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Chronic stress increases pituitary adenylate cyclase-activating peptide (PACAP) and brain-derived neurotrophic factor (BDNF) mRNA expression in the bed nucleus of the stria terminalis (BNST): Roles for PACAP in anxiety-like behavior

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Summary Exposure to chronic stress has been argued to produce maladaptive anxiety-like behavioral states, and many of the brain regions associated with stressor responding also mediate anxiety-like behavior. Pituitary adenylate cyclase activating polypeptide (PACAP) and its specific G protein-coupled PAC_1 receptor have been associated with many of these stress- and anxiety-associated brain regions, and signaling via this peptidergic system may facilitate the neuroplasticity associated with pathological affective states. Here we investigated whether chronic stress increased transcript expression for PACAP, PAC_1 receptor, brain-derived neurotrophic factor (BDNF), and tyrosine receptor kinase B (TrkB) in several nuclei. In rats exposed to a 7 days chronic variate stress paradigm, chronic stress enhanced baseline startle responding induced by handling and exposure to bright lights. Following chronic stress, quantitative transcript assessments of brain regions demonstrated dramatic increases in PACAP and PAC_1 receptor, BDNF, and TrkB receptor mRNA expression selectively in the dorsal aspect of the anterolateral bed nucleus of the stria terminalis (dBNST). Related vasoactive intestinal peptide (VIP) and VPAC receptor, and other stress peptide transcript levels were not altered compared to controls. Moreover, acute PACAP38 infusion into the dBNST resulted in a robust dose-dependent anxiogenic response on baseline startle responding that persisted for 7 days. PACAP/PAC_1 receptor signaling has established trophic functions and its coordinate effects with chronic stress-induced dBNST BDNF and TrkB transcript expression may underlie the maladaptive BNST remodeling and plasticity associated with anxiety-like behavior.

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1. Introduction

Stressors activate several physiological and behavioral systems to promote homeostasis. Many central nervous system (CNS) nuclei that mediate the response to stressors also mediate anxiety-like behavioral responding, and when stressor exposure is chronic, the sustained activation of these nuclei has been argued to lead to the maladaptive morphological and functional changes that underlie pathological affective states (Schulkin et al., 1998; Vyas et al., 2003; Pego et al., 2008). Hence, chronic stress may produce anxiety disorders by promoting neuronal plasticity within these stress-responsive nuclei.

Activation of the bed nucleus of the stria terminalis (BNST) has been argued to mediate both stress-responding and anxiety-like behavioral responding to diffuse and/or unpredictable threat (Walker et al., 2003). Stimulation of the BNST produces many physiological responses that are elicited by anxiogenic stimuli (Casada and Dafny, 1991), anxiogenic pharmacological agents increase BNST neuronal activation (Singewald et al., 2003), and BNST inactivation blocks many anxiogenic behaviors (for review, see Walker et al., 2003). Hence, alterations within the BNST have been argued to underlie anxiety disorders in humans (Walker et al., 2003; Straube et al., 2007). Consistent with this interpretation, chronic exposure to stressors or stress hormone increases anxiety-like behavior and enhances BNST dendritic branching, length and total BNST volume (Stout et al., 2000; Vyas et al., 2003; Pego et al., 2008). Given that stressor exposure is a critical component in the etiology of many anxiety disorders, and that the BNST is a point of confluence between stress and emotion, these data suggest that stress-induced alterations in neuronal function and plasticity in the BNST may underlie some forms of chronic anxiety in humans. Despite provocative evidence, the mechanisms of BNST signaling/plasticity in stress-induced anxiety have not been elucidated.

Pituitary adenylate cyclase activating polypeptides (PACAP) have neurotransmitter and neurotrophic properties, and are also associated with the stress response (Arimura, 1998; Sherwood et al., 2000; Vaudry et al., 2000). In the CNS, higher levels of PACAP and PAC_1 receptor mRNA and immunoreactivity are expressed in stress-associated brain regions, including the hypothalamus, hippocampus, discrete regions of the amygdala, and the BNST (Hashimoto et al., 1996; Jaworski and Proctor, 2000; Hannibal, 2002). PACAP signaling may modulate corticotropin-releasing hormone (CRH) because PACAP-immunoreactive fibers innervate CRH neurons in the hypothalamic paraventricular nucleus (PVN) and BNST (Kozicz et al., 1997; Legradi et al., 1998). PACAP infusion into cerebral ventricles or PVN augments plasma corticosterone levels, activates PVN neurons, and increases PVN CRH expression (Agarwal et al., 2005; Norrholm et al., 2005). However, the role of PACAP in behavioral stress responding is unclear and has so far only been inferred from peptide or receptor knockout studies (Hashimoto et al., 2001; Otto et al., 2001b; Girard et al., 2006). Moreover, only certain stressors regulate PVN PACAP expression (Hannibal et al., 1995).

Here, we demonstrate that chronic stress selectively induces PACAP and PAC_1 receptor transcript expression in the BNST, increases BNST brain-derived neurotrophic factor (BDNF) and TrkB receptor expression, and enhances anxiety-like behavior. Furthermore, PACAP infused into the BNST produces a long-lasting anxiogenic behavioral response.

2. Methods

2.1. Animals

Adult male Sprague—Dawley rats were obtained from Charles River Laboratories (Wilmington, MA), and were allowed to habituate in their home cages for at least 1 week before experimentation. Rats were single-housed, maintained on a 12 h light/dark cycle (lights on at 07:00 h), and 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

Stressed rats were exposed to a 7 days chronic variate stress paradigm. Rats were randomly assigned to control or chronically stressed groups, and for the latter, a single stressor was presented on each day (Table 1).

2.2.1. Oscillation stress

Rats were placed inside a plastic chamber 28 cm × 17 cm × 13 cm ($L \times W \times H$), that was secured to a clinical rotator (Fisher Scientific, Morris Plains, NJ), and oscillated at low to medium speed for 30 min.

2.2.2. Forced swim

Rats were placed in a cylindrical container 29 cm × 37 cm ($D \times H$) that was filled with room temperature water to a depth that prevented the rat tail from touching the bottom. After 5 min of monitored swimming, rats were placed in a holding chamber for 30 min prior to being returned to their home cage.

2.2.3. Footshock

Rats were placed inside a Plexiglas conditioning chamber (Med Associates, St. Albans, VT) 30 cm × 25 cm × 35 cm ($L \times W \times H$). After a 5 min acclimation period, two 1.0 mA 5 s scrambled footshocks were delivered through the grid floor with a 1 min inter-trial interval.

2.2.4. Restraint

Rats were placed in a cylindrical restraining device 9 cm × 15 cm ($D \times H$) for 60 min.

Table 1

Day	Stressor	Duration
1	Oscillation	30 min
2	Swim	5 min
3	Footshock	5 s (×2)
4	Restraint	60 min
5	Pedestal	30 min
6	Swim	5 min
7	Footshock	5 s (×2)