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ACTIVATION OF GASTRIN-RELEASING PEPTIDE RECEPTORS AT THE INFRALIMBIC CORTEX ELICITS GASTRIN-RELEASING PEPTIDE RELEASE AT THE BASOLATERAL AMYGDALA: IMPLICATIONS FOR CONDITIONED FEAR

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Abstract—The basolateral amygdala (BLA) and infralimbic (IL) cortex share strong reciprocal interconnections and are key structures in conditioned fear circuitry. Gastrin-releasing peptide (GRP) or its receptor antagonists can modulate the conditioned fear response when exogenously administered at either of these sites, and increased release of GRP at the BLA occurs in response to conditioned fear recall. The present study sought to determine whether a functional pathway utilizing GRP exists between the IL cortex and BLA and whether this pathway is also influenced by amygdala corticotropin-releasing factor (CRF) release. To this end, we assessed the effects of intra-IL cortex injection of GRP or GRP co-administered with a receptor antagonist, RC-3095, on the downstream release of GRP and/or CRF at the BLA. Results showed that microinjection of GRP at the IL cortex increased the release of GRP, but not CRF, at the BLA, an effect blocked by co-administration of RC-3095. Administration of RC-3095 into the IL cortex on its own, however, also elicited the release of GRP (but not CRF) at the BLA. These findings suggest that a functional pathway utilizing GRP (among other factors) exists between the IL cortex and BLA that may be relevant to conditioned fear, but that GRP and CRF do not interact within this circuitry. Moreover, the finding that the release profile of GRP was similar following administration of either GRP or its receptor antagonist, lends support to the view that RC-3095 has partial agonist properties. Together these findings provide further evidence for the involvement of GRP in fear and anxiety-related disorders.

Key words: bombesin, corticotropin-releasing factor, RC-30-95, microdialysis, learned fear.

INTRODUCTION

The amygdala appears to be involved in all aspects of conditioned fear including the acquisition, expression and extinction of learned fear responses (Maren and Fanselow, 1996; Davis, 1997; Marek et al., 2013), whereas the infralimbic (IL) cortex is more specifically involved in the extinction of conditioned fear (Milad and Quirk, 2002; Milad et al., 2004; Marek et al., 2013). These brain regions share extensive reciprocal neural connections (McDonald et al., 1996; Sah et al., 2003), such that the IL cortex is thought to inhibit neuronal activity within the amygdala (Milad and Quirk, 2002; Milad et al., 2004; Pare et al., 2004). It seems that glutamatergic projections from the IL cortex to the lateral/basolateral amygdala (BLA) synapse on GABA interneurons located both within the BLA and intercalated cells located between the BLA and central nucleus of the amygdala (CeA) (Quirk et al., 2003; Pare et al., 2004), producing a feedward inhibitory mechanism to reduce conditioned fear responses when activated. What is less well understood, however, are the modulatory influences within this circuitry that might impact the excitatory/inhibitory balance and subsequent conditioned fear responses.

Gastrin-releasing peptide (GRP), the mammalian analog of the amphibian peptide bombesin, might have a modulatory influence within this IL-BLA circuitry. GRP (BB_2) receptors are highly expressed at both the anterior cingulate cortex (which encompasses the IL cortex) and the lateral nucleus of the amygdala (LA), specifically on GABAergic interneurons, and the application of GRP to either of these sites causes increased inhibition through release of GABA (Shumyatsky et al., 2002; Cao et al., 2010). Increasing evidence suggests the involvement of GRP in both conditioned fear expression and extinction (Shumyatsky et al., 2002; Roesler et al., 2004, 2012; Mountney et al., 2006, 2008; Martel et al., 2012). For instance, mice

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Abbreviations: BLA, basolateral amygdala; CeA, central nucleus of the amygdala; CRF, corticotropin-releasing factor; GRP, gastrin-releasing peptide; IL, infralimbic; IL-1β, interleukin-1β; IL-1ra, interleukin-1 receptor antagonist; ir-CRF, immunoreactive CRF; ir-GRP, immunoreactive GRP; KRB, Kreb-Ringer buffered saline solution; LA, lateral nucleus of the amygdala.

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lacking BB_2 receptors showed enhanced expression of learned fear responses as well as impaired extinction of a conditioned emotional response (Shumyatsky et al., 2002; Martel et al., 2012), and GRP injected into either the IL cortex, the CeA or the BLA, reduced freezing in a conditioned emotional response paradigm (Mountney et al., 2006, 2008). Unexpectedly, injections of the BB_2 receptor antagonist(s) BW2258U89 or RC-3095 into the IL cortex or the BLA, respectively, also reduced freezing. Injection of BW2258U89 into the CeA had mixed results, as reduced freezing was elicited by a high dose (300 ng), whereas the opposite effect was provoked by a low dose (50 ng) (Mountney et al., 2006). It is possible that at higher doses, these BB_2 receptor antagonists have intrinsic agonist properties, as previously demonstrated with BW2258U89 (Kirkham et al., 1995) as well as other BB_2 receptor antagonists (Wang et al., 1990; Ryan et al., 1999; Gonzalez et al., 2009). Similarly Roesler's group (Dantas et al., 2006) demonstrated opposite effects of low vs. high doses of RC-3095 injected into the dorsal hippocampus on memory retention, with the effects of high-dose administration (10 μg) being more in keeping with agonist activity.

Like GRP, corticotropin-releasing factor (CRF) also appears to be involved in conditioned fear as intra-LA injection of CRF enhanced the expression of learned fear (Isogawa et al., 2012), whereas systemic administration of a CRF antagonist impaired the expression of contextual fear memory (Hikichi et al., 2000). Substantial anatomical overlap exists between GRP and CRF including a high density of CRF1 receptors at the prefrontal cortex (including the IL cortex) and the BLA (De Souza et al., 1985). Moreover, several of the endocrine and behavioral effects of exogenous bombesin (the amphibian counterpart to GRP) administration, such as hypothalamic-pituitary hormone activation and anorectic actions, were blocked by pretreatment with a CRF receptor antagonist (Plamondon and Merali, 1997; Kent et al., 2001b). In addition, central administration of bombesin reduced endogenous CRF levels at several brain sites, including the CeA, while increasing CRF release from the median eminence and anterior pituitary (Kent et al., 2001a). Finally, a protracted and sustained elevation of GRP and CRF release was observed at the BLA 24 h after fear conditioning (Mountney et al., 2011), which may suggest that these two peptidergic systems act in a collaborative manner.

Given the robust anatomical connections between the IL cortex and the BLA, coupled with high BB_2 receptor expression at these two sites, the primary objective of the present study was to determine whether a functional pathway utilizing GRP (and CRF) exists between the IL cortex and the BLA that could be relevant to conditioned fear. To this end, we assessed whether the activation of GRP receptors at the IL cortex (via GRP administration) provokes the downstream release of GRP and/or CRF at the BLA and whether this effect would be blocked by the co-administration of a BB_2 receptor antagonist.

EXPERIMENTAL PROCEDURES

Subjects

Male Sprague–Dawley rats (Charles River Laboratories, St-Constant, Quebec, Canada) weighing approximately 275–300 g upon arrival, were maintained on a 12-h light/dark cycle (lights on at 07:00 h) in a climate-controlled environment (23 °C, relative humidity 60%). Animals were doubly housed in standard plastic cages (45 × 25 × 20 cm) until surgery and had free access to food (Purina Lab Chow; Charles River Laboratories) and water. All experimental procedures were performed in accordance with the guidelines provided by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Ottawa Institute Of Mental Health Research (ethics protocol number: ACC-2011-006). All attempts were made to minimize distress and the number of animals used in the study.

Surgery

Anesthesia was induced with isofluorane in 100% oxygen. Animals were prepared for surgery by shaving the surgical area and applying topical anesthetic. For pain control, rats received oral acetaminophen (Tylenol; 100–200 mg/kg) for 3 days prior to surgery and received rectal Tylenol (50 mg/kg) at the onset of surgery. Saline (5 c.c.) was administered subcutaneously to maintain optimal fluid levels. Animals were subsequently implanted stereotaxically with intracerebral microdialysis probes (MD-2200; Bioanalytical Systems Inc., West Lafayette, IN, USA) in the right-side of the BLA at the following coordinates: A/P −2.9 mm, L +5.4 mm, D/V −9.0 mm (Paxinos and Watson, 1982) and with 22-gauge guide cannulae (Plastics One, Roanoke, VA, USA) implanted bilaterally at the IL: A/P +3.0 mm, L ±0.7 mm, D/V −4.7 mm. The right-side BLA was targeted as there is evidence linking the right hemisphere (as opposed to the left) in stress-regulatory systems (Sullivan and Gratton, 2002; Adamec et al., 2005). Guide cannula aimed at the IL cortex were secured with two stainless steel screws and dental acrylic to allow for removal of the stereotaxic arm and subsequent insertion of injectors, while the microdialysis probes remained in place held by the stereotaxic arm during the course of the experimental procedure. Rats were placed on a heated pad to maintain the core body temperature at 37.5 °C throughout the duration of the experiment. During surgery isofluorane was kept at about 3.0% and during microdialysis sampling isofluorane was maintained at 2.0%.

Drugs and injections

The BB_2 receptor antagonist, RC-3095 (Sigma, St. Louis, MO, USA) and GRP (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) were dissolved in Kreb-Ringer buffered saline solution (KRB) consisting of (in nM; 2.7 K^+, 145 Na^+, 1.35 Ca^{2+}, 1.0 Mg^{2+}, 150 Cl^- ascorbate, pH 7.4). The control (vehicle) animals received an equivalent volume of KRB alone. All drug infusions were delivered via two 28-gauge injectors