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Parabrachial Pituitary Adenylate Cyclase-Activating Polypeptide Activation of Amygdala Endosomal Extracellular Signal–Regulated Kinase Signaling Regulates the Emotional Component of Pain

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ABSTRACT

BACKGROUND: Chronic pain and stress-related psychopathologies, such as depression and anxiety-associated abnormalities, are mutually reinforcing; however, the neuronal circuits and mechanisms that underlie this reinforcement are still not well understood. Pituitary adenylate cyclase-activating polypeptide (PACAP; *Adcyap1*) and its cognate PAC1 receptor (*Adcyap1r1*) are expressed in peripheral nociceptive pathways, participate in anxiety-related responses and have been have been linked to posttraumatic stress disorder and other mental health afflictions.

METHODS: Using immunocytochemistry, pharmacological treatments and behavioral testing techniques, we have used a rodent partial sciatic nerve chronic constriction injury model (n = 5–8 per group per experiment) to evaluate PACAP plasticity and signaling in nociceptive and stress-related behaviors.

RESULTS: We show that chronic neuropathic pain increases PACAP expression at multiple tiers along the spinoparabrachioamygdaloid tract. Furthermore, chronic constriction injury bilaterally augments nociceptive amygdala (in the central nucleus of the amygdala [CeA]) PACAP immunoreactivity, extracellular signal–regulated kinase phosphorylation, and c-Fos activation, in parallel with heightened anxiety-like behavior and nociceptive hypersensitivity. Acute CeA infusions with the PACAP receptor antagonist PACAP(6-38) blocked chronic constriction injury–induced behavioral responses. Additionally, pretreatments with inhibitors of mitogen-activated protein kinase enzymes or endocytosis to block endosomal PACAP receptor extracellular signal–regulated kinase signaling attenuated PACAP-induced CeA neuronal activation and nociceptive responses.

CONCLUSIONS: Our data suggest that chronic pain-induced PACAP neuroplasticity and signaling in spinoparabrachioamygdaloid projections have an impact on CeA stress- and nociception-associated maladaptive responses, which can be ameliorated upon receptor antagonism even during injury progression. Thus, the PACAP pathway provides for an important mechanism underlying the intersection of stress and chronic pain pathways via the amygdala.

Keywords: Amygdala, Nociception, PAC1 receptor, PACAP, Parabrachial nucleus, Stress-related behavior

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Pain carries an aversive emotional component that can severely impact physiological and behavioral responses. Accordingly, chronic pain has been well associated with a number of stress-related psychopathologies, including depression, sleep dysregulation, panic disorders, obsessive compulsive behavior, anxiety abnormalities and posttraumatic stress disorder (1). Although the mechanisms underlying this association are not well understood, the high comorbidity between pain and stress-related behavioral disorders suggests that the two may be interrelated maladaptive processes (2). 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 antinociceptive information is conveyed from the somatosensory cortex and thalamus 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 gray projections for autonomic control and antinociception to dampen pain stimuli (3). Among several direct ascending pathways carrying nociceptive transmission to the CeA, the most prominent is the spinoparabrachioamygdaloid tract (3–6). Peripheral nociceptive signals carried via primary sensory Aδ- and C-fibers terminate on spinal projection neurons in laminae I/II and IV of the dorsal horn

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where the second-order neurons send projections via the spinoparabrachial pathway to pontine lateral parabrachial nuclei (LPBn) (7). In turn, the third-order LPBn neurons relay sensory information to the lateral (CeL) and lateral capsular (CeLC) subdivisions of the CeA. Hence the PBn collects cutaneous (mechanical and thermal), deep (muscular and articular), and visceral nociceptive signals and relays the information in a highly organized topographical manner principally to the nociceptive amygdala.

Although the integration of these inputs with amygdala circuits is a key mechanism underlying the emotional aspects of stress and pain, the neurochemistry, neuroplasticity, and regulatory events that drive the maladaptive responses are still not completely understood. In the CeA, chronic pain can alter glutamate receptor, extracellular signal–regulated kinase (ERK), and c-Fos expression or function; facilitate synaptic transmission to the CeLC; and dysregulate antinociceptive signaling (2,8–12). But in addition to diminished inhibitory neurocircuit function, persistent pain may also augment stimulatory CeA nociceptive neuropeptide levels including corticotropin releasing hormone and calcitonin gene-related peptide as complementary means to facilitate the stress- and pain-induced changes in neural function (6,11,13).

Among brain peptides, there is accumulating evidence implicating pituitary adenylate cyclase-activating polypeptide (PACAP) and its cognate PAC1 receptor in mediating the behavioral and physiological responses to homeostatic challenges (14). Altered PACAP levels and PAC1 receptor polymorphism have been associated with posttraumatic stress disorder (15–19). Mice lacking PACAP or the PAC1 receptor exhibit blunted anxiety-like behavior, show hypothalamic-pituitary-adrenal axis and autonomic system dysregulation, and fail to develop hypersensitivity to nociceptive stimuli in inflammatory pain paradigms (20–27). Furthermore, chronic but not acute stress leads to an upregulation of PACAP and PAC1 receptor transcript expression in the bed nucleus of the stria terminalis (BNST) (28–30). BNST PACAP signaling increases anxiety-like behaviors and hypothalamic-pituitary-adrenal axis activation and mediates many of the behavioral consequences of chronic stress. The BNST and the amygdala share similar circuit connectivity; as in the BNST, dense PACAP immunoreactivity has been identified in the CeLC/CeL, which represented LPBn PACAP projections from the spinoparabrachioamygdaloid tract (31). Importantly, infusions of PACAP or a specific PAC1 receptor agonist directly into the CeA of naive rats produced both anxiety-like behaviors and nociceptive hypersensitivity, suggesting that LPBn PACAP activity via the spinoparabrachioamygdaloid circuit carries signals that may in part alter the emotional responses to pain. Using a partial sciatic nerve ligation chronic constriction injury (CCI) model, we have now examined whether persistent neuropathic pain alters PACAP expression in the spinoparabrachioamygdaloid tract and whether PAC1 receptor antagonism can mitigate CCI-induced nociceptive hypersensitivity and anxiety-like behaviors. As PACAP signaling potently and efficaciously activates mitogen-activated protein kinase/ERK, a central mechanism in synaptic plasticity and CeA-dependent behaviors and pain hypersensitivity, we also assessed CeA PAC1 receptor mechanisms in vivo. The studies in aggregate suggest that endogenous PACAP signaling in the spinoparabrachioamygdaloid pathway and the resulting endosomal PAC1 receptor-stimulated activation of ERK in the CeA mediate the adverse emotional consequences of chronic pain. These results implicate PACAP mechanisms in the comorbidities between chronic pain and other stress-related pathologies.

METHODS AND MATERIALS

Detailed and extended data are available in the Supplement.

Animals

Adult male Sprague Dawley rats were from Charles River Laboratories (Wilmington, MA). PACAP–enhanced green fluorescent protein (EGFP) bacterial artificial chromosome transgenic mice were generated by the GENSAT (Gene Expression Nervous System Atlas, Rockefeller University, New York, NY). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont.

Surgical Procedures

For CCI, rats and PACAP-EGFP mice were anesthetized and loose ties (4-0 chromic gut; Ethicon, West Somerville, NJ) were placed proximal to the trifurcation of the sciatic nerve. Following recovery, only animals that developed thermal hypersensitivity were used for testing. For intra-amygdalar (CeA) infusions, 2 stainless steel cannulae (22-gauge; Plastics-One, Roanoke, VA) were placed bilaterally using coordinates (from bregma in mm) anteroposterior: −2.6, mediolateral: ±4.5, dorsoventral: −7.2. Drug infusions at a rate of 0.5 μL/min (Harvard Apparatus, Holliston, MA) included PACAP38, PACAP(6-38), Pitstop 2, and PD98059.

Histochemistry and Imaging

Anesthetized PACAP-EGFP mice were perfused transcardially with paraformaldehyde and tissue cryosections (30 μm) were mounted on subbed slides for endogenous EGFP fluorescence imaging. Tissues were processed similarly for immunocytochemistry using the procedures detailed in the Supplement. PACAP antibody was from Jens Hannibal (Bisperg Hospital, Copenhagen, Denmark); other antisera included those for phosphorylated ERK (pERK) and β-arrestin 1/2 (Cell Signaling Technology, Danvers, MA); c-Fos (Santa Cruz Biotechnology, Dallas, TX); and vesicular glutamate transporters 1 and 2 (vGlut1, vGlut2) and glutamic acid decarboxylase (all from Millipore Corp., Billerica, MA). Images were acquired under identical settings and parameters using a Nikon E800 scanning confocal (Tokyo, Japan) and Olympus fluorescence (Shinjuku, Japan) microscopes for analyses using ImageJ (National Institutes of Health, Bethesda, MD).

Open Field and Thermal Sensitivity Tests

In open field tests, rats were individually placed into the corner of a 75 cm × 75 cm open arena and center entries and total distance traveled (5 min) were digitally captured for analyses using EthoVision XT (Noldus Information Technology, Wageningen, the Netherlands). A Hargreave apparatus (IITC Life Science, Inc., Woodland Hills, CA) was used to assess thermal