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Parabrachial nucleus (PBn) pituitary adenylate cyclase activating polypeptide (PACAP) signaling in the amygdala: Implication for the sensory and behavioral effects of pain

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ABSTRACT

The intricate relationships that associate pain, stress responses and emotional behavior have been well established. Acute stressful situations can decrease nociceptive sensations and conversely, chronic pain can enhance other pain experiences and heighten the emotional and behavioral consequences of stress. Accordingly, chronic pain is comorbid with a number of behavioral disorders including depression, anxiety abnormalities and associated stress-related disorders including post traumatic stress disorder (PTSD). The central nucleus of the amygdala (CeA) represents a convergence of pathways for pain, stress and emotion, and we have identified pituitary adenylate cyclase activating polypeptide (PACAP) immunoreactivity in fiber elements in the lateral capsular division of the CeA (CeLC). The PACAP staining patterns colocalized in part with those for calcitonin gene related peptide (CGRP); anterograde fiber tracing and excitotoxic lesion studies demonstrated that the CeLC PACAP/CGRP immunoreactivities represented sensory fiber projections from the lateral parabrachial nucleus (LPBn) along the spino-parabrachioamygdaloid tract. The same PBn PACAP/CGRP fiber system also projected to the BNST. As in the BNST, CeA PACAP signaling increased anxiety-like behaviors accompanied by weight loss and decreased feeding. But in addition to heightened anxiety-like responses, CeA PACAP signaling also altered nociception as reflected by decreased latency and threshold responses in thermal and mechanical sensitivity tests, respectively. From PACAP expression in major pain pathways, the current observations are novel and suggest that CeA PACAP nociceptive signaling and resulting neuroplasticity via the spino-parabrachioamygdaloid tract may represent mechanisms that associate chronic pain with sensory hypersensitivity, fear memory consolidation and severe behavioral disorders.

1. Introduction

Chronic neuropathic pain alters sensory responses and carries an emotional subtext that can have severe effects on behavior. Persistent pain can heighten pain experiences from hyperalgesia and allodynia (Rouwette et al., 2012; Veinante et al., 2013). Further, patients suffering from chronic pain are more prone to experience depression, sleep dysregulation, panic disorders, obsessive compulsive behavior, anxiety abnormalities and stress-related disorders including post-traumatic stress disorder (PTSD) (Asmundson and Katz, 2009). The intricate relationship between pain and behavior has been well studied and among brain regions, the amygdala is centrally situated to integrate the many descending and ascending signals to modulate the sensory and emotional components of pain. Highly processed descending polymodal nociceptive information is conveyed from the somatosensory cortex and thalamus to the basolateral amygdala (BLA) which in turn projects to the central nucleus of the amygdala (CeA). The resulting CeA efferents signals are relayed to other central nuclei, including those traveling with hypothalamic – periaqueductal grey projections for autonomic control and anti-

Abbreviations: PACAP, pituitary adenylate cyclase activating polypeptide; PAC1, PACAP selective receptor; CGRP, calcitonin gene related peptide; CRH, corticotropin releasing hormone; PBn, parabrachial nucleus; LPBn, lateral parabrachial nucleus; CeA, central nucleus of the amygdala; CeLC, CeA – lateral capsular division.

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nociception to dampen pain stimuli (Veinante et al., 2013). Among several ascending pathways carrying pain transmission to the CeA, the most prominent is the spino-parabrachioamygdaloid tract (Bernard et al., 1996; Gauriau and Bernard, 2002; Rouwette et al., 2012; Veinante et al., 2013). Peripheral nociceptive signals carried via primary sensory Aδ- and C-fibers terminate in the dorsal horn where second order neurons send projections via the spino-parabrachial pathway to pontine lateral and external medial parabrachial nuclei (PBn) (Todd, 2010). Hence the PBn collects cutaneuous (mechanical and thermal), deep (muscular and articular) and visceral nociceptive signals and relays the information in a highly organized topographical manner principally to lateral capsular division of the CeA (CeLC). The roles of the CeA/CeLC in nociceptive processing have been examined from a number of vantages. *In vivo* electrophysiological studies have shown that noxious stimuli and chronic pain paradigms increase spontaneous and evoked CeA neuronal activity (Bernard et al., 1992; Ji and Neugebauer, 2009; Neugebauer and Li, 2003), and synaptic transmission at PBn-CeA and BLA-CeA synapses (Ikeda et al., 2007; Neugebauer et al., 2003). Visceral, inflammatory and chronic neuropathic pain can induce CeA neuron stress peptide and c-fos expression (Bon et al., 1998; Nakagawa et al., 2003; Suwanprathes et al., 2003; Ulrich-Lai et al., 2006; Rouwette et al., 2011) and increase glutaminergic NR1 receptor phosphorylation in CeA neurons (Bird et al., 2005). Further, human brain imaging studies have implicated the amygdala in pain (Simons et al., 2014). Hence the neurocircuit intersections in the CeA can modulate the sensory, emotional and affective responses to pain.

Pituitary adenylate cyclase activating polypeptide (PACAP) is a well studied neural and endocrine pleiotropic peptide important in the development and homeostatic regulation of many physiological systems (reviewed in Vaudry et al., 2009). In the central and peripheral nervous systems, PACAP is neurotrophic to promote neuronal survival, proliferation and differentiation in development and regeneration, participates in sensory and autonomic signaling, is important in hippocampal learning and memory processes and regulates a variety of hypothalamic/limbic stress-related behavioral responses. PACAP binds to several G protein-couple receptor subtypes (Braas and May, 1999; Harmar et al., 2012; Spengler et al., 1993). PACAP binds selectively at the PAC1 receptor; both PACAP and VIP bind the VPAC receptors with equal high affinity. Recently, the expression of PACAP and its cognate PAC1 receptor has been shown to be upregulated in specific limbic regions by chronic stress (Hammack et al., 2009). PACAP infusions into the bed nucleus of the stria terminalis (BNST) is anxiogenic, and altered blood PACAP levels and PAC1 receptor polymorphism have been associated with PTSD and other stress-related disorders (Almli et al., 2013; Chen et al., 2013; Ressler et al., 2011; Uddin et al., 2013; Wang et al., 2013). In sum, these observations have implicated limbic PACAP/PAC1 receptor signaling in stress- and anxiety-related behaviors.

In evaluating PACAP expression in other limbic structures, we noted high levels of PACAP immunoreactivity in fiber terminals and varicosities the CeLC, suggesting that the CeLC may be a target of distant PACAP projections. The CeLC is heavily innervated by the lateral PBn (LPBn) and PACAP has been localized to many sensory pathways. From these observations, we have hypothesized that LPBn PACAP signaling to the CeLC has both sensory and behavioral consequences. In examining the localization and roles of PACAP to the CeLC, our current work demonstrates that PACAP is a component of the parabrachioamygdaloid pathway and that PACAP/PAC1 receptor signaling in the CeA elicits nociceptive and behavioral responses. The integration of these nociceptive and emotion pathways may represent a set of neural circuits that mediate the adverse sensory and emotional consequences of chronic pain.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were habituated to the animal facility for 1 week before experimentation. Rats were single-housed and maintained on a 12 h light/dark cycle (lights on at 0700 h). Food and water were available ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont.

2.2. Chronic variate stress

Following acclimation, each animal was randomly assigned to either a control or chronically stressed group. Control group animals were handled and remained in their home cages until euthanasia. The chronically stressed group of animals underwent a chronic variate stress paradigm in which rats were exposed to one of 5 different stressors (oscillation, forced swim, restraint, pedestal standing and footshock) each day for 7 days, as described previously (Hammack et al., 2009; Roman et al., 2012, 2014). All animals within the group were exposed to the same order of stressors for the same duration.

2.3. Immunocytochemistry

The brains from perfusion fixed animals were postfixed in 4% paraformaldehyde at 4C for 24 h, washed and equilibrated in 30% surcrose before embedding in Tissue-Tek OCT compound for cryosectioning. The sections (30 μm) were mounted onto subbed slides, permeabilized with 0.3% Triton X-100, blocked with 1% BSA and incubated in primary antibody for 48 h at 4C. CRH immunoreactivity was localized using an affinity purified rabbit antibody (1:100, No. G-019-06, Phoenix Pharmaceuticals, Burlingame, CA). CGRP immunoreactivity was examined using a polyclonal antibody raised against the full length CGRP(1–37) peptide (1:1500, Ian Dickerson, Univ Rochester) for visualization with AlexaFluor 488 conjugated donkey anti-rabbit IgG (1:200, Jackson Immunoresearch). PACAP immunoreactivity was detected using a mouse PACAP monoclonal antibody (1:10, Jens Hannibal, Bisperg Hospital, Copenhagen, Denmark) followed by tyramide signal amplification (Hannibal, 2002). Following primary PACAP antibody incubation, the tissues were incubated in biotinylated horse anti-mouse antibody (1:200, 2 h; Vector Laboratories, Burlingame, CA) and treated streptavidin-HRP (1:200, 30 min) before application of tyramide-biotin reagent (1:100, 10 min; Perkin Elmer, Waltham, MA). After extensive washing, the PACAP immunoreactivity was localized with Cy3-conjugated streptavidin (1:200, 2 h; Jackson Immunoresearch, West Grove, PA). In dual localization studies, the sections were incubated in PACAP and CGRP or CRH antisera concurrently. Tissues sections from BDA anterograde tracing (Section 2.5.1) and excitotoxic lesion (Section 2.5.2) studies were also processed for immunocytochemistry using the same procedures. Images from immunocytochemistry, excitotoxic lesion and anterograde tracing experiments were acquired sequentially with appropriate filter sets using a Nikon E800 point scanning confocal microscope. Image analyses were performed using NIH ImageJ (Schneider et al., 2012) to threshold, determine signal area (pixel number in staining area) and calculate Pearson's and Mander's correlation coefficients. In within subject excitotoxic lesion studies, the area of immunoreactivity on the side of the lesion was compared to the vehicle control contralateral side.

2.4. Transcript analyses

Quantitative PCR (QPCR) was performed exactly as described previously (Girard et al., 2002, 2006; Hammack et al., 2009). Briefly, after euthanasia by rapid decapitation, the coronal rat brain sections were prepared using a rodent brain matrix (Ted Pella, Inc. Redding, CA) and the micropunched amygdala tissues were quickly frozen on dry ice for total RNA extraction using STAT-60 RNA/mRNA isolation reagent (Tel-Test "B", Friendswood, TX). All RNA were reverse transcribed simultaneously using random hexamer primers with the SuperScript II Preamplification System (Invitrogen, Carlsbad, CA) to obviate variability. Real-time QPCR was performed as described using SYBR Green I detection (Girard et al., 2002, 2006; Hammack et al., 2009). Briefly, cDNA templates were diluted 5-fold to minimize the inhibitory effects of the reverse transcription reaction components and assayed on an ABI Prism 7500 Fast Real -Time PCR System (Applied Biosystems, Foster City, CA) using SYBR Green I JumpStartTM Taq ReadyMix (Sigma, St. Louis, MO) containing 3.5 mM MgCl2, 200 μM dATP, dGTP, dCTP and dTTP, 0.64 U Taq DNA polymerase and 300 nM of each primer in a final 25 μl reaction volume. Oligonucleotide primer sequences were: PACAP (S) 5'-CATGTGTAGCGGAGCAAGGTT-3' (AS) 5'-GTCTTGCAGCGGGTTTCC-3'; CRH (S) 5'-TGGATCTCACCTTCCACCTTCTG-3' (AS) 5'-CCGATAATCTCCATCAGTTTCCTG-3'. The melting profiles for amplified DNA fragments were performed to verify unique product amplification in the quantitative PCR assays. For data analyses, a standard curve was constructed by amplification of serially diluted plasmids containing the target sequence (Girard et al., 2002, 2006). The increase in SYBR Green I fluorescence intensity (ΔRn) was plotted as a function of cycle number and the threshold cycle (CT) was determined by the software as the amplification cycle at which the ΔRn first intersects the established baseline. The transcript levels in each sample were calculated from the CT by interpolation from the standard curve to yield the relative changes in expression. For each target