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Neuroanatomical evidence for reciprocal regulation of the corticotrophin-releasing factor and oxytocin systems in the hypothalamus and the bed nucleus of the stria terminalis of the rat: Implications for balancing stress and affect

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Summary Activation of corticotrophin releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus (PVN) is necessary for establishing the classic endocrine response to stress, while activation of forebrain CRF neurons mediates affective components of the stress response. Previous studies have reported that mRNA for CRF2 receptor (CRFR2) is expressed in the bed nucleus of the stria terminalis (BNST) as well as hypothalamic nuclei, but little is known about the localization and cellular distribution of CRFR2 in these regions. Using immunofluorescence with confocal microscopy, as well as electron microscopy, we demonstrate that in the BNST CRFR2-immunoreactive fibers represent moderate to strong labeling on axons terminals. Dual-immunofluorescence demonstrated that CRFR2-fibers co-localize oxytocin (OT), but not arginine-vasopressin (AVP), and make perisomatic contacts with CRF neurons. Dual-immunofluorescence and single cell RT-PCR demonstrate that in the hypothalamus, CRFR2 immunoreactivity and mRNA are found in OT, but not in CRF or AVP-neurons. Furthermore, CRF neurons of the PVN and BNST express mRNA for the oxytocin receptor, while the majority of OT/CRFR2 neurons in the hypothalamus do not. Finally, using adenoviral-based anterograde tracing of PVN neurons, we show that OT/CRFR2-immunoreactive fibers observed in the BNST originate in the PVN. Our results

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strongly suggest that CRFR2 located on oxytocinergic neurons and axon terminals might regulate the release of this neuropeptide and hence might be a crucial part of potential feedback loop between the hypothalamic oxytocin system and the forebrain CRF system that could significantly impact affective and social behaviors, in particular during times of stress.

1. Introduction

Corticotrophin-releasing factor (CRF) containing neurons of the paraventricular nucleus of the hypothalamus (PVN) and the bed nucleus of the stria terminalis (BNST) are critically involved in mediating response to stress (Koob and Heinrichs, 1999; Moore et al., 2000; Shekhar et al., 2005; Hauger et al., 2009). Release of CRF from the PVN activates the hypothalamic—pituitary—adrenal (HPA) axis and initiates the classic endocrine response to stress (Kageyama and Suda, 2009), while CRF BNST neurons mediate the affective response to stressors, including fear and anxiety (Davis, 1998; Alheid, 2003; Walker et al., 2003).

The effects of CRF are mediated by two receptors: CRFR1 and CRFR2 (Chalmers et al., 1995; Lovenberg et al., 1995). In contrast to widely distributed CRFR1, CRFR2 are more regionally restricted and have been found in the lateral septum (LS), ventromedial hypothalamus, raphe nuclei, as well as central amygdala (CeA) and BNST (Chalmers et al., 1995; Chen et al., 2000; Van Pett et al., 2000). Significantly, activation of CRFR1 and CRFR2 appear to mediate opposing behavioral responses (Ji and Neugebauer, 2007; Zhao et al., 2007). Hence, the anxiogenic-like effects of CRF are mediated by CRFR1 (Muller et al., 2003; Nguyen et al., 2006; Sahuque et al., 2006), while CRFR2 activation has been reported to be anxiolytic, anxiogenic, or to have no effect (Bale et al., 2000; Coste et al., 2000; Bale and Vale, 2004; Cooper and Huhman, 2005). This inconsistency suggests that CRFR2 role in modulating the behavioral responses may result from an interaction with other neuromodulatory transmitter systems.

Oxytocin (OT) and arginine-vasopressin (AVP) are nonapeptides synthesized by neurons of the PVN and supraoptic nucleus of the hypothalamus (SON). Both OT and AVP levels are increased in the extended amygdala in response to stress, (Landgraf and Neumann, 2004; Ebner et al., 2005), as a result of release from local fibers (De Vries and Buijs, 1983; Sofroniew, 1983). Like CRFR1 and CRFR2, OT and AVP have generally opposing effects. Hence, AVP acts synergistically with CRF to activate HPA axis and has been shown to enhance anxiety and the consolidation of fear memory (Griebel et al., 2002; Caldwell et al., 2008), while OT acts as an anxiolytic in behavioral paradigms, and dampens the stress response (McCarthy et al., 1996; Windle et al., 1997; Petersson et al., 1999; Neumann et al., 2000; Bale et al., 2001; Amico et al., 2004; Ring et al., 2006; Waldherr and Neumann, 2007).

Intriguingly, the highest levels of OT receptor (OTR) mRNA are found in forebrain regions with high CRF cells density, namely the BNST, CeA, and PVN (Yoshimura et al., 1993; Veinante and Freund-Mercier, 1997). Conversely, CRFR2a mRNA co-localizes with OT mRNA in the rat SON (Arima and Aguilera, 2000), suggesting reciprocal interaction between the CRF and OT systems. However, nothing is known about the relationship between CRFR2 and OT expression in the BNST and hypothalamus, brain regions critically involved in the stress response. In the present study, we addressed this knowledge gap by using immunohistochemical, genetic, and adenoviral-based anterograde tracing techniques to map the regional, cellular, and ultrastructural distribution of CRFR2 in the BNST, PVN and SON in relation to OT, OTR, CRF and AVP.

2. Methods

2.1. Animal subjects

All experiments were performed in tissue from adult (60—70 days old) male, Sprague—Dawley rats (IF, scRT-PCR and WB: Charles River Laboratories, Wilmington, MA; IF and EM — Harlan, Indianapolis, IN). Adenoviral injections were performed on 35-days old Sprague—Dawley rats from Charles River. For stereotaxic surgery for colchicine and rAAV-GFP injections, rats were anaesthetized with an IP injection of Dexdormitor (Orion Pharma, Espoo, Finland) and Ketamine hydrochloride (Bioniche Pharma, Bogart, GA, USA) mixture (0.16 mg/kg Dexdormitor and 48 mg/kg Ketamine). All the procedures used were approved by the Institutional Animal Care and Use Committees (IACUC) of Emory University and University of South Carolina School of Medicine, and were in compliance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. Animals were housed in same sex groups, 4 animals per cage and were maintained on a 12:12-h light—dark cycle with ad libitum access to food and water. Animals were housed in animal facility at least 1 week after arrival prior to experiments.

2.2. Tissue processing for immunofluorescence

Two perfusion protocols were employed to facilitate immunohistochemical analysis: 6 rats received a standard 4% paraformaldehyde fixation procedure and a second cohort of 2 rats received intracerebroventricular (icv) colchicine (Sigma—Aldrich, 120 μg/2 μl) unilaterally 48 h prior to perfusion to maximize CRF peptide content in neuronal cell bodies. Perfusion protocol was described in details elsewhere (Dabrowska and Rainnie, 2010).

2.3. Immunofluorescence experiments

Immunofluorescence experiment protocols were similar to those previously described (Dabrowska and Rainnie, 2010). Briefly, fluorescent immunohistochemistry was performed using the following primary antibodies: rabbit polyclonal anti-CRFR2 antibody directed against the N-terminal (extracellular) domain (1:1000, ab12964, Abcam, Cambridge, MA), rabbit polyclonal anti-CRFR2 antibody against N-terminal domain (1:1000, NLS3570, Novus Biologicals LLC, Littleton,