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The selective PAC1 receptor agonist maxadilan inhibits neurogenic vasodilation and edema formation in the mouse skin

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ABSTRACT

We have earlier shown that PACAP-38 decreases neurogenic inflammation. However, there were no data on its receptorial mechanism and the involvement of its PAC1 and VPAC1/2 receptors (PAC1R, VPAC1/2R) in this inhibitory effect.

Neurogenic inflammation in the mouse ear was induced by topical application of the Transient Receptor Potential Ankyrin 1 (TRPA1) receptor activator mustard oil (MO). Consequent neurogenic edema, vasodilation and plasma leakage were assessed by measuring ear thickness with engineer's micrometer, detecting tissue perfusion by laser Doppler scanning and Evans blue or indocyanine green extravasation by intravital videomicroscopy or fluorescence imaging, respectively. Myeloperoxidase activity, an indicator of neutrophil infiltration, was measured from the ear homogenates with spectrophotometry. The selective PAC1R agonist maxadilan, the VPAC1/2R agonist vasoactive intestinal polypeptide (VIP) or the vehicle were administered i.p. 15 min before MO. Substance P (SP) concentration of the ear was assessed by radioimmunoassay.

Maxadilan significantly diminished MO-induced neurogenic edema, increase of vascular permeability and vasodilation. These inhibitory effects of maxadilan may be partially due to the decreased substance P (SP) levels. In contrast, inhibitory effect of VIP on ear swelling was moderate, without any effect on MO-induced plasma leakage or SP release, however, activation of VPAC1/2R inhibited the increased microcirculation caused by the early arteriolar vasodilation. Neither the PAC1R, nor the VPAC1/2R agonist influenced the MO-evoked increase in tissue myeloperoxidase activity.

These results clearly show that PAC1R activation inhibits acute neurogenic arterial vasodilation and plasma protein leakage from the venules, while VPAC1/2R stimulation is only involved in the attenuation of vasodilation.

1. Introduction

Transient Receptor Potential Ankyrin 1 (TRPA1) is known to mediate pain and inflammatory processes, but its involvement in cold- and somatosensation is still debated (Bautista et al., 2006; Story et al., 2003). These non-selective cation channels are expressed on peripheral and central terminals of capsaicin-sensitive peptidergic primary afferent neurons, where they signal and amplify nociceptive stimuli. Several agents have been shown to activate TRPA1 receptors, including mustard oil (MO, also known as allyl isothiocyanate, AITC), formalin, thio-sulfinate in garlic, α,β-unsaturated aldehydes in cinnamon, air pollutants, nicotine, tear gas components, reactive oxygen species and chlorine (Hinman

Abbrevations: AITC, allyl isothiocyanate; ANOVA, repeated measures analysis of variance; CGRP, calcitonin gene-related peptide; COPD, chronic obstructive pulmonary disease; ICG, indocyanine green; IL, interleukin; i.p., intraperitoneal; i.v., intravenous; MO, mustard oil; MPO, myeloperoxidase; OD, optical density; PACAP, pituitary adenylate cyclase activator polypeptide; PO, paraffin oil; s.c., subcutaneous; SEM, standard error of mean; SP, substance P; TNFα, tumor necrosis factor α; TRPA1, transient receptor potential ankyrin 1; VIP, vasoactive intestinal polypeptide.

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et al., 2006). The natural plant-derived irritant, MO, stimulates TRPA1 on sensory nerve endings through covalent modification of cysteines on the intracellular C-terminal domain of the channel (Bautista et al., 2006; Hinman et al., 2006; Macpherson et al., 2007; McNamara et al., 2007). Sensory neuropeptides, such as calcitonin gene-related peptide (CGRP) and tachykinins (substance P: SP and neurokinins A and B), are released from these stimulated nerve terminals that induce a rapid inflammatory response (arteriolar vasodilation, plasma extravasation, recruitment of leukocytes and mast cell degranulation) locally in the innervated area (Szolcsányi, 1988). Moreover, sensory nerve endings also release neuropeptides, like somatostatin and pituitary adenylate-cyclase activating polypeptide (PACAP), exerting anti-inflammatory actions. Neurogenic inflammation plays a key pathogenetic role in a variety of different acute and chronic inflammatory diseases (Chiu et al., 2012). This is a basically different inflammatory mechanism compared to immune cell-mediated processes, it is often the very early initiation step even in chronic diseases including allergic contact dermatitis, atopic dermatitis, rosacea, migraine, allergic rhinitis, sarcoidosis, rheumatoid arthritis, psoriasis, asthma and COPD (Abad et al., 2006; Anichini et al., 1997; Aubdool and Brain, 2011; Bánvölgyi et al., 2005; Geppetti et al., 2005; O'Connor et al., 2004; Pisi et al., 2009; Raychaudhuri and Raychaudhuri, 2004; Teresiak-Mikołajczak et al., 2013). This triggers and remarkably augments further cellular pathways. Sensory nerve terminal activation and the neurogenic inflammatory component is basically not inhibited by the conventional anti-inflammatory drugs (cyclooxygenase inhibitors), but non-steroidal anti-inflammatory drugs might have an inhibitory action in case of nerve terminal sensitization by prostaglandins in the inflamed tissue (Pethő and Reeh, 2012). Furthermore, glucocorticoids are only moderately effective in extremely high doses in which they exert many severe side-effects that limit their clinical applications (reviewed in: Helyes et al., 2003). Therefore, there is an urgent need to develop a potential candidate which interacts with this crucial pathophysiological mechanism.

Several neuropeptides are known to be involved in the regulation of the neuro-immuno-endocrine system (Ganea and Delgado, 2002). Among these, PACAP is a pleiotropic and multifunctional neuropeptide, which is widely distributed in the brain, peripheral nervous system, cardiovascular, gastrointestinal and respiratory tracts. Besides its diverse effects in these organs, its inhibitory action on cellular and vascular components of inflammation and its vasodilatory actions have also been investigated in numerous studies (reviewed in: Vaudry et al., 2009). Anti-inflammatory properties of the peptide lead to its significant ameliorative effect in animal models of septic shock, stroke, diabetic nephropathy and colitis (Azuma et al., 2008; Banki et al., 2013; Dejda et al., 2011; Martinez et al., 2002). The effects of PACAP are mediated by G protein-coupled receptors: the specific PAC1 receptor with 8 different splice variants, and VPAC1 and VPAC2 receptors which bind PACAP and VIP with the same affinity (reviewed in: Vaudry et al., 2009). PAC1R is mainly expressed on smooth muscle cells, neurons, endothelial cells and peritoneal macrophages. VPAC1 receptor is constitutively expressed in the dorsal horn of the spinal cord, on T-lymphocytes, macrophages, monocytes, mast cells and dendritic cells, while the expression of VPAC2 is inducible on these cells (Delgado et al., 1996, 1999; Delgado and Ganea, 2013; Ganea, 1996; Vaudry et al., 2009).

Similarly to PACAP, the VPAC1/2 agonist VIP and the selective PAC1 agonist maxadilan have also been reported to exert anti-inflammatory and vasodilatory actions. Alterations in the level of the 28 amino acid neuropeptide VIP were shown in several immunological diseases, like sepsis, rheumatoid arthritis, lupus, autoimmune thyroiditis, while its involvement in neurogenic inflammatory disorders has also emerged (Delgado and Ganea, 2013; Lundy and Linden, 2004; Teresiak-Mikołajczak et al., 2013; Wu et al., 2011). We learned from studies with VIP-deficient mice that endogenous VIP exerts anti-inflammatory properties in LPS-induced septic shock, asthma and pulmonary hypertension (Delgado and Ganea, 2013; Hamidi et al., 2006).

The specific PAC1 receptor agonist maxadilan is a vasoactive compound, which was originally isolated from the salivary gland extract of *Lutzomyia longipalpis*, the vector of leishmaniasis (Lerner et al., 1991; Moro and Lerner, 1996). The peptide was named after its potent vasodilating effect, which was found to be endothelium-independent, and was even shown in the human skin by laser Doppler method (Grevelink et al., 1995; Lerner et al., 1991). Vasoactive properties of maxadilan include increase in blood flow, inhibition of platelet aggregation and blood coagulation. Its receptor-binding affinity is high, resulting in prolonged vasoactive effects persisting for 2 days (Grevelink et al., 1995). Maxadilan was also reported to exhibit profound anti-inflammatory properties (Bozza et al., 1998; Qureshi et al., 1996; Soares et al., 1998).

Besides the pivotal role of PACAP in non-neurogenic inflammation, involvement of PACAP in neurogenic inflammation was also investigated. Among several other neuropeptides, PACAP is also released from sensory nerve terminals and exhibits anti-inflammatory properties by inhibiting the stimulated release of neuropeptides including CGRP, SP and somatostatin (Fahrenkrug and Hannibal, 1998; Németh et al., 2006). Németh et al. (2006) reported that mustard oil-induced neurogenic edema and albumin extravasation were diminished by systemic PACAP treatment. However, no studies have been performed to elucidate the contribution of its three receptors to the vascular inflammatory reactions of the neuropeptide. The aim of the present study was to examine the involvement of PAC1 and VPAC1/2 receptors in the anti-inflammatory potential of PACAP in neurogenic inflammation.

2. Materials and methods

2.1. Animals

Experiments were performed using 3-month-old male and female CD1 mice, since we have never found gender difference in this model in earlier experiments (Pozsgai et al., 2010, 2012). Mice were kept in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy of the University of Pécs at 24–25 °C and provided standard mouse chow and water *ad libitum*. All experimental procedures were carried out in accordance with approved protocols (University of Pécs; BA02/2000-15024/2011). All efforts were made to minimize animal suffering and to reduce the number of animals used. The applied solutions were freshly prepared before each experiment.

2.2. Measurement of mustard oil- and formalin-induced neurogenic edema formation in the mouse ear

Mice were treated either with PAC1 receptor agonist maxadilan (100 μg kg^{-1}) or VPAC1/2 agonist VIP (100 μg kg^{-1}) or saline (10 ml kg^{-1}) intraperitoneally (i.p.) 15 min prior to the experiments. Dose of the applied agonists was determined on the basis of earlier experiments with the PAC1 and VPAC1/2 receptor agonist PACAP-38 in the same or similar models (Németh et al., 2006; Helyes et al., 2007), as well as potencies of these peptides are similar to PACAP-38 on isolated primary sensory neurones and nerve terminals (unpublished data). Mice were anesthetised with ketamine and xylazine (100 mg kg^{-1} and 5 mg kg^{-1}, i.p., respectively) before the experiment, and were kept under anesthesia by injecting 1/2–1/3 of the applied initial dose every hour. Either 10 μl of 1 or 5% mustard oil dissolved in paraffin oil (PO) ($n = 4–5$ and 4–6 mice in each experimental group, respectively) or 10 μl of 5% formalin dissolved in distilled water ($n = 4–5$ mice/group) was applied topically on both surfaces of the ear at the beginning of the experiment and 1 h later. Ear thickness was measured with engineer's micrometer (Moore and Wright, Sheffield, UK) with an accuracy of 0.1 mm before the treatment as control and 30 min after the application of mustard oil or formalin, and later every hour until the end of the 6-h period. Data are shown as means ± SEM of percentage increase of ear thickness compared to the initial controls.

2.3. Measurement of Evans blue-bound albumin extravasation in the mouse ear

Intraperitoneal treatment of the mice was performed as described above (Section 2.2.). Mice ($n = 4–5$/group) were anesthetised with urethane (1.2 g kg^{-1}) and their core body temperature was maintained at 38 °C with a heating pad. Evans blue (25 mg kg^{-1})