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Impaired nocifensive behaviours and mechanical hyperalgesia, but enhanced thermal allodynia in pituitary adenylate cyclase-activating polypeptide deficient mice ☆

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ABSTRACT

Pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) and its receptors (PAC1 and VPAC) have been shown in the spinal dorsal horn, dorsal root ganglia and sensory nerve terminals. Data concerning the role of PACAP in central pain transmission are controversial and we have recently published its divergent peripheral effects on nociceptive processes.

The aim of the present study was to investigate acute somatic and visceral nocifensive behaviours, partial sciatic nerve ligation-evoked chronic neuropathic, as well as resiniferatoxin-induced inflammatory thermal and mechanical hyperalgesia in PACAP deficient ($PACAP^{-/-}$) mice to elucidate its overall function in pain transmission. Neuronal activation was investigated with c-Fos immunohistochemistry.

Paw lickings in the early (0–5 min) and late (20–45 min) phases of the formalin test were markedly reduced in $PACAP^{-/-}$ mice. Acetic acid-evoked abdominal contractions referring to acute visceral chemonociception was also significantly attenuated in PACAP knockout animals. In both models, the excitatory role of PACAP was supported by markedly greater c-Fos expression in the periaqueductal grey and the somatosensory cortex. In PACAP-deficient animals neuropathic mechanical hyperalgesia was absent, while c-Fos immunopositivity 20 days after the operation was significantly higher. In this chronic model, these neurons are likely to indicate the activation of secondary inhibitory pathways. Intraplantarly injected resiniferatoxin-evoked mechanical hyperalgesia involving both peripheral and central processes was decreased, but thermal allodynia mediated by only peripheral mechanisms was increased in $PACAP^{-/-}$ mice.

These data clearly demonstrate an overall excitatory role of PACAP in pain transmission originating from both exteroceptive and interoceptive areas, it is also involved in central sensitization. This can be explained by the signal transduction mechanisms of its identified receptors, both PAC1 and VPAC activation leads to neuronal excitation. In contrast, it is an inhibitory mediator at the level of the peripheral sensory nerve endings and decreases their sensitization to heat with presently unknown mechanisms.

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) related to the vasoactive intestinal polypeptide (VIP)/secretin/glucagon family (Miyata et al., 1989) is present in 27 and 38 aminoacid-containing forms. Its actions are mediated by G protein-coupled receptors leading to adenylate cyclase and phospholipase C activation. The PAC1 receptor specifically binds PACAP, the VPAC1/VPAC2 receptors have similar affinity for PACAP and VIP (Laburthe et al., 2007). PAC1 is mainly localized on neurons

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and smooth muscle cells, VPAC1/VPAC2 are expressed in the spinal dorsal horn, sensory nerves, inflammatory and immune cells (Davis-Traber et al., 2008; Ekblad, 1999; Somogyvári-Vígh and Reglődi, 2004; Vaudry et al., 2009; Zhou et al., 2002).

The presence of PACAP in the spinal dorsal horn and capsaicin-sensitive primary sensory neurons (Fahrenkrug and Hannibal, 1998; Moller et al., 1993; Mulder et al., 1994, 1999; Pettersson et al., 2004; Zhang et al., 1996, 1998) proposed its involvement in the transmission of nociceptive information, but *in vivo* studies focusing mainly on its central effects provided contradictory results (Said, 2000; Shimizu et al., 2004). Intrathecal injection of PACAP inhibited spinal nociceptive reflexes (Zhang et al., 1993) and inflammation-induced nociception (Onou et al., 2007; Yamamoto and Tatsuno, 1995; Zhang et al., 1996). Its intracerebroventricular administration was anti-nociceptive in the early phase of the formalin test, but pro-nociceptive in the late phase related to acute inflammatory processes (Shimizu et al., 2004). In contrast, definitive excitatory actions were described for centrally applied PACAP: it decreased thermal stimulation-evoked paw withdrawal latencies and potentiated pain transmission to the dorsal horn (Ohsawa et al., 2002). It facilitated spinal nociceptive flexor reflexes (Sakashita et al., 2001; Xu and Wiesenfeld-Hallin, 1996) and induced hyperalgesia (Narita et al., 1996). We have recently published that PACAP-38 exerts divergent actions on peripheral nociceptive processes depending on the mechanisms of nociceptor activation and the targets of its actions. Peripheral administration of PACAP induced anti-nociceptive, anti-hyperalgesic and anti-allodynic effects in acute somatic and visceral inflammatory pain models, but induced mechanical sensitization of knee joint primary afferents (Sándor et al., 2009). Our earlier data provided evidence that PACAP inhibited the release of pro-inflammatory/pro-nociceptive sensory neuropeptides, substance P and calcitonin gene-related peptide, from peripheral terminals of capsaicin-sensitive nerves and decreased acute inflammatory reactions (Helyes et al., 2007; Németh et al., 2006).

Deletion of PAC1 receptors reduced nocifensive responses evoked by formalin, thermal and mechanical stimulations (Jongsma et al., 2001). Based on all these data, the role of PACAP in pain processes both centrally and peripherally seems to be controversial, it is likely to depend on the site of action, as well as the pathophysiological mechanisms. Therefore, experiments with PACAP-deficient mice are particularly important to clarify its overall significance in nociception (Hashimoto et al., 2006). There is one study showing absent carrageenan-induced inflammatory and spinal nerve transection-evoked neuropathic allodynia in PACAP knockout animals (Mabuchi et al., 2004). Using gene-deleted mice we aimed at elucidating the role of PACAP in acute visceral and somatic nocifensive behaviours, as well as inflammatory and neuropathic hyperalgesia with functional techniques and c-Fos immunohistochemistry.

2. Materials and methods

2.1. Animals

Experiments were performed on PACAP$^{-/-}$, their wildtype (PACAP$^{+/+}$) counterparts and in most models also on heterozygous (PACAP$^{+/-}$) mice. The generation and maintenance of the knockout mice on the CD1 background have been described previously in details (Hashimoto et al., 2006), they were backcrossed for 10 generations with the CD1 strain. Offsprings within the first three generations were used for the experiments. Animals were bred and kept in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy of the University of Pécs at 24–25 °C and provided with standard rat chow and water *ad libitum*.

2.2. Ethics

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, the Helsinki Declaration and ethical quidelines for studying pain in conscious animals (Zimmermann, 1983). The studies were approved by the Ethics Committee on Animal Research of Pécs University according to the Ethical Codex of Animal Experiments and licence was given (licence No.: BA 02/2000-11-2006).

2.3. 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 nocifensive 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 (Tjolsen et al., 1992). Nocifensive behaviour was quantitatively evaluated by the duration of paw liftings and lickings (Bölcskei et al., 2005).

2.4. Writhing test

Acetic acid (3% dissolved in distilled water, 200 μl) was injected i.p. to elicit abdominal constriction response as an indicator of acute visceral chemonocifensive behaviour. The animals were placed in a transparent plastic box right after the challenge and their responses were counted during continuous observation for 20 min (Sándor et al., 2007).

2.5. Resiniferatoxin-induced mechanical and thermal hyperalgesia models

The ultrapotent Transient Receptor Potential Vanilloid 1 receptor agonist resiniferatoxin (RTX) was injected intraplantarly (20 μl, 0.03 mg/ml) into the left hindpaw. This agent has been shown to induce an acute neurogenic inflammatory reaction with the development of a rapid thermal allodynia. This is followed by mechanical hyperalgesia later, after 2 h (Meyer and Campbell, 1981). The noxious heat threshold was measured with an increasing-temperature hot plate (IITC Life Science, Woodland Hills, CA, USA) before and 5, 10, 15 and 20 min after the induction of the inflammation. After habituation, mice were placed onto the plate which was then heated up from room temperature at a rate of 12 °C/min until the animal showed nocifensive behaviour (licking, lifting or shaking of the hindpaw). The corresponding plate temperature was considered the noxious heat threshold. The mechanonociceptive threshold was measured with dynamic plantar aesthesiometry prior to RTX injection and 2, 4, 6 and 24 h afterwards (Ugo Basile 37000, Comerio, Italy). The animals moved about freely in one of the compartments of the enclosure positioned on the metal mesh surface. Following acclimation after cessation of exploratory behaviour, the touch stimulator unit was placed under the animal's paw, using the adjustable angled-mirror to position the filament below the target area of the plantar surface. Then an electrodynamic actuator of proprietary design lifted a straight metal filament, which touched the plantar surface and began to exert an increasing upward force at a preset rate of application until a stop signal (removal of the paw) was attained. The paw withdrawal threshold was expressed in grams. Allodynia/hyperalgesia in both cases was expressed as percentage of initial, pre-injection control values.