Smith, E.; Smith, J.L.; Stapelberg, N.J.; Steer, A.; Steiner, T.; Stolk, W.A.; Stovner, L.J.; Sudfeld, C.; Syed, S.; Tamburlini, G.; Tavakkoli, M.; Taylor, H.R.; Taylor, J.A.; Taylor, W.J.; Thomas, B.; Thomson, W.M.; Thurston, G.D.; Tleyjeh, I.M.; Tonelli, M.; Towbin, J.A.; Truelsen, T.; Tsilimbaris, M.K.; Ubeda, C.; Undurraga, E.A.; van der Werf, M.J.; van Os, J.; Vavilala, M.S.; Venketasubramanian, N.; Wang, M.; Wang, W.; Watt, K.; Weatherall, D.J.; Weinstock, M.A.; Weintraub, R.; Weisskopf, M.G.; Weissman, M.M.; White, R.A.; Whiteford, H.; Wiebe, N.; Wiersma, S.T.; Wilkinson, J.D.; Williams, H.C.; Williams, S.R.; Witt, E.; Wolfe, F.; Woolf, A.D.; Wulf, S.; Yeh, P.H.; Zaidi, A.K.; Zheng, Z.J.; Zonies, D.; Lopez, A.D.; AlMazroa, M.A.; Memish, Z.A., Disability-adjusted life years (DALYs) for 291 diseases and

injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet,* **2012**, *380*, (9859), 2197-2223.

[2] Olesen, J.; Gustavsson, A.; Svensson, M.; Wittchen, H.U.; Jonsson, B., The economic cost of brain disorders in Europe. *Eur J Neurol,* **2012**, *19*, (1), 155-162.

[3] Bloudek, L.M.; Stokes, M.; Buse, D.C.; Wilcox, T.K.; Lipton, R.B.; Goadsby, P.J.; Varon, S.F.; Blumenfeld, A.M.; Katsarava, Z.; Pascual, J.; Lanteri-Minet, M.; Cortelli, P.; Martelletti, P., Cost of healthcare for patients with migraine in five European countries: results from the International Burden of Migraine Study (IBMS). *J Headache Pain,* **2012**, *13*, (5), 361-378.

[4] Silberstein, S.D., Considerations for management of migraine symptoms in the primary care setting. *Postgrad Med,* **2016**, *128*, (5), 523-537.

[5] Russell, M.B.; Ducros, A., Sporadic and familial hemiplegic migraine: pathophysiological mechanisms, clinical characteristics, diagnosis, and management. *Lancet Neurol,* **2011**, *10*, (5), 457-470.

[6] Headache Classification Committee of the International Headache, S., The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia 2013, 33 (9), 629-808.).

[7] Edvinsson, L., Tracing neural connections to pain pathways with relevance to primary headaches. *Cephalalgia,* **2011**, *31*, (6), 737-747.

[8] Liu, Y.; Broman, J.; Zhang, M.; Edvinsson, L., Brainstem and thalamic projections from a craniovascular sensory nervous centre in the rostral cervical spinal dorsal horn of rats. *Cephalalgia,* **2009**, *29*, (9), 935-948.

[9] Goadsby, P.J.; Holland, P.R.; Martins-Oliveira, M.; Hoffmann, J.; Schankin, C.; Akerman, S., Pathophysiology of Migraine: A Disorder of Sensory Processing. *Physiological reviews,* **2017**, *97*, (2), 553-622.

[10] Matharu, M.S.; Bartsch, T.; Ward, N.; Frackowiak, R.S.; Weiner, R.; Goadsby, P.J., Central neuromodulation in chronic migraine patients with suboccipital stimulators: a PET study. *Brain,* **2004**, *127*, (Pt 1), 220-230.

[11] Vincent, M.; Hadjikhani, N., The cerebellum and migraine. *Headache,* **2007**, *47*, (6), 820-833.

[12] Wolff, G.H. Headache and Other Pain, 1st edition, Oxford University Press, New York, 1948.

[13] Strassman, A.M.; Levy, D., Response properties of dural nociceptors in relation to headache. *J Neurophysiol,* **2006**, *95*, (3), 1298-1306.

[14] Derry, S.; Moore, R.A., Paracetamol (acetaminophen) with or without an antiemetic for acute migraine headaches in adults. *Cochrane Database Syst Rev,* **2013**, (4), CD008040.

[15] Derry, S.; Rabbie, R.; Moore, R.A., Diclofenac with or without an antiemetic for acute migraine headaches in adults. *Cochrane Database Syst Rev,* **2013**, (4), CD008783.

[16] Law, S.; Derry, S.; Moore, R.A., Naproxen with or without an antiemetic for acute migraine headaches in adults. *Cochrane Database Syst Rev,* **2013**, (10), CD009455.

[17] Rabbie, R.; Derry, S.; Moore, R.A., Ibuprofen with or without an antiemetic for acute migraine headaches in adults. *Cochrane Database Syst Rev,* **2013**, (4), CD008039.

[18] Cameron, C.; Kelly, S.; Hsieh, S.C.; Murphy, M.; Chen, L.; Kotb, A.; Peterson, J.; Coyle, D.; Skidmore, B.; Gomes, T.; Clifford, T.; Wells, G., Triptans in the Acute Treatment of Migraine: A Systematic Review and Network Meta-Analysis. *Headache,* **2015**, *55 Suppl 4*, 221-235.

[19] Evers, S.; Afra, J.; Frese, A.; Goadsby, P.J.; Linde, M.; May, A.; Sandor, P.S., EFNS guideline on the drug treatment of migraine--revised report of an EFNS task force. *Eur J Neurol,* **2009**, *16*, (9), 968-981.

[20] Dussor, G., Serotonin, 5HT1 agonists, and migraine: new data, but old questions still not answered. *Curr Opin Support Palliat Care,* **2014**, *8*, (2), 137-142.

[21] Carmichael, N.M.; Charlton, M.P.; Dostrovsky, J.O., Activation of the 5-HT1B/D receptor reduces hindlimb neurogenic inflammation caused by sensory nerve stimulation and capsaicin. *Pain,* **2008**, *134*, (1-2), 97-105.

[22] Derry, C.J.; Derry, S.; Moore, R.A., Sumatriptan (all routes of administration) for acute migraine attacks in adults - overview of Cochrane reviews. *Cochrane Database Syst Rev,* **2014**, (5), CD009108.

[23] Derry, C.J.; Derry, S.; Moore, R.A., Sumatriptan (oral route of administration) for acute migraine attacks in adults. *Cochrane Database Syst Rev,* **2012**, (2), CD008615.

[24] O'Quinn, S.; Davis, R.L.; Gutterman, D.L.; Pait, G.D.; Fox, A.W., Prospective large-scale study of the tolerability of subcutaneous sumatriptan injection for acute treatment of migraine. *Cephalalgia,* **1999**, *19*, (4), 223-231; discussion 200.

[25] Limmroth, V.; Kazarawa, Z.; Fritsche, G.; Diener, H.C., Headache after frequent use of serotonin agonists zolmitriptan and naratriptan. *Lancet,* **1999**, *353*, (9150), 378.

[26] Katsarava, Z.; Fritsche, G.; Muessig, M.; Diener, H.C.; Limmroth, V., Clinical features of withdrawal headache following overuse of triptans and other headache drugs. *Neurology,* **2001**, *57*, (9), 1694-1698.

[27] Silberstein, S.D., Preventive treatment of migraine: an overview. *Cephalalgia,* **1997**, *17*, (2), 67-72.

[28] Schaefer, S.M.; Gottschalk, C.H.; Jabbari, B., Treatment of Chronic Migraine with Focus on Botulinum Neurotoxins. *Toxins (Basel),* **2015**, *7*, (7), 2615-2628.

[29] Lee, M.J.; Lee, C.; Choi, H.; Chung, C.S., Factors associated with favorable outcome in botulinum toxin A treatment for chronic migraine: A clinic-based prospective study. *J Neurol Sci,* **2016**, *363*, 51-54.

[30] Szok, D.; Csati, A.; Vecsei, L.; Tajti, J., Treatment of Chronic Migraine with OnabotulinumtoxinA: Mode of Action, Efficacy and Safety. *Toxins (Basel),* **2015**, *7*, (7), 2659-2673.

[31] Tajti, J.; Szok, D.; Tuka, B.; Csati, A.; Kuris, A.; Majlath, Z.; Lukacs, M.; Vecsei, L., [Botulinum neurotoxin--a therapy in migraine]. *Ideggyogy Sz,* **2012**, *65*, (3-4), 77-82.

[32] Chiu, I.M.; von Hehn, C.A.; Woolf, C.J., Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. *Nat Neurosci,* **2012**, *15*, (8), 1063-1067.

[33] Xanthos, D.N.; Sandkuhler, J., Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. *Nat Rev Neurosci,* **2014**, *15*, (1), 43-53.

[34] Tajti, J.; Szok, D.; Majlath, Z.; Tuka, B.; Csati, A.; Vecsei, L., Migraine and neuropeptides. *Neuropeptides,* **2015**, *52*, 19-30.

[35] Corrigan, F.; Mander, K.A.; Leonard, A.V.; Vink, R., Neurogenic inflammation after traumatic brain injury and its potentiation of classical inflammation. *J Neuroinflammation,* **2016**, *13*, (1), 264.

[36] Sweitzer, S.M.; Hickey, W.F.; Rutkowski, M.D.; Pahl, J.L.; DeLeo, J.A., Focal peripheral nerve injury induces leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. *Pain,* **2002**, *100*, (1-2), 163-170.

[37] Minami, M.; Kuraishi, Y.; Satoh, M., Effects of kainic acid on messenger RNA levels of IL-1 beta, IL-6, TNF alpha and LIF in the rat brain. *Biochem Biophys Res Commun,* **1991**, *176*, (2), 593-598.

[38] De Simoni, M.G.; Perego, C.; Ravizza, T.; Moneta, D.; Conti, M.; Marchesi, F.; De Luigi, A.; Garattini, S.; Vezzani, A., Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. *Eur J Neurosci,* **2000,** *12,* (7), 2623-2633.

[39] Buture, A.; Gooriah, R.; Nimeri, R.; Ahmed, F., Current Understanding on Pain Mechanism in Migraine and Cluster Headache. *Anesth Pain Med,* **2016,** *6,* (3), e35190.

[40] Moskowitz, M.A.; Reinhard, J.F., Jr.; Romero, J.; Melamed, E.; Pettibone, D.J., Neurotransmitters and the fifth cranial nerve: is there a relation to the headache phase of migraine? *Lancet,* **1979,** *2,* (8148), 883-885.

[41] Markowitz, S.; Saito, K.; Moskowitz, M.A., Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. *J Neurosci,* **1987,** *7,* (12), 4129-4136.

[42] Buzzi, M.G.; Moskowitz, M.A., The antimigraine drug, sumatriptan (GR43175), selectively blocks neurogenic plasma extravasation from blood vessels in dura mater. *Br J Pharmacol,* **1990,** *99,* (1), 202-206.

[43] Edvinsson, L.; Uddman, R., Neurobiology in primary headaches. *Brain research. Brain research reviews,* **2005,** *48,* (3), 438-456.

[44] Buzzi, M.G.; Sakas, D.E.; Moskowitz, M.A., Indomethacin and acetylsalicylic acid block neurogenic plasma protein extravasation in rat dura mater. *Eur J Pharmacol,* **1989,** *165,* (2-3), 251-258.

[45] Buzzi, M.G.; Moskowitz, M.A., Evidence for 5-HT1B/1D receptors mediating the antimigraine effect of sumatriptan and dihydroergotamine. *Cephalalgia,* **1991,** *11,* (4), 165-168.

[46] Ahn, A.H.; Basbaum, A.I., Where do triptans act in the treatment of migraine? *Pain,* **2005,** *115,* (1-2), 1-4.

[47] Goadsby, P.J.; Edvinsson, L., The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. *Ann Neurol,* **1993,** *33,* (1), 48-56.

[48] Kristiansen, K.A.; Edvinsson, L., Neurogenic inflammation: a study of rat trigeminal ganglion. *J Headache Pain,* **2010,** *11,* (6), 485-495.

[49] Edvinsson, L.; Ekman, R.; Jansen, I.; McCulloch, J.; Uddman, R., Calcitonin gene-related peptide and cerebral blood vessels: distribution and vasomotor effects. *J Cereb Blood Flow Metab,* **1987,** *7,* (6), 720-728.

[50] Steenbergh, P.H.; Hoppener, J.W.; Zandberg, J.; Visser, A.; Lips, C.J.; Jansz, H.S., Structure and expression of the human calcitonin/CGRP genes. *FEBS Lett,* **1986,** *209,* (1), 97-103.

[51] Mulderry, P.K.; Ghatei, M.A.; Bishop, A.E.; Allen, Y.S.; Polak, J.M.; Bloom, S.R., Distribution and chromatographic characterisation of CGRP-like immunoreactivity in the brain and gut of the rat. *Regul Pept,* **1985,** *12,* (2), 133-143.

[52] Watkins, H.A.; Rathbone, D.L.; Barwell, J.; Hay, D.L.; Poyner, D.R., Structure-activity relationships for alpha-calcitonin gene-related peptide. *Br J Pharmacol,* **2013,** *170,* (7), 1308-1322.

[53] Fluhmann, B.; Muff, R.; Hunziker, W.; Fischer, J.A.; Born, W., A human orphan calcitonin receptor-like structure. *Biochem Biophys Res Commun,* **1995,** *206,* (1), 341-347.

[54] Chang, C.P.; Pearse, R.V., 2nd; O'Connell, S.; Rosenfeld, M.G., Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron,* **1993,** *11,* (6), 1187-1195.

[55] McLatchie, L.M.; Fraser, N.J.; Main, M.J.; Wise, A.; Brown, J.; Thompson, N.; Solari, R.; Lee, M.G.; Foord, S.M., RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature,* **1998,** *393,* (6683), 333-339.

[56] Choksi, T.; Hay, D.L.; Legon, S.; Poyner, D.R.; Hagner, S.; Bloom, S.R.; Smith, D.M., Comparison of the expression of calcitonin receptor-like receptor (CRLR) and receptor activity modifying proteins (RAMPs) with CGRP and adrenomedullin binding in cell lines. *Br J Pharmacol,* **2002,** *136,* (5), 784-792.

[57] Hoare, S.R., Mechanisms of peptide and nonpeptide ligand binding to Class B G-protein-coupled receptors. *Drug Discov Today,* **2005,** *10,* (6), 417-427.

[58] Walker, C.S.; Conner, A.C.; Poyner, D.R.; Hay, D.L., Regulation of signal transduction by calcitonin gene-related peptide receptors. *Trends Pharmacol Sci,* **2010,** *31,* (10), 476-483.

[59] Eftekhari, S.; Salvatore, C.A.; Calamari, A.; Kane, S.A.; Tajti, J.; Edvinsson, L., Differential distribution of calcitonin gene-related peptide and its receptor components in the human trigeminal ganglion. *Neuroscience,* **2010,** *169,* (2), 683-696.

[60] Eftekhari, S.; Warfvinge, K.; Blixt, F.W.; Edvinsson, L., Differentiation of nerve fibers storing CGRP and CGRP receptors in the peripheral trigeminovascular system. *J Pain,* **2013,** *14,* (11), 1289-1303.

[61] Eftekhari, S.; Edvinsson, L., Calcitonin gene-related peptide (CGRP) and its receptor components in human and rat spinal trigeminal nucleus and spinal cord at C1-level. *BMC Neurosci,* **2011,** *12,* 112.

[62] Edvinsson, L.; Eftekhari, S.; Salvatore, C.A.; Warfvinge, K., Cerebellar distribution of calcitonin gene-related peptide (CGRP) and its receptor components calcitonin receptor-like receptor (CLR) and receptor activity modifying protein 1 (RAMP1) in rat. *Mol Cell Neurosci,* **2011,** *46,* (1), 333-339.

[63] Doods, H.; Hallermayer, G.; Wu, D.; Entzeroth, M.; Rudolf, K.; Engel, W.; Eberlein, W., Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br J Pharmacol,* **2000,** *129,* (3), 420-423.

[64] Petersen, K., Birk S, Lassen LH, Kruuse C, Jonassen O, Lesko L, Olesen J, The CGRP-antagonist, BIBN4096BS does not affect cerebral or systemic haemodynamics in healthy volunteers. *Cephalalgia,* **2005,** *2005 Feb;25(2):139-47.*

[65] Rudolf, K.; Eberlein, W.; Engel, W.; Pieper, H.; Entzeroth, M.; Hallermayer, G.; Doods, H., Development of human calcitonin gene-related peptide (CGRP) receptor antagonists. 1. Potent and selective small molecule CGRP antagonists. 1-[N2-[3,5-dibromo-N-[[4-(3,4-dihydro-2(1H)-oxoquinazolin-3-yl)-1-piperidinyl]car bonyl]-D-tyrosyl]-l-lysyl]-4-(4-pyridinyl)piperazine: the first CGRP antagonist for clinical trials in acute migraine. *J Med Chem,* **2005,** *48,* (19), 5921-5931.

[66] Paone, D.V.; Shaw, A.W.; Nguyen, D.N.; Burgey, C.S.; Deng, J.Z.; Kane, S.A.; Koblan, K.S.; Salvatore, C.A.; Mosser, S.D.; Johnston, V.K.; Wong, B.K.; Miller-Stein, C.M.; Hershey, J.C.; Graham, S.L.; Vacca, J.P.; Williams, T.M., Potent, orally bioavailable calcitonin gene-related peptide receptor antagonists for the treatment of migraine: discovery of N-[(3R,6S)-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl]-4- (2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin- 1-yl)piperidine-1-carboxamide (MK-0974). *J Med Chem,* **2007,** *50,* (23), 5564-5567.

[67] Salvatore, C.A.; Hershey, J.C.; Corcoran, H.A.; Fay, J.F.; Johnston, V.K.; Moore, E.L.; Mosser, S.D.; Burgey, C.S.; Paone, D.V.; Shaw, A.W.; Graham, S.L.; Vacca, J.P.; Williams, T.M.; Koblan, K.S.; Kane, S.A., Pharmacological characterization of MK-0974 [N-[(3R,6S)-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl]-4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin-1-yl)piperidine-1-carboxamide], a potent and orally active

calcitonin gene-related peptide receptor antagonist for the treatment of migraine. *J Pharmacol Exp Ther,* **2008**, *324,* (2), 416-421.

[68]   Connor, K.M.; Aurora, S.K.; Loeys, T.; Ashina, M.; Jones, C.; Giezek, H.; Massaad, R.; Williams-Diaz, A.; Lines, C.; Ho, T.W., Long-term tolerability of telcagepant for acute treatment of migraine in a randomized trial. *Headache,* **2011**, *51,* (1), 73-84.

[69]   Edvinsson, L., CGRP receptor antagonists and antibodies against CGRP and its receptor in migraine treatment. *Br J Clin Pharmacol,* **2015**, *80,* (2), 193-199.

[70]   Zeller, J.; Poulsen, K.T.; Sutton, J.E.; Abdiche, Y.N.; Collier, S.; Chopra, R.; Garcia, C.A.; Pons, J.; Rosenthal, A.; Shelton, D.L., CGRP function-blocking antibodies inhibit neurogenic vasodilatation without affecting heart rate or arterial blood pressure in the rat. *Br J Pharmacol,* **2008**, *155,* (7), 1093-1103.

[71]   Bigal, M.E.; Walter, S.; Rapoport, A.M., Therapeutic antibodies against CGRP or its receptor. *Br J Clin Pharmacol,* **2015**, *79,* (6), 886-895.

[72]   Baumann, A., Early development of therapeutic biologics--pharmacokinetics. *Curr Drug Metab,* **2006**, *7,* (1), 15-21.

[73]   Dodick, D.W.; Goadsby, P.J.; Spierings, E.L.; Scherer, J.C.; Sweeney, S.P.; Grayzel, D.S., Safety and efficacy of LY2951742, a monoclonal antibody to calcitonin gene-related peptide, for the prevention of migraine: a phase 2, randomised, double-blind, placebo-controlled study. *Lancet Neurol,* **2014**, *13,* (9), 885-892.

[74]   Giamberardino, M.A.; Affaitati, G.; Curto, M.; Negro, A.; Costantini, R.; Martelletti, P., Anti-CGRP monoclonal antibodies in migraine: current perspectives. *Intern Emerg Med,* **2016**, *11,* (8), 1045-1057.

[75]   Dodick, D.W.; Goadsby, P.J.; Silberstein, S.D.; Lipton, R.B.; Olesen, J.; Ashina, M.; Wilks, K.; Kudrow, D.; Kroll, R.; Kohrman, B.; Bargar, R.; Hirman, J.; Smith, J., Safety and efficacy of ALD403, an antibody to calcitonin gene-related peptide, for the prevention of frequent episodic migraine: a randomised, double-blind, placebo-controlled, exploratory phase 2 trial. *Lancet Neurol,* **2014**, *13,* (11), 1100-1107.

[76]   Bigal, M.E.; Dodick, D.W.; Krymchantowski, A.V.; VanderPluym, J.H.; Tepper, S.J.; Aycardi, E.; Loupe, P.S.; Ma, Y.; Goadsby, P.J., TEV-48125 for the preventive treatment of chronic migraine: Efficacy at early time points. *Neurology,* **2016**, *87,* (1), 41-48.

[77]   Sun, H.; Dodick, D.W.; Silberstein, S.; Goadsby, P.J.; Reuter, U.; Ashina, M.; Saper, J.; Cady, R.; Chon, Y.; Dietrich, J.; Lenz, R., Safety and efficacy of AMG 334 for prevention of episodic migraine: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Neurol,* **2016**, *15,* (4), 382-390.

[78]   Moskowitz, M.A., Neurogenic inflammation in the pathophysiology and treatment of migraine. *Neurology,* **1993**, *43,* (6 Suppl 3), S16-20.

[79]   Lee, W.S.; Moussaoui, S.M.; Moskowitz, M.A., Blockade by oral or parenteral RPR 100893 (a non-peptide NK1 receptor antagonist) of neurogenic plasma protein extravasation within guinea-pig dura mater and conjunctiva. *Br J Pharmacol,* **1994**, *112,* (3), 920-924.

[80]   Diener, H.C., RPR100893, a substance-P antagonist, is not effective in the treatment of migraine attacks. *Cephalalgia,* **2003**, *23,* (3), 183-185.

[81]   Goldstein, D.J.; Offen, W.W.; Klein, E.G.; Phebus, L.A.; Hipskind, P.; Johnson, K.W.; Ryan, R.E., Jr., Lanepitant, an NK-1 antagonist, in migraine prevention. *Cephalalgia,* **2001**, *21,* (2), 102-106.

[82]   Goadsby, P.J.; Edvinsson, L.; Ekman, R., Release of vasoactive peptides in the extracerebral circulation of

humans and the cat during activation of the trigeminovascular system. *Ann Neurol,* **1988**, *23,* (2), 193-196.

[83]   Goadsby, P.J.; Edvinsson, L.; Ekman, R., Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol,* **1990**, *28,* (2), 183-187.

[84]   Goldstein, D.J.; Wang, O.; Saper, J.R.; Stoltz, R.; Silberstein, S.D.; Mathew, N.T., Ineffectiveness of neurokinin-1 antagonist in acute migraine: a crossover study. *Cephalalgia,* **1997**, *17,* (7), 785-790.

[85]   Guix, F.X.; Uribesalgo, I.; Coma, M.; Munoz, F.J., The physiology and pathophysiology of nitric oxide in the brain. *Prog Neurobiol,* **2005**, *76,* (2), 126-152.

[86]   Abu-Soud, H.M.; Stuehr, D.J., Nitric oxide synthases reveal a role for calmodulin in controlling electron transfer. *Proc Natl Acad Sci U S A,* **1993**, *90,* (22), 10769-10772.

[87]   Stuehr, D.J., Mammalian nitric oxide synthases. *Biochim Biophys Acta,* **1999**, *1411,* (2-3), 217-230.

[88]   Ebadi, M.; Sharma, S.K., Peroxynitrite and mitochondrial dysfunction in the pathogenesis of Parkinson's disease. *Antioxid Redox Signal,* **2003**, *5,* (3), 319-335.

[89]   Tajti, J.; Uddman, R.; Moller, S.; Sundler, F.; Edvinsson, L., Messenger molecules and receptor mRNA in the human trigeminal ganglion. *J Auton Nerv Syst,* **1999**, *76,* (2-3), 176-183.

[90]   Taffi, R.; Vignini, A.; Lanciotti, C.; Luconi, R.; Nanetti, L.; Mazzanti, L.; Provinciali, L.; Silvestrini, M.; Bartolini, M., Platelet membrane fluidity and peroxynitrite levels in migraine patients during headache-free periods. *Cephalalgia,* **2005**, *25,* (5), 353-358.

[91]   Lassen, L.H.; Ashina, M.; Christiansen, I.; Ulrich, V.; Grover, R.; Donaldson, J.; Olesen, J., Nitric oxide synthase inhibition: a new principle in the treatment of migraine attacks. *Cephalalgia,* **1998**, *18,* (1), 27-32.

[92]   Alderton, W.K.; Angell, A.D.; Craig, C.; Dawson, J.; Garvey, E.; Moncada, S.; Monkhouse, J.; Rees, D.; Russell, L.J.; Russell, R.J.; Schwartz, S.; Waslidge, N.; Knowles, R.G., GW274150 and GW273629 are potent and highly selective inhibitors of inducible nitric oxide synthase *in vitro* and *in vivo. Br J Pharmacol,* **2005**, *145,* (3), 301-312.

[93]   Van der Schueren, B.J.; Lunnon, M.W.; Laurijssens, B.E.; Guillard, F.; Palmer, J.; Van Hecken, A.; Depre, M.; Vanmolkot, F.H.; de Hoon, J.N., Does the unfavorable pharmacokinetic and pharmacodynamic profile of the iNOS inhibitor GW273629 lead to inefficacy in acute migraine? *J Clin Pharmacol,* **2009**, *49,* (3), 281-290.

[94]   Bhatt, D.K.; Gupta, S.; Jansen-Olesen, I.; Andrews, J.S.; Olesen, J., NXN-188, a selective nNOS inhibitor and a 5-HT1B/1D receptor agonist, inhibits CGRP release in preclinical migraine models. *Cephalalgia,* **2013**, *33,* (2), 87-100.

[95]   Hougaard, A., Hauge, AW., Guo, S., Tfelt-Hansen, P. , The nitric oxide synthase inhibitor and serotonin-receptor agonist NXN-188 during the aura phase of migraine with aura: A randomized, double-blind, placebo-controlled cross-over study. *Scandinavian Journal of Pain,* **2013**, *4,* 1.

[96]   Onoue, S.; Ohmori, Y.; Matsumoto, A.; Yamada, S.; Kimura, R.; Yajima, T.; Kashimoto, K., Structure-activity relationship of synthetic truncated analogues of vasoactive intestinal peptide (VIP): an enhancement in the activity by a substitution with arginine. *Life Sci,* **2004**, *74,* (12), 1465-1477.

[97]   Rahmann, A.; Wienecke, T.; Hansen, J.M.; Fahrenkrug, J.; Olesen, J.; Ashina, M., Vasoactive intestinal peptide causes marked cephalic vasodilation, but does not induce migraine. *Cephalalgia,* **2008**, *28,* (3), 226-236.

[98]   Cernuda-Morollon, E.; Martinez-Camblor, P.; Alvarez, R.; Larrosa, D.; Ramon, C.; Pascual, J., Increased VIP levels in peripheral blood outside migraine attacks as a potential biomarker of cranial parasympathetic activation in chronic migraine. *Cephalalgia,* **2015**, *35*, (4), 310-316.

[99]   Miyata, A.; Arimura, A.; Dahl, R.R.; Minamino, N.; Uehara, A.; Jiang, L.; Culler, M.D.; Coy, D.H., Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun,* **1989**, *164*, (1), 567-574.

[100]   Vaudry, D.; Falluel-Morel, A.; Bourgault, S.; Basille, M.; Burel, D.; Wurtz, O.; Fournier, A.; Chow, B.K.; Hashimoto, H.; Galas, L.; Vaudry, H., Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacol Rev,* **2009**, *61*, (3), 283-357.

[101]   Bourgault, S.; Vaudry, D.; Dejda, A.; Doan, N.D.; Vaudry, H.; Fournier, A., Pituitary adenylate cyclase-activating polypeptide: focus on structure-activity relationships of a neuroprotective Peptide. *Curr Med Chem,* **2009**, *16*, (33), 4462-4480.

[102]   Laburthe, M.; Couvineau, A.; Tan, V., Class II G protein-coupled receptors for VIP and PACAP: structure, models of activation and pharmacology. *Peptides,* **2007**, *28*, (9), 1631-1639.

[103]   Couvineau, A.; Laburthe, M., VPAC receptors: structure, molecular pharmacology and interaction with accessory proteins. *Br J Pharmacol,* **2012**, *166*, (1), 42-50.

[104]   Tajti, J.; Uddman, R.; Edvinsson, L., Neuropeptide localization in the "migraine generator" region of the human brainstem. *Cephalalgia,* **2001**, *21*, (2), 96-101.

[105]   Uddman, R.; Tajti, J.; Hou, M.; Sundler, F.; Edvinsson, L., Neuropeptide expression in the human trigeminal nucleus caudalis and in the cervical spinal cord C1 and C2. *Cephalalgia,* **2002**, *22*, (2), 112-116.

[106]   Tuka, B.; Szabo, N.; Toth, E.; Kincses, Z.T.; Pardutz, A.; Szok, D.; Kortesi, T.; Bagoly, T.; Helyes, Z.; Edvinsson, L.; Vecsei, L.; Tajti, J., Release of PACAP-38 in episodic cluster headache patients - an exploratory study. *J Headache Pain,* **2016**, *17*, (1), 69.

[107]   Nemeth, J.; Reglodi, D.; Pozsgai, G.; Szabo, A.; Elekes, K.; Pinter, E.; Szolcsanyi, J.; Helyes, Z., Effect of pituitary adenylate cyclase activating polypeptide-38 on sensory neuropeptide release and neurogenic inflammation in rats and mice. *Neuroscience,* **2006**, *143*, (1), 223-230.

[108]   Mabuchi, T.; Shintani, N.; Matsumura, S.; Okuda-Ashitaka, E.; Hashimoto, H.; Muratani, T.; Minami, T.; Baba, A.; Ito, S., Pituitary adenylate cyclase-activating polypeptide is required for the development of spinal sensitization and induction of neuropathic pain. *J Neurosci,* **2004**, *24*, (33), 7283-7291.

[109]   Tuka, B.; Helyes, Z.; Markovics, A.; Bagoly, T.; Szolcsanyi, J.; Szabo, N.; Toth, E.; Kincses, Z.T.; Vecsei, L.; Tajti, J., Alterations in PACAP-38-like immunoreactivity in the plasma during ictal and interictal periods of migraine patients. *Cephalalgia,* **2013**, *33*, (13), 1085-1095.

[110]   Schytz, H.W.; Birk, S.; Wienecke, T.; Kruuse, C.; Olesen, J.; Ashina, M., PACAP38 induces migraine-like attacks in patients with migraine without aura. *Brain,* **2009**, *132*, (Pt 1), 16-25.

[111]   Vecsei, L.; Tuka, B.; Tajti, J., Role of PACAP in migraine headaches. *Brain,* **2014**, *137*, (Pt 3), 650-651.

[112]   Tajti, J.; Szok, D.; Nagy-Grocz, G.; Tuka, B.; Petrovics-Balog, A.; Toldi, J.; Vecsei, L., Kynurenines and PACAP in migraine: medicinal chemistry and pathogenetic aspects. *Curr Med Chem,* **2017**.

[113]   Baun, M.; Pedersen, M.H.; Olesen, J.; Jansen-Olesen, I., Dural mast cell degranulation is a putative mechanism for

[114]   headache induced by PACAP-38. *Cephalalgia,* **2012**, *32*, (4), 337-345.

[114]   Baeres, F.M.; Moller, M., Origin of PACAP-immunoreactive nerve fibers innervating the subarachnoidal blood vessels of the rat brain. *J Cereb Blood Flow Metab,* **2004**, *24*, (6), 628-635.

[115]   Chan, K.Y.; Baun, M.; de Vries, R.; van den Bogaerdt, A.J.; Dirven, C.M.; Danser, A.H.; Jansen-Olesen, I.; Olesen, J.; Villalon, C.M.; MaassenVanDenBrink, A.; Gupta, S., Pharmacological characterization of VIP and PACAP receptors in the human meningeal and coronary artery. *Cephalalgia,* **2011**, *31*, (2), 181-189.

[116]   Schytz, H.W.; Olesen, J.; Ashina, M., The PACAP receptor: a novel target for migraine treatment. *Neurotherapeutics,* **2010**, *7*, (2), 191-196.

[117]   Akerman, S.; Goadsby, P.J., Neuronal PAC1 receptors mediate delayed activation and sensitization of trigeminocervical neurons: Relevance to migraine. *Sci Transl Med,* **2015**, *7*, (308), 308ra157.

[118]   Lerner, E.A.; Ribeiro, J.M.; Nelson, R.J.; Lerner, M.R., Isolation of maxadilan, a potent vasodilatory peptide from the salivary glands of the sand fly Lutzomyia longipalpis. *J Biol Chem,* **1991**, *266*, (17), 11234-11236.

[119]   Lerner, E.A.; Iuga, A.O.; Reddy, V.B., Maxadilan, a PAC1 receptor agonist from sand flies. *Peptides,* **2007**, *28*, (9), 1651-1654.

[120]   Moro, O.; Wakita, K.; Ohnuma, M.; Denda, S.; Lerner, E.A.; Tajima, M., Functional characterization of structural alterations in the sequence of the vasodilatory peptide maxadilan yields a pituitary adenylate cyclase-activating peptide type 1 receptor-specific antagonist. *J Biol Chem,* **1999**, *274*, (33), 23103-23110.

[121]   Schytz, H.W.; Hargreaves, R.; Ashina, M., Challenges in developing drugs for primary headaches. *Prog Neurobiol,* **2016**.

[122]   Jansen-Olesen, I.; Baun, M.; Amrutkar, D.V.; Ramachandran, R.; Christophersen, D.V.; Olesen, J., PACAP-38 but not VIP induces release of CGRP from trigeminal nucleus caudalis *via* a receptor distinct from the PAC1 receptor. *Neuropeptides,* **2014**, *48*, (2), 53-64.

[123]   Slominski, A.; Semak, I.; Pisarchik, A.; Sweatman, T.; Szczesniewski, A.; Wortsman, J., Conversion of L-tryptophan to serotonin and melatonin in human melanoma cells. *FEBS Lett,* **2002**, *511*, (1-3), 102-106.

[124]   Amori, L.; Guidetti, P.; Pellicciari, R.; Kajii, Y.; Schwarcz, R., On the relationship between the two branches of the kynurenine pathway in the rat brain *in vivo. J Neurochem,* **2009**, *109*, (2), 316-325.

[125]   Vamos, E.; Pardutz, A.; Klivenyi, P.; Toldi, J.; Vecsei, L., The role of kynurenines in disorders of the central nervous system: possibilities for neuroprotection. *J Neurol Sci,* **2009**, *283*, (1-2), 21-27.

[126]   Curto, M.; Lionetto, L.; Fazio, F.; Mitsikostas, D.D.; Martelletti, P., Fathoming the kynurenine pathway in migraine: why understanding the enzymatic cascades is still critically important. *Intern Emerg Med,* **2015**, *10*, (4), 413-421.

[127]   Vecsei, L.; Szalardy, L.; Fulop, F.; Toldi, J., Kynurenines in the CNS: recent advances and new questions. *Nat Rev Drug Discov,* **2013**, *12*, (1), 64-82.

[128]   Curto, M.; Lionetto, L.; Negro, A.; Capi, M.; Fazio, F.; Giamberardino, M.A.; Simmaco, M.; Nicoletti, F.; Martelletti, P., Altered kynurenine pathway metabolites in serum of chronic migraine patients. *J Headache Pain,* **2015**, *17*, 47.

[129]   Kaszaki, J.; Erces, D.; Varga, G.; Szabo, A.; Vecsei, L.; Boros, M., Kynurenines and intestinal neurotransmission:

the role of N-methyl-D-aspartate receptors. *J Neural Transm (Vienna),* **2012**, *119*, (2), 211-223.

[130] Kessler, M.; Terramani, T.; Lynch, G.; Baudry, M., A glycine site associated with N-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. *J Neurochem,* **1989**, *52*, (4), 1319-1328.

[131] Prescott, C.; Weeks, A.M.; Staley, K.J.; Partin, K.M., Kynurenic acid has a dual action on AMPA receptor responses. *Neurosci Lett,* **2006**, *402*, (1-2), 108-112.

[132] Rozsa, E.; Robotka, H.; Vecsei, L.; Toldi, J., The Janus-face kynurenic acid. *J Neural Transm (Vienna),* **2008**, *115*, (8), 1087-1091.

[133] MacKenzie, C.R.; Gonzalez, R.G.; Kniep, E.; Roch, S.; Daubener, W., Cytokine mediated regulation of interferon-gamma-induced IDO activation. *Adv Exp Med Biol,* **1999**, *467*, 533-539.

[134] Pardutz, A.; Fejes, A.; Bohar, Z.; Tar, L.; Toldi, J.; Vecsei, L., Kynurenines and headache. *J Neural Transm (Vienna),* **2012**, *119*, (2), 285-296.

[135] Knyihar-Csillik, E.; Chadaide, Z.; Okuno, E.; Krisztin-Peva, B.; Toldi, J.; Varga, C.; Molnar, A.; Csillik, B.; Vecsei, L., Kynurenine aminotransferase in the supratentorial dura mater of the rat: effect of stimulation of the trigeminal ganglion. *Exp Neurol,* **2004**, *186*, (2), 242-247.

[136] Shigemoto, R.; Ohishi, H.; Nakanishi, S.; Mizuno, N., Expression of the mRNA for the rat NMDA receptor (NMDAR1) in the sensory and autonomic ganglion neurons. *Neurosci Lett,* **1992**, *144*, (1-2), 229-232.

[137] Knyihar-Csillik, E.; Toldi, J.; Mihaly, A.; Krisztin-Peva, B.; Chadaide, Z.; Nemeth, H.; Fenyo, R.; Vecsei, L., Kynurenine in combination with probenecid mitigates the stimulation-induced increase of c-fos immunoreactivity of the rat caudal trigeminal nucleus in an experimental migraine model. *J Neural Transm (Vienna),* **2007**, *114*, (4), 417-421.

[138] Vamos, E.; Pardutz, A.; Varga, H.; Bohar, Z.; Tajti, J.; Fulop, F.; Toldi, J.; Vecsei, L., l-kynurenine combined with probenecid and the novel synthetic kynurenic acid derivative attenuate nitroglycerin-induced nNOS in the rat caudal trigeminal nucleus. *Neuropharmacology,* **2009**, *57*, (4), 425-429.

[139] Fulop, F.; Szatmari, I.; Vamos, E.; Zadori, D.; Toldi, J.; Vecsei, L., Syntheses, transformations and pharmaceutical applications of kynurenic acid derivatives. *Curr Med Chem,* **2009**, *16*, (36), 4828-4842.

[140] Fulop, F.; Szatmari, I.; Toldi, J.; Vecsei, L., Modifications on the carboxylic function of kynurenic acid. *J Neural Transm (Vienna),* **2012**, *119*, (2), 109-114.

[141] Lukacs, M.; Haanes, K.A.; Majlath, Z.; Tajti, J.; Vecsei, L.; Warfvinge, K.; Edvinsson, L., Dural administration of inflammatory soup or Complete Freund's Adjuvant induces activation and inflammatory response in the rat trigeminal ganglion. *J Headache Pain,* **2015**, *16*, 564.

[142] Lukacs, M.; Warfvinge, K.; Kruse, L.S.; Tajti, J.; Fulop, F.; Toldi, J.; Vecsei, L.; Edvinsson, L., KYNA analogue SZR72 modifies CFA-induced dural inflammation-regarding expression of pERK1/2 and IL-1beta in the rat trigeminal ganglion. *J Headache Pain,* **2016**, *17*, (1), 64.

[143] Csati, A.; Edvinsson, L.; Vecsei, L.; Toldi, J.; Fulop, F.; Tajti, J.; Warfvinge, K., Kynurenic acid modulates experimentally induced inflammation in the trigeminal ganglion. *J Headache Pain,* **2015**, *16*, 99.

[144] Fejes-Szabo, A.; Bohar, Z.; Vamos, E.; Nagy-Grocz, G.; Tar, L.; Veres, G.; Zadori, D.; Szentirmai, M.; Tajti, J.; Szatmari, I.; Fulop, F.; Toldi, J.; Pardutz, A.; Vecsei, L., Pre-treatment with new kynurenic acid amide dose-dependently prevents the nitroglycerine-induced neuronal activation and sensitization in cervical part of trigemino-cervical complex. *J Neural Transm (Vienna),* **2014**, *121*, (7), 725-738.

[145] Veres, G.; Fejes-Szabo, A.; Zadori, D.; Nagy-Grocz, G.; Laszlo, A.M.; Bajtai, A.; Mandity, I.; Szentirmai, M.; Bohar, Z.; Laborc, K.; Szatmari, I.; Fulop, F.; Vecsei, L.; Pardutz, A., A comparative assessment of two kynurenic acid analogs in the formalin model of trigeminal activation: a behavioral, immunohistochemical and pharmacokinetic study. *J Neural Transm (Vienna),* **2017**, *124*, (1), 99-112.

[146] Mandi, Y.; Vecsei, L., The kynurenine system and immunoregulation. *J Neural Transm (Vienna),* **2012**, *119*, (2), 197-209.

[147] Greco, R.; Demartini, C.; Zanaboni, A.M.; Redavide, E.; Pampalone, S.; Toldi, J.; Fulop, F.; Blandini, F.; Nappi, G.; Sandrini, G.; Vecsei, L.; Tassorelli, C., Effects of kynurenic acid analogue 1 (KYNA-A1) in nitroglycerin-induced hyperalgesia: Targets and anti-migraine mechanisms. *Cephalalgia,* **2016**.