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Role of Pituitary Adenylate-Cyclase-Activating Polypeptide in nociception and migraine

Running title: PACAP: nociception and migraine

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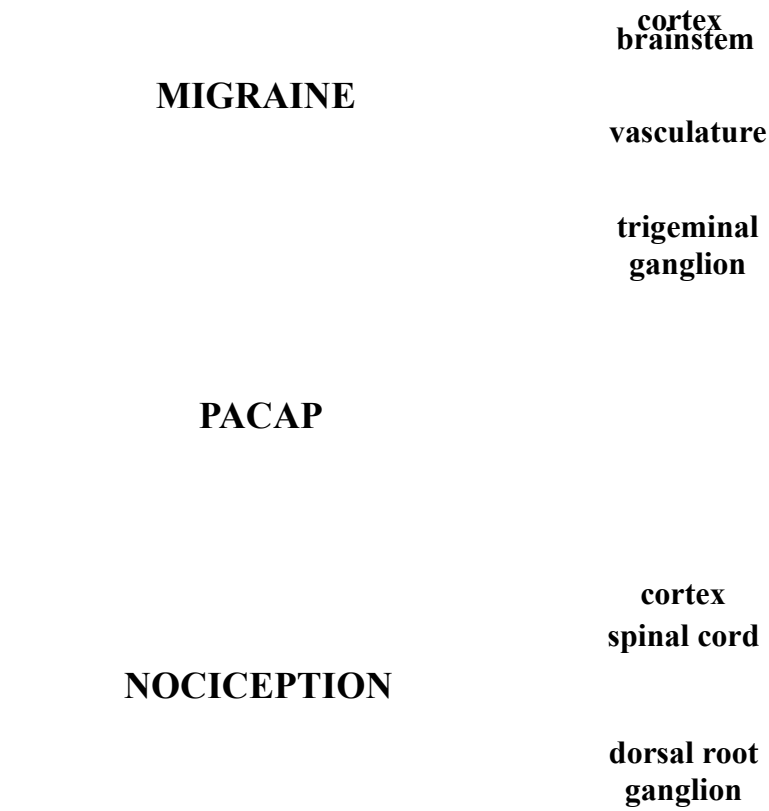
Abstract

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and its receptors are widely distributed at different levels of the pain-processing pathway. Its action at the peripheral sensory nerve terminals has been found to be divergent; it can exert both pro- and anti-nociceptive effects, depending on the mode of administration (local or systemic) and the mechanism of the pain process (acute or chronic, inflammatory or neuropathic). In the central nervous system it exerts mainly neuronal excitation, leading to increased nociceptive signalling. Since the clinical data strongly suggest the involvement of PACAP in the pathophysiology of migraine, special emphasis is placed on examinations of its role and the mechanisms of activation of the trigeminovascular system (TS). The intravenous administration of PACAP to migraineurs induces migraine-like headache and extracranial arterial dilatation. Furthermore, an increased PACAP concentration has been detected in the peripheral blood of patients during a migraine attack. Animal experiments have also revealed that PACAP elicits peripheral and central sensitization of the neuronal elements of the TS and evokes meningeal vasodilatation. This review summarizes data relating to the expression of PACAP and its receptors, and the main effects and mechanisms in the nociceptive pathways, with special emphasis on migraine. It is clear that PACAP plays an excitatory role in migraine, but its target and signalling pathways have not yet been elucidated due to the lack of non-peptide, selective agonists and antagonists. Identification of its up- and downstream regulations and receptorial molecular mechanisms might open up future perspectives for the development of novel analgesic drugs.

Graphical abstract



Keywords

primary sensory neuron, nociception, pain matrix, pituitary adenylate cyclase-activating polypeptide, sensitization, trigeminovascular system, migraine

Introduction

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a member of the Vasoactive Intestinal Polypeptide (VIP)/secretin/glucagon neuropeptide superfamily and is considered to be a "brain-gut peptide" due to its widespread expression and divergent biological effects through the peripheral (PNS) and central (CNS) nervous systems [1; 2]. Since PACAP is expressed at several levels of the pain transmission pathways, recent research activities have focused extensively on its roles in nociception. Despite the considerable interest and huge amount of data relating to this topic [2-5], the overall impact of PACAP in pain is still debated. There are many reasons for the contradictory results, as the effects of PACAP depend on both the underlying mechanisms and the site of action. Delineating its role in nociception and pain perception has proved to be challenging because of its numerous downstream targets, and also the considerable overlap of its receptorial pathways with those of VIP, to which it is closely related.

Among the several pain conditions, special emphasis has been placed on migraine in consequence of the important clinical findings. Migraine is a primary headache disorder with a high socio-economic and personal impact, affecting almost 16% of the adult population [6]. It was ranked by a WHO report as the 19th cause of disability worldwide [6-8], but the precise pathophysiological mechanisms are still unclear [9]. Several hypotheses have been proposed to explain the initiation of migraine pain. Although the predisposition to its development is presumably genetically determined, certain environmental factors (alcohol, alimentary factors, stress, hormonal changes, etc.) can trigger the headache. The factors assumed in the background include neurovascular alterations, sensory neuropeptide release and consequent neurogenic inflammation, plasma protein extravasation, peripheral and central sensitization, cortical spreading depression (CSD), a brain energy deficit and lesions in the white matter, as separately or simultaneously occurring phenomena. Since the 1990s, the central theme of migraine research has been the trigeminovascular theory [10]. The trigeminovascular system (TS) provides an important link between the vascular and neuronal elements, because this is the major afferent pain pathway between the cranial vessels and the brainstem [11]. The TS consists of the pseudounipolar primary sensory neurons whose cell bodies are located in the trigeminal ganglia (TRIG), their terminals innervating the cranial vessels, the supratentorial mater, the dural vasculature and the pial arteries and central endings projecting to the trigeminal nucleus caudalis (TNC). The third-order neurons are located in the thalamus. The descending pathways from the monoaminergic nuclei (the nuclei raphe, the periaqueductal grey matter (PAG) and the locus coeruleus (LC)), which are considered to be migraine

generators, control the central components of the TS. During activation of the TS, peripheral and central sensitization develops [9; 11-14]. The consequences of peripheral sensitization are the throbbing nature and worsening of the headache pain due to intracranial hypersensitivity in response to physical activity [15]. Cephalic and extracephalic cutaneous allodynia and extracranial tenderness are the results of the central sensitization [15; 16].

One of the leading current hypotheses is based on the activation of the TS, which consists in vascular alterations in the dura mater and the cerebral cortex, and also neuronal sensitization [9]. Human and preclinical experimental studies have revealed that PACAP has a crucial role in the activation of the TS [17]. Intravenous PACAP administration causes immediate headache in healthy volunteers, and delayed migraine-like strong pain in migraineurs without aura, and additionally induces pronounced dilatations of the extracranial arteries [18-20]. In spontaneous migraine attacks, increased concentrations of PACAP are observed in the peripheral blood relative to the headache-free period [21]. Electrical and chemical stimulation of the rat TS elevates the PACAP concentrations in the extracranial bloodflow and the TNC [22]. In a model of chemical TS activation, the light aversive behaviour (mimicking the symptom of photophobia), meningeal vasodilatation and c-fos activation in the TRIG and TNC are significantly decreased in PACAP-deficient mice [23].

Distribution of PACAP and its receptors in the CNS and PNS

PACAP was discovered thanks to its ability to increase the adenylate cyclase activity in rat pituitary cells, and it was first isolated from the ovine hypothalamus in 1989 [24]. The PACAP gene (ADCYAP1) is localized on the short arm of chromosome 18 [25]. The peptide exists in two biologically active amidated forms, containing 38 and 27 amino acids, PACAP-38 and PACAP-27 [2; 26]; the longer one is the predominant form in most mammalian tissues, accounting for 90% of the total PACAP content. Both forms are rapidly metabolized and theirs plasma elimination half-lives are less than 5 min [27].

It was established relatively early that PACAP is expressed in diverse regions of the CNS and PNS. Pronounced PACAP expression was demonstrated in the superficial layer of the spinal dorsal and ventral horns, in the dorsal root ganglia (DRG) and the TRIG, and also in peripheral nerve fibres [28-30]. Strong PACAP immunoreactivity was detected in the dorsal horn of the human spinal cord and the PAG [31]. Low to moderate amounts of PACAP-immunoreactive (-ir) fibres were observed in the C_1 and C_2 levels of Rexed's laminae I and II and in the tract of Lissauer [32; 33]. A closer inspection of the PACAP-38 immunoreactivity within the spinal cord revealed that not only the dorsal horn, but also the lateral horn, numerous regions of the medulla and DRG demonstrate a considerable expression of this peptide [34]. In the human TRIG, 20% of the neurons show PACAP immunoreactivity [35], similarly as in the rat TRIG [28; 29]. Double staining revealed that approximately 68% of the nociceptin-positive cells contained PACAP [36]. In the human DRG, the majority of the ganglion cells (59%) were also PACAP-ir [33]. *In situ* hybridization in the rat DRG indicated approximately 10% PACAP-containing neurons [29], while about 30-45% of the lumbar DRG neurons were PACAP-ir [37; 38]. PACAP is also expressed in capsaicin-sensitive primary sensory neurons [39]; pretreatment with capsaicin reduces the PACAP immunoreactivity [28]. Furthermore, a noteworthy co-localization has been observed with calcitonin gene-related peptide (CGRP), VIP and substance P (SP) in the porcine sensory distal ganglion of the vagus (nodose ganglion), implicating its involvement in vagal nociceptive transmission [40]. Only very few PACAP-ir fibres have been detected in the rat dura mater, showing co-expression with CGRP [41]. In the human skin, PACAP is localized in the dermal nerves in connection with the sweat glands [42], mast cells and hair follicles [43; 44].

As concerns the brain, in human cerebral cortical areas the highest concentrations of PACAP-38 have been detected in the cingulate, insular, temporal, parahippocampal and somatosensory cortex, the LC, the TNC and the dorsal vagal complex [31]. About 40% of the

human LC neurons are PACAP-ir, while there is little expression in the PAG [45], and only moderate expression in the TNC [32] (Fig. 1, Table 2).

Besides the CNS, PACAP occurs widely in a broad range of peripheral organs [46] and endocrine [47; 48] and exocrine glands [31; 49; 50]. In the PNS, it was first described in the parasympathetic system [24] and later in sensory neurons [28; 35]. It functions as a pleiotropic peptide [51; 52]: it is a hypophysiotrophic hormone [53], a neurotransmitter and a neuromodulator in the CNS [54] that exerts neuroprotective [55], anti-apoptotic [56] and differentiation-promoting effects [57; 58]. Furthermore, it serves important regulatory and protective roles in the gastrointestinal [59], cardiovascular [60-62], reproductive [63; 64] and respiratory systems [65].

PAC_1, $VPAC_1$ and $VPAC_2$ receptors

The effects of PACAP are mediated through three receptors: $VPAC_1$ (previously designated the VIP, VIP1 or PACAP type II receptor), $VPAC_2$ (known as the VIP2 or PACAP type III receptor) and PAC_1 (formerly known as the PACAP type I receptor). The latter displays a 1000-fold higher specificity for both forms of PACAP than for VIP [66; 67]. The binding of PACAP to its receptors induces two main signal transduction pathways. Through Gs- or Gq/11-protein activation, a number of kinases exert a variety of physiological and pathophysiological effects [2; 66].

In the rodent and human brain, pronounced PACAP-specific binding has been identified in the olfactory bulb, the cerebral cortex, the septum and amygdala, the hippocampus, the thalamus, the hypothalamus, the substantia nigra, the cerebellum and the area postrema [2]. High and moderate PACAP binding relating to migraine has also been found in the raphe nuclei, the LC, the nuclei of the trigeminal complex and the spinal trigeminal nucleus [2]. In the rat, the $VPAC_1$ and $VPAC_2$ receptors occur mainly in the olfactory bulb, the cerebral cortex, the dentate gyrus, the pineal gland and the thalamus. Although PACAP and VIP binding sites have been demonstrated in certain regions of the human brain (the cortex, basal ganglia, hypothalamus, cerebellum and brainstem), PACAP has a greater affinity than VIP for these sites. All three receptors are present both in neurons and in glial cells [2]. In the second- order neurons, the presence of $VPAC_1$ and PAC_1 receptors has been shown [68], while $VPAC_1$, $VPAC_2$ and PAC_1 receptors have been located in the rat middle cerebral artery by immunohistochemistry and Western blotting [69]. Multiple variants of the PAC_1 receptor have been found besides the $VPAC_2$ receptors in the rat middle meningeal arteries [70]. In another human study the $VPAC_1$ receptors have been detected in the meningeal arteries, as

relevant factors of migraine [71]. Furthermore, $VPAC_2$ receptor expression has been identified in human mast cells [72]. All three receptors are also present in the spinal dorsal horn. $VPAC_1$ is the most widely expressed (laminae II-IV), whereas the $VPAC_2$ expression is lower in these regions. PAC_1 mRNA is expressed in both the ventral and dorsal horns and in the DRG, but its expression is unaltered following unilateral sciatic nerve transection [37]. $VPAC_2$ and several PAC_1 receptor splice variants have been found in small-diameter neurons in the rat TRIG [73]. $VPAC_1$, $VPAC_2$ and PAC_1 receptor mRNAs are present in the human TRIG [74], while PAC_1 receptors are expressed in the primary sensory neurons and Schwann cells of the monkey TRIG [75] (Fig. 1, Table 2).

Early mechanistic insight: PACAP expression is influenced by peripheral nerve injury

Sciatic nerve transection increases the number of PACAP-ir neurons in the respective DRGs, in contrast with the expression and mRNA levels of CGRP or other neurotransmitters [37; 38; 76]. An elevated PACAP expression has also been observed in a population of large ventral horn motoneurons following sciatic nerve transection, suggesting its role in motor neuron regeneration and repair [30]. On the basis of these results, PACAP was proposed as an important protective factor in the CNS that is upregulated following nerve injury [77]. Since then, numerous studies have confirmed that PACAP is an endogenous neuroprotective factor in a variety of conditions [78-81].

PACAP plays a key role in peripheral nociceptive signalling

PACAP-38 is released from the stimulated peripheral endings of capsaicin-sensitive sensory neurons and diminishes the release of both pro-inflammatory/pronociceptive (SP and CGRP) and anti-inflammatory peptides (somatostatin) [82]. The PACAP immunoreactivity increased in the systemic circulation in response to systemic, but local excitation of cutaneous afferents by the ultrapotent capsaicin analogue resiniferatoxin (RTX). Intraperitoneal PACAP-38 ameliorated capsaicin/RTX-induced purely neurogenic plasma protein extravasation in the paw skin, and also carrageenan-induced mixed neurogenic/non-neurogenic hindlimb edema. These results suggest that PACAP released from the capsaicin-sensitive sensory terminals into the circulation reduces inflammatory reactions and pro-inflammatory mediator production [83] (Table 1). Later results revealed that intraplantar PACAP-38 was able to inhibit carrageenan-induced mechanical hyperalgesia, noxious heat-induced thermal hyperalgesia, and both the immediate and tonic phase of the nociceptive responses in the formalin test in rats, without influencing the baseline mechano- or thermonociceptive thresholds. In mice,

PACAP-38 ameliorated acetic acid-induced visceral pain, but did not influence neuropathic mechanical hyperalgesia in the partial sciatic nerve ligation model. These effects of PACAP-38 were blocked by pretreatment with $VPAC_{1/2}$, but not by the PAC1 receptor antagonist [84].

PACAP was also linked to inflammatory pain, as the number of PACAP mRNA-expressing sensory neurons was significantly upregulated in the rat L5 DRG early after an intraplantar challenge with complete Freund's adjuvant, but not in the later phase of the inflammation [85]. Intravitreal injection of PACAP-27 or -38 elicited an inflammation-like response characterized by conjunctival hyperaemia, swelling and flare in rabbits. A topical challenge by other noxious stimulants (e.g. formaldehyde or endotoxin) resulted in elevated PACAP immunoreactivity in the aqueous humour [86]. PACAP-containing nerve fibres were previosuly identified in several parts of the rat temporomandibular joint, including those innervating the synovial membrane, the joint capsule, and the articular disc [87]. In the adjuvant-induced paw inflammation model of the rat, an increased expression was shown in tyrosine kinase A-expressing small/medium-sized DRG neurons. The systemic administration of anti-nerve growth factor (NGF) resulted in diminished PACAP expression in responsive neuronal populations. Moreover, higher doses of anti-NGF were capable of decreasing PACAP expression in the non-inflamed DRGs. NGF a known mediator of inflammatory pain, is therefore likely to be an endogenous positive modulator of PACAP besides other pronociceptive peptides such as SP [88].

Local injection of PACAP-38 increased rotation-induced afferent firing in the acute carrageenan/kaolin-induced synovitis model of the rat, which suggests its role in peripheral mechanical sensitization of the knee joint primary afferents, and the induction of joint mechanosensitivity [84], similarly to VIP [89]. However, it remains an open question as to whether this is due to the direct action of PACAP, or the effect of secondary sensitizing mediators released, for instance, during mast cell degranulation.

Only minimal information is available concerning the effect of PACAP on pain perception in humans. In healthy volunteers, intradermal injection of PACAP-38 (200 pmol) into the forearm induces cutaneous pain, similarly to VIP. VIP evokes a more pronounced skin blood flow increase and flare than does PACAP-38. These results suggest that the acute peripheral pronociceptive effect of PACAP-38 is not $PAC_{1,}$ but rather $VPAC_{1}/_{2}$-receptor-mediated [44] (Table 1). The observed hyperaemia and flare together with the pain mirror the classical picture of neurogenic inflammation (Fig. 2).

PACAP, as a regulator of central pain transmission

It was observed relatively early that capsaicin treatment induces PACAP release from the C-fibres of the spinal cord, suggesting its potential modulator role in nociception [90]. In acute models of somatic or visceral inflammatory pain conditions, PACAP decreases pain transmission. Intrathecal (i.t.) injections of both PACAP-27 and 38, even in small concentrations, produce a long-lasting suppression of the C-fibre-evoked flexor reflex, though PACAP-38 was found to be less potent [91]. In the formalin test of the rat, both forms of PACAP elicited an antinociceptive effect in a high dose (~15.5 nmol i.t.), but also induced a motor impairment [4; 92]. Later data with lower doses of PACAP-27 (0.06-5 pmol) in the formalin test also revealed a clear antinociceptive effect, but without observable motor incapacitation [93]. PACAP-27 (3 pmol–3 nmol i.t.) elicited a dose-dependent facilitation of the spinal nociceptive flexor reflex, with no inhibitory effect at any of the applied doses that cause an excitatory effect in the spinal cord [94].

PACAP-38 administration in low doses (10–100 pmol i.t.) decreased noxious heat-induced tail-flick latency, whereas higher doses (0.2-2 nmol) triggered pain behaviour immediately following injection [95]. Later investigations confirmed the presence of an aversive behavioural response following i.t PACAP-38, which became pronounced above a dosage of 50 pmol. Immediately upon administration (30–90 s), a brief increase in tail-flick latency was observed, followed by a long-lasting algesia. The PACAP-38-induced aversive behaviour had a relatively slow onset, but long duration, in contrast with SP, for example [96]. Other researchers have observed that PACAP-38 (0.05-0.5 μg i.t.) diminishes the paw-withdrawal latencies induced by thermal stimulation in a dose-dependent manner, while it augments the aversive behaviour (licking and biting) induced by i.t. NMDA. Pretreatment with either the PAC_1 receptor antagonist PACAP6-38 or PACAP-antiserum did not influence the immediate nociceptive response in the formalin test, but diminished the late-phase algesia. PACAP-38 also potentiated NMDA receptor-mediated currents in the spinal dorsal horn neurons, and it was therefore suggested that PACAP might increase the NMDA receptor-mediated nociceptive responses by the sensitization of dorsal horn neurons [97].

Recent results suggest that PACAP is a key player in central pain processing by modifying a variety of neurochemical mechanisms through divergent pathways. There is limited evidence that PACAP is able to influence the central opioidergic tone. PAC_1-receptor activation by PACAP-38 inhibited, whereas antagonism by PACAP6-38 increased dynorphin 1-17 (Dyn) release in the rat spinal cord. This implies that PACAP inhibits Dyn release in the CNS via PAC_1 activation, and antagonism of this effect results in antinociceptive action. The

frequnetly observed pronociceptive central effect of PACAP is therefore at least partially attributed to the tonic inhibition of opioid release by PAC_1 activation [98]. This points to the possibility of a bidirectional connection between opioid mediators and transmitters released from the primary afferent terminals, such as PACAP. The well-known inhibitory effect of opioids on the release of these transmitters works both ways, and PACAP, and also related mediators, are likely to exert an inhibitory effect on opioid release. It has been verified that PACAP-ir terminals synapse onto Dyn-expressing neurons [98]. PACAP can therefore be suggested to be a regulator of the opioidergic antinociceptive system, which is mostly silent under normal conditions.

The central nucleus of the amygdala, an important junction of nociception-related pathways (besides stress and emotion), has been found to display considerable PACAP immunoreactivity in its lateral capsular division, partially colocalized with CGRP. The immunoreactive regions represent sensory projections from the lateral parabrachial nucleus along the spino-parabrachioamygdaloid tract. Intra-amygdalar infusion of PACAP led to a marked pronociceptive effect (besides increased anxiety and weight loss), demonstrated by decreased thermo- and mechanonociceptive thresholds in rats. Importantly, administration of the PAC_1-specific agonist maxadilan yielded similar results, implicating the involvement of the PAC_1 receptor in this phenomenon. In view of these results, PACAP signalling was suggested to be a link between chronic pain and negative behavioural changes (anxiety and depression) [99] (Table 1, Fig. 2).

Knockout studies: a PACAP deficiency diminishes nociceptive behaviour

Interestingly, PACAP gene-deficient mice demonstrate an absence of mechanical hyperalgesia in inflammatory (carrageenan) and neuropathic (L5 spinal nerve transection and partial sciatic nerve ligation) pain models [100-102], while their normal nocifensive behaviour remains unaffected. Despite the significantly reduced hyperalgesia observed in PACAP knockouts in the chronic mononeuropathy model, the early marker of neuronal activation, c-fos in the PAG and the somatosensory cortex was markedly higher, suggesting a potential inhibitory action of PACAP on inhibitory neuronal circuits [101]. The NADPH-dependent nitric oxide (NO) synthase (NOS) activity was increased in the superficial layer of the spinal dorsal horn in the wild-type, but not in PACAP gene-deficient neuropathic mice. I.t. NMDA injection-induced mechanical hyperalgesia was also absent in PACAP knockouts, but could be restored by exogenous PACAP administration [100]. A PACAP deficiency reduced nocifensive behaviour in both the acute (0-5 min) and the late phase (20-45 min) of the

formalin test, and additionally the number of abdominal contractions in the acetic acid-evoked acute visceral nociception model. Intraplantar RTX-induced mechanical hyperalgesia (which incorporates both central and peripheral components) was diminished, whereas thermal hyperalgesia (a primarily peripheral process) was elevated in PACAP gene-deficient mice [101]. PACAP-deleted animals also exhibited diminished mechanical hyperalgesia and overall inflammation severity in the K/BxN model of immune-mediated chronic arthritis [103] (Table 1).

Role of the PAC_1 receptor in nociceptive transmission

I.t. administration of the PAC_1 receptor antagonist PACAP6-38 decreased the flinching behaviour of the rat following intraplantar formalin injection, diminished the mechanociception in the spinal nerve ligation-evoked neuropathy model and also ameliorated the thermal hyperalgesia in the carrageenan-induced paw inflammation model in a dose-dependent manner, without any detrimental effect on motor coordination. These results suggest that central (spinal cord) activation of PAC_1 is pronociceptive [104]. It is important to note, however, that the antagonistic nature of PACAP6-38 has been questioned, as in numerous tissues/cell types it has been found to exert agonistic actions similar to those of PACAP-38 [105]. Others have verified the i.t. antinociceptive effect of PACAP6-38, and observed that spinal κ-opioid antagonism by nor-binaltorphimine administration abolished this effect [98]. PAC_1 antagonism by PACAP6-38 is therefore likely to trigger spinal Dyn release, resulting in κ-opioid receptor activation and hence antinociception. Earlier investigations on PAC_1 gene-deficent mice also revealed a significant reduction of writhing behaviour in the late phase of the formalin test, but their thermo- and mechanonociceptive thresholds were unaltered under normal conditions [106; 107]. The lack of difference in the tail pressure, tail flick and hot plate tests supported these findings, as PAC_1 signalling apparently did not contribute to acute somatic pain perception at the periphery. It was further observed that, while there was a dramatic decrease in the abdominal writhing test in another PAC_1 knockout strain, forebrain-specific PAC_1-deleted ($PAC_{1\,CaMKCre2}$) mice did not display a similar reduction of acute visceral nocifensive reactions (PAC_1 deletion affected the hippocampus, the cortical regions of the forebrain, and the olfactory bulbs in these mice) [107] (Table 1).

The currently available results well substantiate the PAC_1 receptor as a mediator of the pronociceptive effects in the CNS, whereas its role in peripheral nociceptive transmission remains poorly understood.

The role of the $VPAC_1$ and $VPAC_2$ receptors in nociceptive transmission

As both PACAP and VIP are potent agonists at these receptors, elucidation of their individual effects and importance remains a challenge. To exclude interferences caused by PAC_1-mediated effects, most of the results relating to the role of VPAC receptors in nociception were obtained by using VIP and its antagonists.

Early studies with receptor-specific antagonists revealed that, in the chronic constriction nerve injury (CCI)-evoked neuropathy model, $VPAC_1$, $VPAC_2$ and PAC_1 receptor antagonism inhibited the activity of the dorsal horn neurons induced by the Transient Potential Ankyrin receptor 1 agonist mustard oil, but did not influence the effect of mechanical stimulation (brush). Furthermore, the neuronal activity evoked by cold stimuli was ameliorated by $VPAC_1$ and PAC_1, but not $VPAC_2$ receptor antagonists. Experimental CCI-induced neuropathy decreased $VPAC_1$ and increased $VPAC_2$ mRNA expression in the ipsilateral spinal dorsal horn (laminae III-IV), but in contrast, the PAC_1 receptor expression remained unchanged [108]. Administration of VIP into the basolateral amygdala diminished the heat-induced tail flick reflex in anaesthetized rats, presumably by exciting neurons originating in the amygdala and synapsing on inhibitory cells of the PAG [109]. Spinal $VPAC_2$ receptor activation in the rat neuropathy model induced a downstream activation of p38 and p42/44 mitogen-activated protein (MAP) kinases in spinal glial cells by phosphorylation. Antagonism of these MAP kinases resulted in diminished sensitization of the dorsal horn neurons following a mustard oil challenge, and also reduced thermal and mechanical reflex sensitization. I.t. administration of a $VPAC_2$, but not a $VPAC_1$ or PAC_1 receptor antagonist was able to diminish the sensitization to thermal and mechanical stimulation in neuropathic rats. In contrast, i.t. administration of a $VPAC_2$ receptor agonist increased thermal hyperalgesia, which could be blocked by coadministration of a p38 inhibitor [110]. The $VPAC_{1/2}$ receptor antagonist VIP6-28 inhibited peripheral sensitization by VIP upon topical administration around the osteoarthritic joints of rats [111]. These results suggest a similar effect of PACAP to that of VIP acting at the common VPAC receptors, which have been implicated as potential targets in the treatment of neuropathic pain [112].

Activation of these receptors exerts a mostly pronociceptive effect in the CNS, while at the periphery their role appears to be more ambiguous. Human studies have demonstrated that both PACAP-38 and VIP generate a painful neurogenic inflammatory reaction upon intradermal administration, which can be attributed to the VPAC receptors [44]. In mouse pain models, where the peripheral administration of PACAP-38 resulted in diminished nociception, coadministered $VPAC_{1/2}$ antagonists abolished this effect [84], suggesting that

these receptors also contribute to antinociceptive signalling at the periphery. However, the algesic effect of PACAP is likely to be caused by a local mast cell degranulating potential of the peptide, unrelated to its action on the nociceptors. $VPAC_2$, but not $VPAC_1$, is present on human mast cells [113], and both VIP and PACAP potently induce their degranulation [72; 114]. This is supported by the finding that topical PACAP-38-induced dermal oedema is completely abolished in mast cell-deficient mice [114]. These results suggest that the observed algesia is likely to be caused by $VPAC_2$-mediated mast cell degranulation and secondary neurogenic inflammation. Moreover, the reported pain-inducing effect of PACAP-38 is extremely short-lasting (it ceased within 2 min) [44], and this transient pronociceptive impact of the peptide therefore does not contradict the observed permanent analgesic effect of peripherally administered PACAP-38 (presumably acting on sensory afferent endings) in murine models (Table 1).

PACAP and migraine

Preclinical and human data point to the involvement of PACAP in the pathophysiological mechanisms of migraine.

Nitroglycerol (NTG) is a NO donor which causes an immediate and a delayed migraine-like attack in migraine patients without aura [115]. It is therefore a useful tool for chemical activation of the TS in animal models that mimic the symptoms of migraine. NTG-evoked pathophysiological changes in the TS, such as light-aversive behaviour, meningeal vasodilatation and c-fos activation in the TRIG and TNC, were significantly reduced in PACAP-deficient mice. Moreover, the systemic administration of PACAP-38 elicited light aversion, similarly to that of NTG in wild-type mice, but not in PACAP-deficient ones [23] showing that PACAP plays a crucial role in both the vascular and neuronal pathophysiological mechanisms of migraine.

Another model of the migraine attack is the electrical stimulation of the TRIG [116; 117]. Both chemical (NTG) and electrical (square pulses) stimulation of the TRIG in rats resulted in significantly elevated concentrations of PACAP-27 and -38 in the TNC. The plasma PACAP-38 immunoreactivity increased following electrical stimulation [22], suggesting that these peptides are closely related to TS activation and the nociceptive transmission in the TNC.

Intravenously administered PACAP-38 induces headache in healthy volunteers and delayed migraine-like attacks in patients with migraine without aura [18], similarly to the action of NTG in migraineurs [115; 118]. A decreased mean blood flow velocity in the middle cerebral artery and an increased diameter of the superficial temporal artery were observed [18].

Further, pronounced dilation of the extracranial, but not the intracranial arteries was revealed [19]. Recent findings have revealed that a PACAP-38 infusion causes dilation of the middle meningeal artery in rats, which is diminished in mast cell-depleted and antihistamine-pretreated rats [119]. The observed vasodilating effects are therefore likely to be indirect and mediated by the PACAP-38-induced histamine release from the mast cells [119]. In another human study, significantly lower interictal plasma PACAP-38 immunoreactivity was detected in migraineurs as compared with the healthy control group. In contrast, elevated plasma PACAP-38 and CGRP levels were measured in the ictal phase relative to the attack-free period. A negative correlation was detected between the PACAP-38 plasma concentration in the attack-free period and the disease duration. The plasma changes were independent of various disease conditions [21]. These results suggest a correlation between the attack/headache-free period and the plasma PACAP-38 levels.

Presumably, PACAP can be released from the peripheral and central terminals of the pseudounipolar primary sensory neurons of the TRIG in response to stimulation. It acts locally, but also enters the systemic circulation. Activation of PAC_1 and $VPAC_2$ receptors can elicit vasodilation in the meningeal vessels; the $VPAC_1$ receptor is important in the vascular responses of the cerebellar arteries [70]. The PAC_1 receptor is also involved in the activation of the second-order sensory neurons [23], which appears consistent with previous interpretations implicating PAC_1 receptors in migraine [18; 120]. This is in agreement with the results that intradermal injections of PACAP-38 or VIP elicit mild, short-lasting cutaneous pain and increased skin blood flow, flare and wheal in healthy volunteers, supporting the concept that primarily the VPAC, and not the PAC_1 receptors mediate these alterations. There is experimental evidence that PACAP induces neurogenic inflammation, mast cell degranulation, neuronal activation and sensitization [18; 121]. The participation of PACAP in the processes of migraine may be confirmed by the finding that PACAP-induced dilation of the middle meningeal artery and headache pain are attenuated by the serotonin 5-HT1B/D receptor agonist sumatriptan [20]. Overall, there is strong clinical and experimental evidence of an important mediator role of PACAP in many components of migraine through the initiation, promotion of the development and/or aggravation of the severity of headache attacks (Fig. 3).

The relevance and potential future therapeutic applications of PACAP

The broad range of clinical and experimental evidence described above clearly reveals that

PACAP is an important mediator of pain processing and a key player in migraine headache.

Since most of the effects of PACAP on nociception are different from those of VIP [19; 122], it may be proposed that the main actions in pain are mediated via the specific PAC1 receptor. However, due to the lack of availability of stable, receptor-selective non-peptide agonists and antagonists with a good blood-brain-barrier (BBB) penetration ability, its target(s) and signalling mechanisms have not been elucidated. Several data show that a fragment of the full peptide, PACAP6-38, acting as a potent PAC_1/$VPAC_2$ antagonist in a variety of model systems [123-128], behaves as an agonist on rat primary TRIG cultures, similarly to PACAP itself [129], and on the peripheral terminals of primary sensory neurons both *in vitro* [105; 130] and *in vivo* [131]. It can be therefore suggested that primary sensory neurons do not express the same PAC_1 receptor as previously cloned, but either a PAC_1-related receptor or a different PAC1 splice variant. Unravelling the molecular pathways and deciphering the downstream mechanisms could open up promising perspectives for the development of novel analgesic drugs.

Maxadilan, a 61 AA PAC_1 receptor-specific peptide agonist, is already available; deletion between the 25 and 41 amino acids yielded the specific PAC_1 receptor antagonist Maxa-65 [132]. As concerns our current understanding of the role of PACAP in migraine, and available mechanistic insights, the immediate aim can be defined as the development of a non-peptide specific PAC_1 receptor antagonist capable of efficient BBB penetration, thereby of future clinical use in the treatment of migraine-induced headache, and potentially in a plethora of other pain conditions too.

Conflict of interest

The authors declare that they have no conflict of interest and have received no payment in preparation of their manuscript.

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List of abbreviations

BBB: blood brain barrier

CCI: chronic constriction injury

CGRP: calcitonin gene-related peptide

CNS: central nervous system

CSD: cortical spreading depression

DRG: dorsal root ganglion

Dyn: dynorphin

i.t.: intrathecal

ir: immunoreactive

LC: locus coeruleus

MAP: mitogen-activated kinase

NGF: nerve growth factor

NO: nitric oxide

NOS: nitric oxide synthase

NTG: Nitroglycerol

PAC_1: PACAP type I receptor

PACAP: pituitary adenylate cyclase-activating polypeptide

PAG: periaqueductal grey matter

PNS: peripheral nervous system

RTX: resiniferatoxin

SP: substance P

TNC: trigeminal nucleus caudalis

TRIG: trigeminal ganglion

TS: trigeminovascular system

VIP: vasoactive intestinal polypeptide

$VPAC_1$: VIP1 or PACAP type II receptor

$VPAC_2$: VIP2 or PACAP type III receptor

Figures

Figure 1. Scheme of the PACAP-related components in the trigeminal system (Modified ref. 14).

Abbreviations:

C_2: cervical 2 segments of spinal cord; DRG: dorsal root ganglion; DRN: dorsal raphe nucleus; NRM: nucleus raphe magnus; LC: locus coeruleus; PAG: periaqueductal grey matter; TNC: trigeminal nucleus caudalis; TRIG: trigeminal ganglion; +: presence

Figure 2. Effects of PACAP in nociceptive transmission

Figure 3. Putative mechanism of PACAP in migraine headache

Abbreviations:

PACAP: pituitary adenylate cyclase-activating polypeptide; TNC: trigeminal nucleus caudalis; TRIG: trigeminal ganglion

Tables

Table 1. The effects of PACAP on nociception/pain perception.

Localization	Effect on nociception	Model(s)	Species studied	Readout	Receptor(s) implicated	Ref.
i.pl./s.c. PACAP-38	↓ (acute models) - (neuropathy model)	i.pl. carrageenan, i.pl. formalin, i.p. acetic acid, partial sciatic nerve ligation	mouse	mechanical and thermal nociceptive threshold, writhing test	$VPAC_{1/2}$	[84]
i.d. PACAP-38	↑	study on healthy volunteers	human	visual analogue scale	$VPAC_{1/2}$ on mast cells	[44]
i.t. PACAP-27/-38 (high dose)	↓	i.pl. formalin	rat	flinching behaviour	n.a.	[92]
i.t. PACAP-27	↓	i.pl. formalin	rat	flinching behaviour	n.a.	[93]
i.t. PACAP-38	↑	thermonociception, spontaneous pain behaviour	rat	tail-flick latency, behavioural observation	n.a.	[95]
i.t. PACAP-38	↓(early phase) ↑(late phase)	thermonociception, spontaneous pain behaviour	mouse	tail-flick latency, behavioural observation	n.a.	[96]
i.t. PACAP-38	↑	thermonociception, i.t. NMDA, i.pl. formalin	mouse	paw-withdrawal latency, behavioural observation	n.a.	[97]
intraamygdalar PACAP-38	↑	thermo- and mechanonoception	rat	Paw- withdrawal threshold	PAC_1	[99]
global PACAP knockout	↓	spinal nerve transection, i.pl. carrageenan, i.t. NMDA	mouse	mechanical and thermal nociceptive threshold	n.a.	[100]
global PACAP knockout	↓	i.pl. formalin, intraplantar resiniferatoxin partial sciatic nerve ligation, i.p. acetic acid		behavioural observation, mechano- and thermonociceptive threshold, abdominal writhing	n.a.	[101]
global PACAP knockout	↓	partial sciatic nerve ligation	mouse	mechanonociceptive threshold	n.a.	[102]
global PACAP knockout	↓	K/BxN serum-transfer arthritis	mouse	mechanonociceptive threshold	n.a.	[103]
i.t. PACAP antagonist (6-38)	↓	i.pl. formalin, spinal nerve ligation, intraplantar carrageenan monoiodo-acetate-induced osteoarthritis	rat	flinching behaviour, mechano- and thermonociceptive threshold, hind limb weight-bearing difference	PAC_1	[104]

i.t. PACAP (6-38) antagonist	↓	i.pl. formalin	rat	behavioural observation	PAC_1	[98]
global PAC_1 knockout	↓ (only in the formalin test)	i.pl. formalin, thermo- and mechanonociception	mouse	behavioural observation, mechano- and thermonociceptive threshold	PAC_1	[106]
global and forebrain-specific PAC_1 knockout	↓ in global knockouts (only visceral nociception)	thermo- and mechanonociception, i.p. acetic acid	mouse	mechano- and thermonociceptive threshold, abdominal writhing	PAC_1	[107]

Abbreviations:
i.d. = intradermally, i.t. = intrathecally, i.p. = intraperitoneally, i.pl. = intraplantarly, s.c. = subcutaneously, n.a. = not available

Table 2. Distribution of PACAP and its receptors in the trigeminovascular system

	PACAP	PAC1	VPAC1	VPAC2	References
Dura mater	+	+	+	+	[70; 71]
Cortex	+	+	+	+	[2; 31]
Raphe nuclei	+	+	no data	no data	[2; 31]
LC	+	+	no data	no data	[2; 31; 45]
PAG	+	+	no data	no data	[2; 31; 45]
TNC	+	+	+	no data	[2; 31; 32; 68]
TRIG	+	+	+	+	[2; 31; 73-75]

Abbreviations:

C_2: cervical 2 segments of spinal cord; DRG: dorsal root ganglion; DRN: dorsal raphe nucleus; NRM: nucleus raphe magnus; LC: locus coeruleus; PAG: periaqueductal grey matter; TNC: trigeminal nucleus caudalis; TRIG: trigeminal ganglion; +: presence

Figure legends

Figure 1.

PACAP and its receptors are expressed in the different levels of the trigeminovascular system.

Figure 2.

The divergent effects of PACAP on nociceptive signalling and related functions in the central nervous system and on the periphery. 1. Sensory nerve terminal, 2. Dorsal root ganglion, 3. Spinal dorsal horn, 4. Amygdala

Figure 3.

The activation of TRIG may lead to the release of PACAP-38 from the peripheral and central terminals of the sensory nerves. Meningeal vasodilation, mast cell degranulation and neuronal activation and sensitization in the TNC may be consequences of the extra neuropeptide release. Clinically it may explain the throbbing headache, which is aggravated by physical activity, and also the cranial and extracranial cutaneous allodynia during migraine attack.

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