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REVIEW

Targeted Pituitary Adenylate Cyclase-Activating Peptide Therapies for Migraine

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Abstract

Here, we review the role of pituitary adenylate cyclase-activating peptide-38 (PACAP38) in migraine pathophysiology and data implicating PAC_1 receptor as a future drug target in migraine. Much remains to be fully elucidated about migraine pathophysiology, but recent attention has focused on signaling molecule PACAP38, a vasodilator able to induce migraine attacks in patients who experience migraine without aura. PACAP38, with marked and sustained effect, dilates extracerebral arteries but not the middle cerebral artery. The selective affinity of PACAP38 to the PAC_1 receptor makes this receptor a highly interesting and potential novel target for migraine treatment. Efficacy of antagonism of this receptor should be investigated in randomized clinical trials.

Keywords PACAP38 · PAC_1 receptor · Vasoactive intestinal polypeptide · Human provocation models · Primary headaches · Migraine

Introduction

Since its discovery in 1989 [1], pituitary adenylate cyclase-activating peptide (PACAP) has gained considerable interest as a key molecule in migraine [2–4]; importantly, more recently, one of its receptors has been implicated as a treatment target for this debilitating neurological disease [5]. PACAP hails from the same superfamily as structurally related vasoactive intestinal peptide (VIP) [6]. Two bioactive forms exist: a 38-amino acid peptide (PACAP38) and a 27-amino acid peptide (PACAP27) [1]; the former accounting for 90% of mammalian tissue PACAP. In the nervous system, PACAP38 acts as a hormone, a neurotransmitter, and a neuromodulator [6, 7]. PACAP38 crosses the blood–brain barrier (BBB) via a protein transport system, which is responsible for transport in both directions, that is, from blood to brain and from brain to blood. While PACAP38 transport over the BBB occurs in a saturable manner, PACAP27 influx is nonsaturable. Although, both PACAPs cross the BBB, their effect on the central nervous system is questionable owing to 1) rapid efflux back to the blood from brain, and 2) degradation of the peptide [8]. PACAP38 is found in several important structures of interest in migraine pathophysiology—notably in sensory and parasympathetic perivascular fibers [9, 10], the trigeminal [11] and sphenopalatine [12] ganglia, and in the trigeminal nucleus caudalis (TNC) (see Fig. 1) [34]. In mast cells in human skin, PACAP38 causes degranulation and histamine release [35]. PACAP38 exerts its effects through at least 3 different receptors—the $VPAC_1$, $VPAC_2$, and PAC_1 receptors [36], which are all G protein-coupled receptors that increase intracellular cyclic adenosine monophosphate [37]. PACAP38 shares affinity for the $VPAC_{1–2}$ receptors with the structurally similar parasympathetic signaling molecule VIP [36], but surpasses VIP 300 to 1000-fold in affinity for the PAC_1 receptor. mRNA of these receptors is found in trigeminal (sensory) and otic (parasympathetic) ganglia, with only $VPAC_1$ found in sphenopalatine ganglia [38], and all 3 receptors are found in cerebral and cranial vessels (see Fig. 2) [38, 48]. Vasodilation and mast-cell degranulation are mediated by $VPAC_{1–2}$ receptors. The PAC_1 receptor is involved in multiple physiological functions [49], including chronic pain.

Here, we review studies implicating PACAP38 in migraine pathophysiology and discuss data sparking interest in the PAC_1 receptor as a future drug target in migraine.

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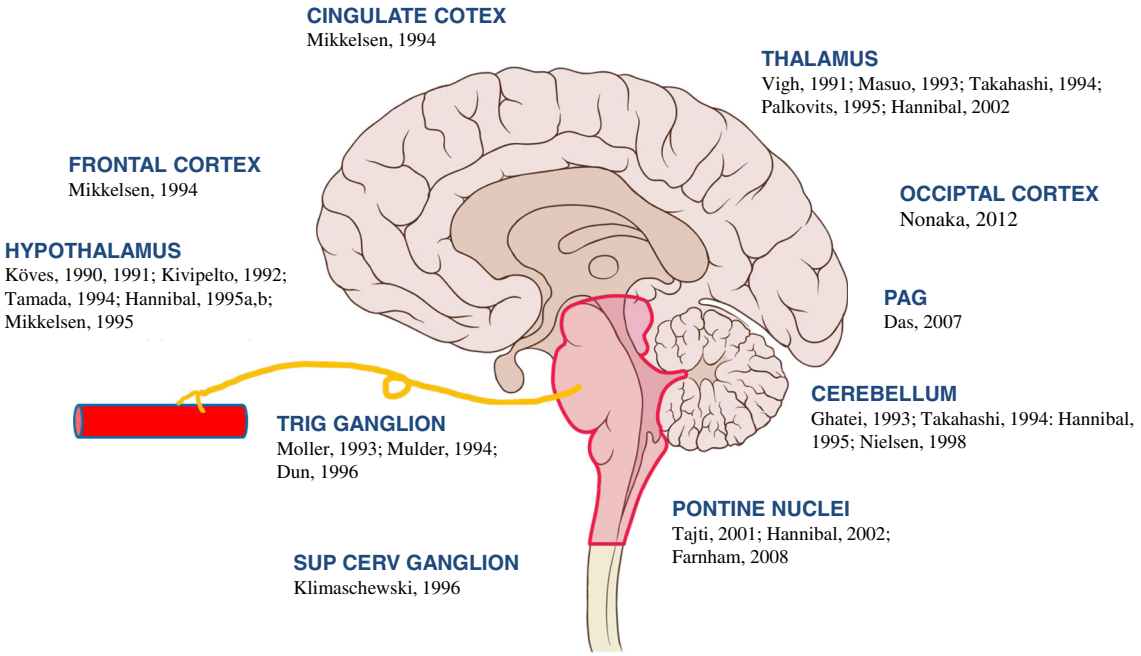

Fig. 1 Schematic illustration of distribution of pituitary adenylate cyclase activating peptide (PACAP) in cingulate cortex [13], frontal cortex [13], hypothalamus [14–20], trigeminal ganglion (trig ganglion) [21–23], superior cervical ganglion (sup cerv ganglion) [13], thalamus [15, 24–27], occipital cortex [28], periaqueductal grey (PAG) [29], cerebellum [13, 19, 25, 30, 31], and pontine nuclei [27, 32, 33]

PACAP38 Provocation Studies

The continued development of human experimental models of migraine have helped elucidate migraine pathophysiology [50]. Recent advances include combining provocation studies with advanced imaging techniques such as structural and functional magnetic resonance imaging [51, 52].

A first systematic study investigated the effect of PACAP38 on cerebral hemodynamics in healthy volunteers and reported no effect on regional cerebral blood flow (when

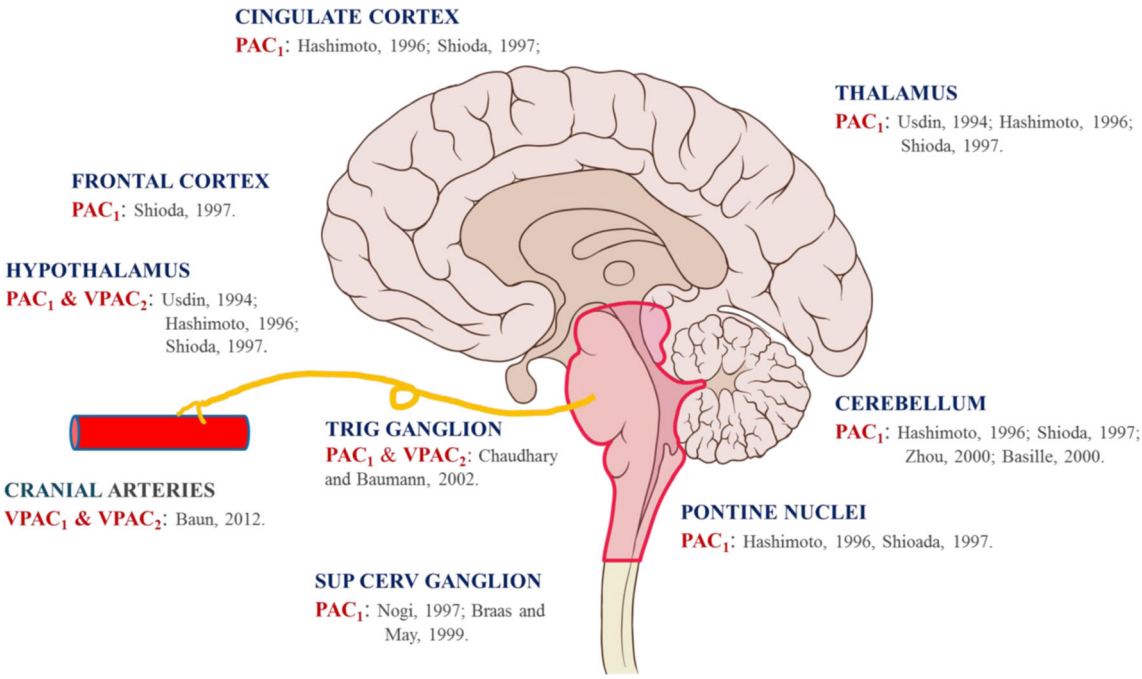

Fig. 2 Schematic illustration of distribution of pituitary adenylate cyclase activating peptide (PACAP) receptors PAC_1, $VPAC_1$, and $VPAC_2$ in cingulate cortex [39, 40], frontal cortex [40], hypothalamus [39–41], trigeminal ganglion (trig ganglion) [42], superior cervical ganglion (sup cerv ganglion) [43, 44], thalamus [39–41], cerebellum [39, 40, 45, 46], pontine nuclei [39, 40], and cranial arteries[47]

corrected for PACAP38-induced hypocapnia) [53]. Mild headache was noted as a side effect of infusion of PACAP38 at doses of 10 pmol/kg/min in 10 of 12 patients (83%), as well as a heart rate increased by 40% to 50%. Owing to the latter and other dose-dependent changes in vital signs a maximum dose of 10 pmol/kg/min was chosen for future provocation studies—a finding that was later upheld after investigation of headache inducing abilities of PACAP38 at lower doses [54].

The first study specifically investigating the headache and migraine-inducing abilities of PACAP38 elucidated responses in 12 healthy volunteers and 12 patients with migraine without aura (MO) [2]. In both groups, participants completed a randomized, double-blind, placebo-controlled crossover study comparing 20-min intravenous infusion of PACAP38 (10 pmol/kg/min over 20 min) to saline. Healthy volunteers reported 100% incidence of headache after PACAP38 and, interestingly, 2 of 12 reported migraine-like attack within 1 h of PACAP38 infusion. In patients with MO, 58% reported migraine attacks after PACAP38 versus none after placebo. In the same study a modest dilation of the middle cerebral artery (MCA) by 9.5% was calculated based on velocity measurements in the MCA and the assumption that cerebral blood flow was unchanged [53]. Moreover, the superficial temporal artery (STA) was dilated by 37.5%, which lasted throughout the 90-min observation period. Interestingly, migraine attacks occurred at a mean time of 6 h after the start of the infusion, suggesting the link between sustained vasodilation and PACAP38-induced delayed migraine attacks. To explore this a double-blind, placebo-controlled study investigated vascular responses after PACAP38 and placebo in 14 healthy volunteers by high-resolution magnetic resonance angiography (MRA) [55]. The major outcome was that PACAP38 dilated the middle meningeal artery (MMA; extracranial segment of the artery) over the 5-h observation period. In contrast, PACAP38 did not dilate MCA (intracerebral artery). Furthermore, migraine abortive treatment (sumatriptan injection 6 mg) had no effect on the MCA but reversed the MMA dilation and reduced the headache accompanying dilation.

To further elucidate mechanisms underlying PACAP38-induced migraine, a double-blind, randomized, crossover study in 22 patients with MO examined vascular responses after 20-min infusion of PACAP38 and VIP by MRA [52]. This study demonstrated that migraine induction was higher after PACAP38 (73% of patients) than with VIP (18% of patients). Both peptides dilated the STA and MMA but not the MCA, which is in contrast to findings of MCA increase and no change in STA and MMA in spontaneous migraine attacks in patients with migraine [56]. The discrepancy between arterial circumference during spontaneous and PACAP-induced migraine attack may be caused by the difference in time. In PACAP38-induced migraine experiments, patients underwent MRA scans at a very early time point, when PACAP38 had a marked dilatory effect on the extracranial arteries. During spontaneous attacks patients were scanned many hours into the attacks. Therefore, direct comparison of arterial circumference during provoked and spontaneous migraine is not possible. Interestingly, MMA dilation returned to baseline 2 h after start of VIP infusion but sustained (> 2 h) following PACAP38. PACAP38 receptors are found in both human meningeal and cerebral vessels [38, 48]. The vasodilator effect of PACAP38 is suggested to be mediated via the $VPAC_{1-2}$ receptors, which are shared with VIP [48, 57, 58]. Although *in vivo* rat studies have yielded contradictory results reporting the $VPAC_{1-2}$ receptors as mainly responsible for the PACAP38-induced meningeal vasodilation [57, 58] neither of the two *in vivo* studies reported any significant inhibition of this vasodilation by a PAC_1 receptor antagonist. Previous studies pointed out the importance of mast-cell degranulation and histamine release in PACAP38-induced prolonged vasodilation [59, 60]. However, a human provocation study found no increase in serum tryptase, a marker for mast-cell degranulation, after PACAP38 [52]. It is important to note that possible mast-cell degranulation localized in dura might not adequately be reflected in antecubital vein blood.

To investigate the differential impact of PACAP38 and VIP on intrinsic functional brain connectivity, one study employed resting-state functional magnetic resonance imaging, a method analyzing the functional connectivity between the various parts of the brain [51]. PACAP38, but not VIP, was associated with altered connectivity in the three networks investigated. These networks, the salience, sensorimotor, and default network, have been implicated in cognition, emotional processing [61–64], photo- and phonophobia [65], and pain processing [66]. Whether the reported alterations are specific for PACAP38-induced migraine attacks is unknown. A similar study in other pain conditions than migraine may further elucidate this.

To investigate a possible genetic component in susceptibility to migraine after PACAP38, a double-blind human provocation study [67] compared PACAP38 migraine induction in patients with high (≥2 first-degree relatives with MO) and low (≤1 first-degree relative with MO) family load of migraine. In addition, based on previous genotyping, patients were stratified on presence of 2 or 0 risk alleles of a single nucleotide polymorphism, rs2274316, known as a risk factor in migraine and located within the *MEF2D* gene, which is involved in regulating PACAP expression [68]. Results showed no difference in migraine induction between groups—either based on family load or allele status. Thus, no apparent genetic susceptibility to PACAP38 was found.

The PAC_1 Receptor and Future Directions

Several studies have shed light on the fluctuations of PACAP38 in ictal and interictal phases of migraine and in comparison to healthy volunteers. While PACAP38 levels in patients with migraine are higher ictally than interictally [69, 70], these increases are actually not statistically higher than PACAP38 levels in healthy controls [69]. Furthermore, conflicting results on interictal PACAP38 levels in patients with migraine have been reported [71]. Two studies reported *lower* PACAP levels in patients with migraine interictally than in healthy controls [69, 72], whereas one recent study reports comparable PACAP levels interictally in patients with migraine and controls [73]. This discrepancy can be explained by interassay differences. Interictally lower PACAP levels in patients with migraine could indicate chronic depletion of PACAP38 in the trigeminovascular system caused by repeated attack activity [74]. In an animal model, Han et al. [74] established a model of chronic migraine by repeated dural exposure to an inflammatory soup. In both plasma and trigeminal ganglion (TG), rats subsequently showed decreased PACAP38 levels. Interestingly, increased PAC_1 receptor mRNA expression in TG but not TNC was reported. The mRNA expression of $VPAC_{1-2}$ receptors, which PACAP38 and VIP share equal affinity for, was not significantly increased in TG and TNC [74]. Given that VIP did not induce migraine [75] and that PAC_1 receptor mRNA was expressed in meningeal arteries [48], TG neurons [38], and TNC [34], the PAC_1 receptor should be considered as a viable candidate for targeted migraine treatment. Interestingly, one *in vitro* study reported that administration of the selective PAC_1 receptor agonist maxadilan and the PAC_1 receptor antagonist M65 on TG neurons increased intracellular free calcium concentration [76]. The authors suggested that the PAC_1 receptor antagonist may also act as agonists on primary sensory neurons and that unknown receptors or splice variants linked to distinct signal transduction pathways might explain this effect [76].

PACAP38 signaling in migraine could be blocked in a number of ways. Small molecules or monoclonal antibodies could target the PAC_1 receptor. Alternatively, targeting PACAP38 itself with monoclonal antibodies would be an option [77]. These strategies also hail from emerging results of targeting similar peptide calcitonin gene-related peptide (CGRP) in large-scale clinical trials showing promising results in migraine prevention [78–80]. At present, it is difficult to speculate on the possible mechanism of the treatment of monoclonal antibodies against PACAP itself or PAC_1 receptor. Anti-CGRP antibody trials revealed no difference between compounds developed against CGRP or its receptor [78–80], and the exact site of action for anti-CGRP agents remains to be fully elucidated [81]. Whether targeting the PACAP38 signaling pathway will be efficacious in migraine treatment is unknown. Future phase II trials investigating this are currently planned [82, 83].

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