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Pituitary adenylate cyclase-activating polypeptide (PACAP) in the bed nucleus of the stria terminalis (BNST) increases corticosterone in male and female rats

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Summary Single nucleotide polymorphisms (SNP) in the genes for pituitary adenylate cyclase-activating polypeptide (PACAP) and the PAC1 receptor have been associated with several psychiatric disorders whose etiology has been associated with stressor exposure and/or dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. In rats, exposure to repeated variate stress has been shown to increase PACAP and its cognate PAC1 receptor expression in the bed nucleus of the stria terminalis (BNST), a brain region implicated in anxiety and depression-related behaviors as well as the regulation of HPA axis activity. We have argued that changes in BNST PACAP signaling may mediate the changes in emotional behavior and dysregulation of the HPA axis associated with anxiety and mood disorders. The current set of studies was designed to determine whether BNST PACAP infusion leads to activation of the HPA axis as determined by increases in plasma corticosterone. We observed an increase in plasma corticosterone levels 30 min following BNST PACAP38 infusion in male and female rats, which was independent of estradiol (E2) treatment in females, and we found that plasma corticosterone levels were increased at both 30 min and 60 min, but returned to baseline levels 4 h following the highest dose. PACAP38 infusion into the lateral ventricles immediately above the BNST did not alter plasma corticosterone level, and the increased plasma corticosterone following BNST PACAP was not blocked by BNST corticotropin releasing hormone (CRH) receptor antagonism. These results support others suggesting that BNST PACAP plays a key role in regulating stress responses.

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1. Introduction

We have reported that levels of pituitary adenylate cyclase-activating polypeptide (PACAP) and a single nucleotide polymorphism (SNP) within an estrogen response element (ERE) for the PAC1 receptor gene were correlated with post-traumatic stress disorder (PTSD) in women, while PAC1 gene methylation correlated with PTSD in both men and women (Ressler et al., 2011). More recent studies linking PTSD (Almli et al., 2013; Uddin et al., 2013; Wang et al., 2013), major depressive disorder (Hashimoto et al., 2010) and schizophrenia (Hashimoto et al., 2007) with PACAP gene SNP associations have also contributed to a growing interest in understanding a role for PACAP systems in stress-related psychopathologies.

PACAP has been called a "master regulator" of stress responses (for review see Stroth et al., 2011a). PACAP is highly expressed in the several regions that play a key role in stress responding and emotional behavior, including the bed nucleus of the stria terminalis (BNST, (Kozicz et al., 1997; for review see Vaudry et al., 2009)). However, the brain circuits by which central PACAP regulates the physiological and behavioral consequences of stressor exposure are still being determined.

The BNST plays a key role in regulating behavioral responses associated with anxiety and depression in animals and humans (Hammack et al., 2012; Kalin et al., 2005; Somerville et al., 2010; Walker et al., 2009), and tightly regulates the output of the hypothalamic-pituitary-adrenal (HPA) axis (Choi et al., 2007; Herman et al., 2005; Radley and Sawchenko, 2011). HPA dysregulation is a characteristic feature of several anxiety and mood disorders, and maladaptive BNST function may underlie both the behavioral features and the disrupted HPA-function associated with these disorders.

The BNST has been subdivided into multiple subnuclei (Dong et al., 2001), and different BNST subregions regulate HPA activity in different ways (for review, see (Herman et al., 2005)). For example, Radley and colleagues (2009) found that ablation of gamma-aminobutyric acid (GABA) neurons in the anteroventral BNST enhanced the glucocorticoid response to restraint stress; however, lesions to this region have also been shown to attenuate the glucocorticoid response to restraint stress, suggesting an excitatory role that may be dependent on the activation of corticotropin releasing hormone (CRH; Choi et al., 2007). Hence, the relationship between the BNST and HPA axis is complex, and BNST activation can inhibit or excite HPA activity depending on the BNST subregion and/or neuronal population targeted. Together, these data suggest that the BNST tightly regulates HPA activity. Moreover, as noted above, the BNST is sexually dimorphic, and the mechanisms through which the BNST modulates anxiety and stress responding has been largely studied in males. Therefore, it is unknown if this area functions in a similar manner for females in response to stress.

We have shown that PACAP and PAC1 receptor expression in the BNST is sensitive to stressor exposure (Hammack et al., 2009, 2010); exposure to repeated variate stress increased anxiety-related behavior and PACAP and PAC1 receptor transcript levels in the dorsolateral BNST (BNSTld) (Hammack et al., 2009). Moreover, intra-BNST PACAP infusion increased anxiety-like behavior (Hammack et al., 2009), and produced anorexia and weight loss (Kocho-Schellenberg et al., 2014). The current set of studies examined corticosterone levels following BNST PACAP infusion in both male and female rats and how BNST PACAP and CRH, and BNST PACAP and circulating estradiol (E2) may interact to influence corticosterone levels.

2. Methods

2.1. Subjects

Adult male or overiectomized female Sprague-Dawley rats weighing 250—300 g at the start of experimentation (male rats weighed ~350—400 g and female rats ~275—325 g on the day of infusion for all experiments) were obtained from Charles River Laboratories (Canada). Rats were allowed to habituate in their home cages at least one week before experimentation. Rats were single-housed and 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. Surgery

Rats were secured by blunted earbars in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) under isofluorane vapor (1.5—3.5%) anesthesia. Following a midline incision, 4 screws were put into the skull and bilateral guide cannulae were aimed at an angle of 20° just dorsal to the oval BNST (in respect to bregma: AP = −0.1, ML = +3.8, DV = −5.3 from dura) or at an angle of 20° in the dorsal aspect of the lateral ventricles immediately next to the BNST (respect to bregma: AP = −0.3, ML = +3.0, DV = −3.4). Placements for intra-cerebroventricular (ICV) surgeries were verified during surgery by observing the movement of a small amount of sterile isotonic saline in a length of tubing attached to the cannulae. For both BNST and ICV surgeries, stylettes were inserted to maintain cannulae patency, and a skull cap was created with dental acrylic to secure cannulae and stylettes. Immediately following surgery, rats were administered Ringer's Solution to provide hydration and analgesic care via Meloxicam or Carprofen (Pfizer), as well as additional analgesic care 24 h following surgery. Post-operative care was performed for seven days following surgery. Handling, consisting of light restraint, was performed for at least 3 days prior to experimentation in order to habituate rats to the infusion procedure.

For experiments involving female rats, BNST cannulae were implanted in the same manner as previously described. Females also received subcutaneous implants of silicone sealed silastic capsules containing 10 mm length of 10% estradiol/90% cholesterol, or cholesterol alone (100%). In order to determine the efficacy of the implants, trunk blood was collected at the time of perfusion to be later analyzed for blood estradiol levels via an estradiol enzyme linked immunoassay. Trunk blood was allowed to clot at room temperature for one hour and then subsequently spun at 4 °C at 4000 rpm for one hour to separate serum. Serum samples were stored at −80 °C for later processing.