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Predator threat induces behavioral inhibition, pituitary-adrenal activation and changes in amygdala CRF-binding protein gene expression

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Summary
Behavioral inhibition (BI) is an adaptive defensive response to threat; however, extreme BI is associated with anxiety-related psychopathology. When rats are exposed to a natural predator they display stress- and anxiety-related behavioral alterations and physiological activation. To develop a preclinical rodent model to study mechanisms underlying human BI and anxiety, we examined the extent to which ferret exposure elicits anxiety-related BI and HPA and amygdala activation of the CRF system. In the first experiment, BI and other behaviors were assessed in the presence or absence of a ferret. In the second experiment, ferret-induced corticosterone release and changes in brain c-fos expression were assessed. In the final experiment, gene chip and quantitative real time-PCR analyses were performed on amygdala tissue from control and ferret-exposed rats. Ferret exposure increased BI and submissive posturing, as well as plasma corticosterone and the number of Fos-positive cells in several brain regions including the amygdala. Gene expression analysis revealed increased amygdalar mRNA for CRF-binding protein, but not the CRF_1 receptor, CRF_2 receptor or CRF. In rodents, ferret exposure can be used to elicit anxiety-related BI, which is associated with HPA and amygdala activation. Since the amygdala and the CRF system have been implicated in adaptive and maladaptive anxiety responses in humans, these data support use of our rodent model to further investigate mechanisms underlying anxiety-related psychopathology in humans.

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1. Introduction

While physical stressors are commonly used in rodent stress studies, the most relevant stressors in humans are of a psychological or social nature. Furthermore, psychosocial stressors are known to precipitate stress-induced psychopathology (Dunner et al., 1979; Hammen et al., 1992). Therefore, when performing preclinical studies to understand mechanisms relevant to human psychopathology, it is important to use species-specific psychosocial stressors (Albeck et al., 1997; Kollack-Walker et al., 1997; Blanchard et al., 1998). Exposure of rats to a natural predator such as a ferret represents a potent form of psychosocial stress. Ferret predator stress has been used in recent years to study behavioral and hormonal stress responses, and is a potent stimulus to which rats display high levels of stress-like behaviors (Anisman et al., 1997; Merali et al., 2001; Masini et al., 2006). In addition, ethologically relevant stressors, such as predator exposure, produce long-lasting increases in stress-related behavior and plasma corticosterone. It has also been shown that habituation is less likely to occur with repeated exposure to a predator than with repeated exposure to other stressors such as restraint (Plata-Salaman et al., 2000).

The CRF peptide system is one of the major systems that integrates the response to psychological stress (Dunn and Berridge, 1990). CRF is produced by the hypothalamus in response to stress resulting in the release of adrenocorticotropic hormone (ACTH) from the pituitary and ultimately cortisol (corticosterone in rats) secretion from the adrenal glands (Vale et al., 1981; Berne and Levy, 1993). CRF is also produced in a variety of brain regions including the amygdala where it acts as a neurotransmitter and is thought to participate in the psychological and autonomic response to stressful stimuli (Dunn and Berridge, 1990). The CRF system is composed of at least seven components including the 41-amino acid peptide CRF, and three related urocortin peptides (urocortin 1–3). These peptides can interact with three different proteins, the two CRF receptors (CRF_1 and CRF_2) and the CRF-binding protein (CRF-BP). The CRF receptors are G protein-linked seven transmembrane domain receptors that are positively coupled to adenylate cyclase as well as other second messenger systems (Chen et al., 1993; Perrin et al., 1995). The CRF-BP binds CRF with an affinity equal to or higher than that of the receptors, and is thought to buffer the action of CRF by preventing interaction with the receptor and possibly targeting the peptide for degradation (Behan et al., 1995). Interestingly, recent work suggests that when CRF is bound to CRF-BP, this complex may act as a cellular signaling molecule (Ungless et al., 2003).

The amygdala is a medial temporal lobe structure that is important in identifying and interpreting cues that are associated with threatening stimuli (Davis and Whalen, 2001; Amaral, 2002; LeDoux, 2003). Considerable evidence implicates the amygdala in conditioned and unconditioned fear responses (Blanchard and Blanchard, 1972; LeDoux, 2000; Davis and Whalen, 2001; Amaral, 2003; Kalin et al., 2004), and human functional imaging studies have demonstrated increased amygdala activation in some patients with anxiety and depressive disorders (Drevets, 2003; Rauch et al., 2003). Furthermore, evidence supports a role for the amygdala CRF system in mediating anxiety and fear. For example, ferret exposure increases release of CRF in the Central amygdala (CeA) (Merali et al., 2001), and administration of a CRF receptor antagonist into the CeA blocks foot shock stress-induced freezing (Swiergiel et al., 1993).

Due to its ethological and psychological relevance, its potency, and the failure of rats to habituate to it after repeated exposure, ferret predator stress provides an excellent method to study the consequences of long-term and short-term psychosocial stress exposure. While this model has been used in rodents, few studies have focused on changes in the amygdala, second messenger systems and the CRF system. In the present study we characterized the behavioral, endocrine and amygdala gene expression changes following 10 min of ferret exposure. We report that brief ferret exposure induced intense fearful responses, caused a 7-fold increase in plasma corticosterone, altered expression of the c-fos gene in numerous brain regions including nuclei within the amygdala, and altered the levels of CRF-BP mRNA as determined by gene chip and quantitative real time-PCR (qRT-PCR) analyses.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 275–300 g were housed in pairs with lights on from 0700 to 1900 h. To control for possible diurnal variability in behavioral or biochemical indices, all testing and sacrificing occurred between 1000 and 1300 h. Food and water were available ad libitum. All rats were handled for several days prior to testing to minimize subsequent handling-related stress. Six ferrets (Marshall Farms, North Rose, NY) were used in these studies and were housed in pairs; food and water were available ad libitum. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80–23, revised 1978).

3. Experiment 1

3.1. Ferret exposure

Rats ($N = 8$) were exposed to a ferret for 10 min by being placed within a protective wire cage (7.75 in long $\times$ 6 in wide $\times$ 5.5 in high), inside the ferret's homecage. The bottom and ends of the cage were made of solid black plastic, whereas the top and sides were made of a black metal wire mesh. Control rats ($N = 8$) were placed inside an identical protective cage within a separate room. All rats were videotaped during the 10 min exposure period for subsequent behavioral scoring.

3.2. Behavioral scoring

All videotapes were analyzed by a single individual who scored the duration of the following behaviors: rearing (raising both front paws off the floor of the test cage), grooming (licking and rubbing with paws of any accessible