Divergent peripheral effects of pituitary adenylate cyclase-activating polypeptide-38 on nociception in rats and mice

Katalin Sándor a, Kata Bölcskei b, Jason J. McDougall c, Niklas Schuelert c, Dóra Reqlödi d, Krisztán Elekes a, Gábor Pethő a, Erika Pintér a, János Szolcsányi a, Zsuzsanna Helyes a,∗

a Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, H-7624, Pécs, Szigeti u. 12., Hungary
b Analgesic Research Laboratory, University of Pécs and Gedeon Richter Plc. (Budapest, Hungary), H-7624, Pécs, Szigeti u. 12., Hungary
c Department of Physiology and Biophysics, University of Calgary, 3330 Hospital Drive NW, Calgary, Alta., Canada T2N 4N1
d Department of Anatomy, Faculty of Medicine, University of Pécs, H-7624, Pécs, Szigeti u. 12., Hungary

A R T I C L E   I N F O

Article history:
Received 19 February 2008
Received in revised form 11 October 2008
Accepted 30 October 2008

Keywords:
Thermal hyperalgesia
Mechanical allodynia
Formalin test
Carrageenan
Writhing test
Electrophysiological recordings
Knee joint afferents

A B S T R A C T

Pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) and its receptors have been shown in the spinal dorsal horn, on capsaicin-sensitive sensory neurons and inflammatory cells. The role of PACAP in central pain transmission is controversial, and no data are available on its function in peripheral nociception. Therefore, the aim of the present study was to analyze the effects of locally or systemically administered PACAP-38 on nociceptive behaviors, inflammatory/neuropathic hyperalgesia and afferent firing. Intraplantar PACAP-38 (0.2 nmol) injection inhibited carrageenan-evoked inflammatory mechanical allodynia, mild heat injury-induced thermal hyperalgesia, as well as nociceptive behaviors in the early and late phases of the formalin test in rats. However, the above dose did not alter basal mechanical or heat thresholds. In mice, PACAP-38 (0.2 nmol/kg s.c.) significantly diminished acetin acid-induced abdominal contractions, but exerted no effect on sciatic nerve ligation-induced neuropathic mechanical hyperalgesia. In contrast, local PACAP-38 injection markedly increased rotation-induced afferent firing in the inflamed rat knee joint clearly demonstrating a peripheral sensitization in this organ. These actions were blocked by VPAC1/VPAC2 receptor antagonist pretreatment, but were not altered by PAC1 receptor antagonism. This paper presents the first data for the peripheral actions of PACAP-38 on nociceptive transmission mediated by VPAC receptors. These effects seem to be divergent depending on the mechanisms of nociceptor activation and the targets of PACAP actions. In acute somatic and visceral inflammatory pain models, PACAP exerts anti-nociceptive, anti-hyperalgesic and anti-allodynic effects. It has no significant peripheral role in traumatic mononeuropathy, but induces mechanical sensitization of knee joint afferents.

© 2008 International Association for the Study of Pain. Published by Elsevier B.V. All rights reserved.

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP-38) is a member of the vasoactive intestinal peptide (VIP)/secretin/glucagon peptide family that was originally isolated from ovine hypothalamus [28]. Highest concentrations of PACAP are found in the nervous system and endocrine organs. PACAP serves as a sensory neurotransmitter based on the presence of PACAP-like immunoreactivity in the superficial dorsal spinal horn layers, cell bodies [29,30] and peripheral terminals of capsaicin-sensitive primary sensory neurons [10,60]. Furthermore, PACAP [36] and its receptors have also been detected in the dorsal horn of the spinal cord [6,8] as well as in the articular capsule [52]. On the basis of these morphological and molecular biological results, PACAP has been suggested to be involved in pain transmission, but very few functional data are available to support this theory. All in vivo experiments studying its role in nociception focused on its central effects, and the results are contradictory [43]. Intrathecally injected PACAP inhibited spinal nociceptive reflexes [61] and inflammation-induced nociception [35,57,60]. Administration of PACAP intracerebroventricularly also resulted in analgesia in the early phase and algesia in the late phase [43]. On the other hand, central application of PACAP dose-dependently decreased paw withdrawal latencies induced by thermal stimulation and potentiated nociceptive transmission to the spinal dorsal horn by interacting primarily with N-methyl-D-aspartate (NMDA) receptors [34]. It also facilitated spinal nociceptive flexor reflexes [39,56] and induced hyperalgesia [32].
PACAP acts via G protein-coupled receptors mainly associated with the adenylate cyclase and phospholipase C: the PAC1 receptor which specifically binds PACAP and the VPAC1/VPAC2 receptors which have a similar binding affinity for PACAP and VIP. Both receptors have been described on neurons, smooth muscle cells and several inflammatory cells [5,46,53,63].

Our earlier data provided evidence that PACAP inhibits the release of pro-inflammatory/pro-nociceptive sensory neuropeptides: substance P (SP) and calcitonin gene-related peptide (CGRP) from peripheral terminals of capsaicin-sensitive nerves [33]. PACAP also inhibited acute neurogenic and non-neurogenic inflammatory processes in both mice and rats [13,33]. Based on these results, it was tempting to assume that this peptide might be involved in peripheral mechanisms of nociception.

Since there were no data available on the peripheral actions of PACAP-38 in nociceptive processes, the present study aimed at examining its effects on acute visceral and somatic nociceptive behaviors, as well as on inflammatory and neuropathic mechanical allodynia and heat injury-evoked thermal hyperalgesia after local or subcutaneous/intraperitoneal administration in different rat and mouse models. Since PACAP-38 is a large peptide, it does not cross the blood–brain barrier, therefore, even following systemic administration the observed effects can be considered as peripheral. Despite its short plasma elimination half-life (few minutes), the dose applied in our experiments for systemic administration was 50 ng rat^{-1} min^{-1}. PACAP acts via G protein-coupled receptors mainly associated with adenylate cyclase and phospholipase C: the PAC1 receptor which specifically binds PACAP and the VPAC1/VPAC2 receptors which have a similar binding affinity for PACAP and VIP. Both receptors have been described on neurons, smooth muscle cells and several inflammatory cells [5,46,53,63].

To study the involvement of specific receptors in the PACAP-induced effects, 2 nmol (40 μM) of the PAC1 receptor-selective M65 (Max.d.4 kindly provided by Prof. E.A. Lerner, Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital, Charlestown, MA, USA) was applied. The applied dose was chosen on the basis of the literature, where PACAP-38-induced effects were blocked by a 10 times higher dose of M65 in different in vitro systems [12,44]. In separate experimental groups, the VPAC1/VPAC2 receptor antagonist VIP6-28 (Sigma, St. Louis, MO, USA) [1,27,45] was administered into the plantar region 5 min prior to 0.2 nmol PACAP-38 (50 μM 4 μM). PACAP-38 as well as both antagonists was dissolved in 0.9% saline, and the effect of PACAP was evaluated by comparing with the solvent (saline)-treated control group.

2. Materials and methods

2.1. Animals

Experiments were performed on Wistar rats of both sexes and on male CD1 mice, bred and kept in the Laboratory Animal Centre of the University of Pécs or the University of Calgary at 24–25 °C, and were provided with standard rat chow and water ad libitum.

All experimental procedures were carried out according to the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (243/1988) and complied with the recommendations of the International Association for the Study of Pain [64] and the Helsinki Declaration. The studies were approved by the Ethics Committee on Animal Research of the University of Pécs and the University of Calgary according to the Ethical Codex of Animal Experiments and license was given (license No.: BA 02/2000-11-2006).

2.2. Formalin test

Formalin (Formaldehydum solutum 37%; Ph.Hg. VII.; 50 μl, 2.5%-injected s.c. into the plantar surface of the left hindpaw induces nociceptive reactions in two phases, the first of which (0–5 min) is thought to be due to a direct chemonociceptive effect of formalin, while the second one (20–45 min) is mainly mediated by inflammatory reactions [51]. Nocifensive behavior of male Wistar rats (180–220 g) was quantitatively evaluated by the duration of paw liftings and the duration of paw licks, and “Composite Pain Score” (CPS = (1 × duration of paw liftings + 2 × duration of paw licks in sec)/duration of examination period in sec) was calculated [54]. PACAP-38 (0.2 nmol; 2 μM 100 μl; Sigma, St. Louis, MO, USA) or its solvent (saline) was also injected intraplantarly 5 min before formalin administration.

2.3. Heat injury-induced thermal hyperalgesia model

The noxious heat threshold of female Wistar rats (150–200 g) was determined with a recently validated [4] increasing temperature water bath, which was developed in our laboratory in cooperation with Experimetria Ltd. (Budapest, Hungary). The equipment is suitable for the determination of the behavioral noxious heat threshold of rats defined as the lowest temperature at which the animal withdraws its hindpaw immersed into the water bath. A starting temperature of 30 °C and a heating rate of 24 °C/min were employed, and the cut-off temperature was set to 53 °C. Rats were lightly restrained and one of the hindpaws was immersed into the water, and the heating process was started afterwards. At the moment when the animal withdrew its paw, heating was immediately stopped by the foot switch, and the corresponding water temperature was recorded as the noxious heat threshold of the examined paw. For habituation purposes, two heat threshold measurements were performed the day before the experiment. In each experiment, the same assistant handled all the animals with a special emphasis on minimalizing the restraint stress and pain.

After control threshold measurements rats were anaesthetised with diethyl ether, and one of the hindpaws was immersed in a constant, 51 °C hot water bath for 20 s in order to evoke a mild burn injury. Following recovery from anaesthesia, heat threshold determinations were repeated 10 and 20 min after heat injury to confirm the development of hyperalgesia. PACAP-38 (0.2 nmol; 2 μM 100 μl) was administered intraplantarily after the 20-min measurement, which was followed by repeated heat threshold measurements at 10 min intervals. The effect of the drug was examined by comparing with the solvent (saline)-treated control group. For studying the involvement of specific receptors in the PACAP-induced effects, 2 nmol (40 μM 50 μl) of the PAC1 receptor-selective M65 (Max.d.4) or the VPAC1/VPAC2 receptor antagonist VIP6-28 was administered into the plantar region 5 min prior to 0.2 nmol PACAP-38 (4 μM 50 μl).

2.4. Carrageenan-evoked mechanical alldynia model

Mechan nociceptive threshold of the hindpaw of male Wistar rats (180–250 g) was determined by aesthesiometry (Ugo Basile Dynamic Plantar Aesthesiometer 37400, Comerio, Italy). Previous studies have revealed that this method is the most appropriate to study mechanosensitivity in inflammatory models [14]. Measurements were performed before, 120 and 180 min after the induction of an acute inflammation with an injection of λ-carrageenan (3%, 50 μl; Sigma, St. Louis, MO, USA) into the plantar surface of one hindpaw. PACAP-38 (0.2 nmol; 2 μM 100 μl) was administered intraplantarly 5 min before both measurements. For examining the involvement of the VPAC receptors in the PACAP-induced effect, the VPAC1/VPAC2 receptor antagonist VIP6-28 was administered into the plantar region 5 min prior to 0.2 nmol PACAP-38 (4 μM 50 μl).
2.5. Writhing test

Acetic acid (3% dissolved in distilled water, 200 μl) was injected i.p. to elicit abdominal constriction responses in male CD1 mice (20–25 g). The animals were placed in a transparent plastic box right after the challenge, and their responses were counted during continuous observation for 20 min. PACAP-38 (0.2 nmol/kg) or its solvent (saline) was administered s.c. 30 min before the acetic acid treatment. For examining the involvement of the VPAC receptors in the PACAP-induced effect, mice were pretreated with the VPAC1/VPAC2 receptor antagonist VIP6–28 s.c. 5 min before PACAP-38 administration.

2.6. Electrophysiological recording of joint primary afferents

Experiments were performed on 15 male Wistar rats (250–450 g). Animals were deeply anaesthetised by an intraperitoneal injection of urethane (25% stock solution; 2 g/kg, i.p.), and depth of anaesthesia was confirmed by the absence of a pedal pinch withdrawal reflex. An acute synovitis was induced in the right knee joint by an intra-articular injection of 2% kaolin followed by 2% carrageenan. Total injected volume was 200 μl, and inflammation gradually developed over the succeeding 3–6 h. Following inflammation induction, animals were placed supine on a thermostatically controlled heating blanket, and core body temperature was maintained at 37°C. The right hip joint was immobilised by attaching a specially designed clamp to the femur at mid-thigh and by connecting it to a stereotaxic frame. The right hindpaw was placed into a plastic boot and the knee joint was rotated. The amount of torque generated during the rotational movement of the knee was measured by a torque meter (MVD2510; Hottinger-Baldwin Messtechnik, Darmstadt, Germany). Finally, a longitudinal skin incision was made along the medial aspect of the hindlimb, and the resulting skin flaps were secured to a metal “O” ring to create a pouch which was filled with warm paraffin oil.

The saphenous nerve was cut distal to the knee joint to prevent sensory input from the hindpaw. The nerve was also isolated in the inguinal region of the right hindlimb and centrally transected to prevent spinal reflex arcs. Axon bundles of the proximal nerve stump were hooked over a platinum electrode, and an electrical activity in the nerve was recorded. Action potentials were amplified via a pre-amplifier (DAM80, World Precision Instruments, Sarasota, FL, USA. Settings: gain X10000, low filter 300 Hz, high filter 1 kHz) and amplifier (gain X10). Afferent nerve fibres originating from the knee joint were positively identified by the elicitation of an electrical response to gentle probing of the knee joint with a thin, blunt glass rod. The conduction velocity of the recorded nerve fibres was ascertained by measuring the latency to an evoked potential produced by electrically stimulating the receptive field (0.2 Hz, 100 ms pulse width, 6–12 V).

A total of 40 fibres were recorded in this study. Afferent activity was recorded during the normal outward rotation of the knee joint (14–22 mNm), and the amount of torque applied to the individual knees was held constant for 10 s. Initially, 3 movement cycles were performed to acquire control baseline nerve activity which was set at 100%. PACAP was directly dissolved in Tyrode solution and then administered to the knee by close intra-arterial injection via the saphenous artery (0.02 and 0.2 nmol; 0.1 ml bolus). Subsequent movement cycles were carried out at 1, 3, 5, 7, 9 and 11 min after drug administration. Further electrophysiology experiments were carried out in which PACAP was co-administered with the VPAC receptor antagonist VIP6–28 (10 μM). Recorded nerve activity was collected by a data acquisition system (CED1401, Cambridge Electronic Design, Cambridge, UK) and stored on a microcomputer for off-line analysis. The number of action potentials/movement was determined using Spike 2 software (Cambridge Electronic Design, Cambridge, UK), and the % change in afferent activity following PACAP administration was calculated compared to control (baseline = 100%).

2.7. Traumatic mononeuropathy model

Mice were anaesthetised with a combination of 100 mg/kg ketamine i.p. and 5 mg/kg xylazine i.m. The common sciatic nerve was exposed unilaterally high in the thigh, and 1/3–1/2 of the nerve trunk was carefully separated and tightly ligated using a siliconised silk suture (Ethicone 9–0). Then the wound was closed, and the animals were allowed to survive for 8 days. During this period, signs of spontaneous pain (holding the legs in elevated position) and mechanonociceptive hyperalgesia developed. Mechano-nociception of the hindpaws was measured with aesthesiometry, and hyperalgesia was expressed in % compared to the initial control values. Significant decrease in mechanical threshold developed 7 days after the surgery. PACAP-38 (0.02 and 0.2 nmol/kg) or its solvent (saline) was administered i.p. 30 min before the measurement.

2.8. Statistical analysis

For statistical evaluation of the heat injury-induced thermal, neuropathic and carrageenan-evoked mechanical nociceptive threshold/hyperalgesia data analysis of variance (one-way ANOVA) followed by Bonferroni’s modified t-test was used. Results of the formalin and writhing tests were evaluated by Student’s t-test for unpaired comparison, and the electrophysiological data were evaluated by two-way ANOVA. In all cases P < 0.05 was considered significant.

3. Results

3.1. Effect of PACAP-38 on formalin-induced acute nocifensive behavior of the rat

The anti-nociceptive effect of intraplantarly administered PACAP-38 (0.2 nmol) was assessed on the characteristic two phases of the formalin test [52]. Nocifensive behavior expressed as CPS calculated from paw lickings and liftings was significantly inhibited by i.pl. injection of PACAP-38 both in the early phase (0–5 min) referring to acute chemonociception and in the late phase (20–45 min) evoked by the inflammatory reaction (Fig. 1). Local administration of the selective PAC1 receptor antagonist M65 (2 nmol) 5 min prior to PACAP-38 (0.2 nmol) into the plantar region did not alter the PACAP-induced anti-nociceptive effect in either phase. Meanwhile, preinjection of the VPAC1/2 receptor antagonist VIP6–28 (2 nmol i.pl.) abolished the PACAP-evoked inhibitory action on the CPS in phase II, but did not influence PACAP action in phase I (Fig. 1).

3.2. Effect of PACAP-38 on heat injury-evoked thermal hyperalgesia of the rat

After the heat injury (51°C, 20 s), rats recovered from anaesthesia within minutes. There were no signs of spontaneous nocifensive behavior in any of the animals. On average, control noxious heat threshold was 42.8 ± 0.2°C. Upon heat threshold measurements following heat injury, the threshold dropped to an average of 34.4 ± 0.5°C, and this heat hyperalgesia was maintained at an even level for at least an hour. PACAP-38 (0.2 nmol i.pl.) administered after the 20-min measurement markedly reduced heat injury-induced thermal hyperalgesia at 30 and 40 min after heat injury as compared to the solvent-treated group. At the 40-min
3.3. Effect of PACAP-38 on carrageenan-induced mechanical allodynia of the rat

Carrageenan injection resulted in 26.5 ± 1.6% and 32.2 ± 1.9% decrease of the mechanonociceptive threshold of the paw 2 and 3 h after its intraplantar injection in the control, saline-treated group. PACAP-38 (0.2 nmol i.pl.) administered 5 min before both time points markedly diminished carrageenan-induced inflammatory mechanical allodynia, its inhibitory action was 66.1% and 80.3% at 120 min and 180 min, respectively. Pretreatment with the VPAC1/2 receptor antagonist VIP6.28 (2 nmol) abolished the anti-allodynic action of PACAP-38 (Fig. 3).

3.4. No effect of PACAP-38 on mechano- and thermonociceptive thresholds of the rat hindpaw

The mechanonociceptive threshold of the rat hindpaw determined with a dynamic plantar aesthesiometry was 46.6 ± 1.5 g before and 44.1 ± 1.5 g 10 min after i.pl. injection of PACAP-38 (0.2 nmol). The respective thermonociceptive threshold values measured with the increasing temperature water bath were 42.4 ± 0.2 °C and 42.3 ± 0.4 °C. These data show that this dose of PACAP-38 did not alter nociceptive thresholds (Fig. 4).

3.5. Effect of PACAP-38 on acetic acid-evoked writhing behavior of the mouse

Pretreatment with PACAP-38 30 min before the acetic acid exposure significantly diminished writhing behavior in mice. The number of abdominal contractions was 54.5 ± 2.93 after s.c. injection of 0.2 nmol/kg PACAP compared to the control 63.3 ± 2.84 measured during 20 min in the solvent (saline)-treated group. Pretreatment with the VPAC1/2 receptor antagonist VIP6.28 (2 nmol/kg) abolished the anti-nociceptive action of PACAP-38 (Fig. 5).

3.6. Electrophysiological recordings

Knee joint diameter was significantly increased after kaolin/carrageenan injection confirming an inflammatory reaction in the joint (P < 0.001; paired Student’s t-test). Between 1 and 3, afferent fibres were examined per animal such that a total of 27 units were recorded in this study. The mechanical threshold required to initiate afferent firing in these inflamed knees was between 4 and 17 mNm, and the conduction velocities of the recorded fibres ranged from 0.78 to 2.20 m/s. As such all recorded fibres were classified as type IV knee joint primary afferents.
Local administration of 0.2 nmol PACAP-38 caused a transient but significant increase in afferent firing rate compared to vehicle ($P < 0.01$). The mean afferent firing frequency before PACAP-38 administration was 49.9 ± 8.5 action potentials/movement and 67.2 ± 3.4 action potentials/movement following PACAP-38 injection. The sensitizing effect of PACAP-38 was found to be concentration-dependent, since 0.02 nmol of the peptide had no significant effect on joint mechanosensitivity. Percent change in firing frequency for all time points is shown in Fig. 6A. The sensitizing effect of PACAP-38 was significantly attenuated by co-administration of the VPAC receptor antagonist VIP6/C028 ($P < 0.0001$; Fig. 6B).

3.7. No effect of PACAP-38 on neuropathic mechanical hyperalgesia in the mouse

After recovering from surgery, all mice showed guarding of the operated limb. The mechanonociceptive threshold values of the paw was 8.44 ± 0.78 g before the operation, which decreased to 4.64 ± 0.32 on the 8th postoperative day showing the development of a 45% mechanical hyperalgesia in average. This hyperalgesia was not significantly altered by either 0.02 nmol/kg or 0.2 nmol/kg i.p. PACAP administration (Fig. 7).

4. Discussion

Although immunolocalization of PACAP has been described in capsaicin-sensitive neurons [29,30], there are only few scattered data concerning its role in nociception. This study provides the first data on the peripheral actions of PACAP-38 on nociceptive processes. We have shown that peripherally administered PACAP-38 inhibits acute somatic and visceral chemonociception, as well as inflammatory mechanical allodynia and heat injury-induced thermal hyperalgesia in both rats and mice. On the contrary, it did not alter neuropathic mechanical hyperalgesia in the mouse paw and even induced mechanical sensitization of rat knee joint afferents.

PACAP acts on a family of three G protein-coupled receptors: the PAC1 receptor which has much higher affinity for PACAP than VIP and the VPAC1/VPAC2 receptors which similarly bind both
PACAP and VIP [21,46,53]. PAC1 receptors have been described mainly on neurons and smooth muscle cells [9,16,18,41], and VPAC1/VPAC2 receptors are expressed in the dorsal horn of the spinal cord, on knee joint afferents, inflammatory and immune cells including mast cells and fibroblast-like synoviocytes [27,50,53]. The complex peripheral role of these receptors is also supported by the data on the alteration in the number of both receptor types in models of peripheral mononeuropathy [8], sciatic nerve transection [17] and inflammatory conditions such as cystitis [3]. These receptors are coupled to several signal transduction mechanisms, e.g. activation of adenylate cyclase and increasing cAMP concentration, stimulation of phospholipase C leading to inositol triphosphate (IP3)-mediated Ca2+ mobilization and Ca2+- and diacylglycerol-dependent protein kinase C activation or stimulation of NO synthesis [6,24,53].

Several previous results obtained after injecting PACAP into the central nervous system reported on its diverse effects on nociceptive transmission [6,38,43,61]. Intrathecally administered PACAP decreased formalin-induced nocifensive behaviors in the rat without impairing motor functions [58,60]. Meanwhile, others found pro-nociceptive actions for PACAP in the mouse tail flick test [32]. Decreased paw withdrawal latencies induced by thermal stimulation and potentiated nociceptive transmission to the spinal dorsal horn have been described in rats [34]. Extracellularly recorded electrophysiological activity of dorsal horn neurons was markedly increased by PACAP-38 [7]. PACAP also facilitated flexor reflexes in spinalized rats [56].

The strong upregulation of PACAP in dorsal root ganglia following adjuvant-induced inflammation suggests a role for this peptide in inflammatory pain conditions, although during chronic processes [59]. PACAP functions as an immuno-modulator, and the majority of the studies report on its anti-inflammatory actions [1,11] by modulating several inflammatory soluble factors [2]. The PACAP-induced decreased cytokine or transcriptional factor synthesis [2,5] cannot be considered as an explanation of the peripheral inhibitory effects we observed within 30–45 min in the acute inflammation models, but VPAC receptor-mediated diminished release of other membrane-derived or granular inflammatory/nociceptive mediators is possible even during this short time period.

We have recently shown that not only the cellular, but also the acute neurogenic components of the inflammatory reactions are decreased by systemic PACAP application. It attenuated mustard oil-, capsaicin- and resiniferatoxin-induced plasma protein extravasation in the rat paw skin and mustard oil-evoked ear swelling of the mouse [13,33]. We have also provided direct in vitro evidence that PACAP-38 inhibits the release of pro-inflammatory sensory neuropeptides (SP and CGRP) from the peripheral terminals of capsaicin-sensitive sensory fibres, which serves as an explanation for its ability to decrease plasma protein extravasation of exclusively neurogenic origin [33]. These sensory neuropeptides are also involved in nocifensive behaviors and in the development of inflammatory hyperalgesia/allodynia [37,52]. Although the precise mechanism for the observed anti-nociceptive and anti-hyperalgesic actions of PACAP-38 is not known, our results with selective PAC1 and VPAC1/VPAC2 receptor antagonists clearly showed that both the inhibitory and excitatory peripheral actions are mediated by the activation of VPAC receptors. The observed anti-nociceptive and anti-hyperalgesic effect is not likely to be a direct inhibitory action on the nociceptors, since these receptors are connected to Gs and Gq proteins. Although they are linked to several signal transduction pathways, they all increase intracellular cAMP and Ca2+ levels [21,52], therefore, seem to be stimulatory in neurons [47–49]. Meanwhile, elevation of intracellular cAMP attenuates the release of inflammatory mediators from mast cells and granulocytes [19,55]. The peripheral anti-nociceptive and anti-hyperalgesic actions of PACAP in the acute inflammation-associated models might be explained by the decreased release of these pro-nociceptive neuropeptides as well as other sensitizing agents (bradykinin, prostaglandins, leukotriens, serotonin, etc.) from cellular sources. The finding that neither the basal mechanical nor the thermal nociceptive thresholds were altered by local PACAP injection suggests that it does not inhibit voltage-gated sodium channels, therefore local anaesthetic-like effect is not involved in these peripheral anti-nociceptive actions. Surprisingly, PACAP exerted a marked anti-nociceptive effect in phase I of the formalin test, which is due to the direct chemical stimulation of sensory nerves and usually not affected by classical analgesics. This inhibition was not altered either by PAC1 or VPAC receptor antagonism, therefore, nonspecific, non-receptor-mediated mechanisms are likely to be involved, but the existence of a presently unknown receptor for PACAP-38 or an overlapping action on other inhibitory receptors such as cannabinoid, opioid or somatostatin receptors cannot be excluded either [31]. Formalin has been shown to directly activate the ankyrin-repeat transient receptor potential 1 (TRPA1) receptors on sensory nerves [23], which induces the acute chemonocifensive behaviors in phase I. On the basis of the present findings, the ability of PACAP-38 to negatively modulate or antagonize the activity of this ligand-gated ion channel is also possible.

In contrast to the observed inhibitory effects of PACAP-38 on visceral and somatic nociceptive reactions in the paw, in the acutely inflamed rat knee joint, electrophysiological results clearly showed a sensitizing action. These results are in a complete agreement with the previous studies examining the effect of VIP on joint nociception [26,40]. Similar to PACAP, local administration of VIP led to the sensitization of rat joint primary afferents, which was blocked by the VPAC receptor antagonist VIP6-26 [40]. Thus, it is very likely that PACAP-38 also activates VPAC1/VPAC2 receptors located on nociceptive nerve terminals within the articular capsule, which leads to enhanced joint mechanosensitivity. The intracellular mechanisms which could explain this sensitizing action might be related to cAMP accumulation in response to adenylyl cyclase activation and enhanced protein kinase A activity, but phospholipase C activation can also be involved [47–49]. Besides these direct neural mechanisms of action, VPAC receptors have also been shown on human synoviocytes [50]. Fibroblast-like and macrophage-like synoviocytes are unique cells in the joints, which are able to secrete several sensitizing inflammatory mediators including IL-1, PGE2 and TNF-α [62]. Furthermore, histamine released from synovial mast cells also act as an algogenic substance in the
joints [15]. Evidence has been given for the ability of VIP to cause mast cell degranulation [45]. Therefore, an indirect action of PACAP to activate synoviocytes and mast cells and to induce the release of sensitizing substances in the acutely inflamed joints is also possible [27]. The contradicting data found between the acutely inflamed paw and joint might be explained by the following points: (1) mechanosensory responses to various mediators could be organ specific due to the differences in the receptor expression or disparate second messenger pathways, (2) the mechanisms responsible for formalin-induced paw inflammation and kaolin-carrageenan-evoked joint inflammation are distinct with different inflammatory cells and mediators being involved, (3) the nocifensive reaction we observe in the formalin test is a complex behavioral response that involves central mechanisms (i.e. central sensitization and/or spinal reflexes), whereas single fibre recordings from knee joint primary afferents reflect only the activity of peripheral nociceptors, and (4) although the inhibition of mediator release from mononuclear cells and granulocytes which could explain the anti-nociceptive effects in the paw also might exist in the joint, the direct neural stimulation as well as mast cell and synoviocyte activation (which is specific in the joint model) are likely to override this mechanism. Similar diverse peripheral effects on nociceptive transmission have also been found with opioids [20].

We found no change in mechanical hyperalgesia after the systemic administration of PACAP in the mouse mononeuropathy model, despite the electrophysiological evidence for the ability of PACAP as well as VIP to enhance the activity of dorsal horn neurons in rat experimental mononeuropathy [6]. Similarly, in PACAP-deficient mice no neuropathic thermal hyperalgesia and mechanical allodynia developed suggesting that PACAP is required for spinal sensitization and induction of neuropathic pain [22]. Although these data suggest a significant pro-nociceptive role of PACAP under neuropathic conditions at the spinal cord level, we could not confirm this function in the periphery, on sensory nerve terminals.

In conclusion, this paper presents the first data for the peripheral actions of PACAP-38 on nociceptive transmission. These effects seem to be divergent depending on the mechanisms of nociceptor activation and the targets of PACAP actions. In acute visceral and somatic inflammatory pain models, PACAP exerts anti-nociceptive, anti-hyperalgesic and anti-allodynic effects, while it causes mechanical sensitization in the acutely inflamed knee joint. Further studies are needed to completely elucidate both neural and non-neural factors in order to define the exact molecular mechanisms of PACAP-ergic effects on peripheral nociception.

Conflict of interest

The authors have no conflicts of interest regarding this manuscript.

Acknowledgements

This work was sponsored by Hungarian Grants: T049027, T046589, K72592, K73044, NRDP1A/005/2004, RET-008/2005, ETT-06-348/2006 and ETT-06-284/2006. Zs. Heyes and D. Reglodi are supported by Janos Bolyai Postdoctoral Research Fellowship. J. J. McDougall is an Alberta Heritage Foundation for Medical Research Senior Scholar and an Arthritis Society Investigator. N. Schuelert receives postdoctoral funding from the Alberta Heritage Foundation for Medical Research and the Canadian Arthritis Network. The authors are grateful to Prof. Ethan A. Lerner (Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital, Charlestown, MA, USA) for the generous gift of the PAC1 receptor-selective antagonist M65 (Max.d.4).

References


K. Sándor et al. / PAIN 141 (2009) 143–150 149