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Age-specific threats induce CRF expression in the paraventricular nucleus of the hypothalamus and hippocampus of young rats

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Abstract

Young animals respond to threatening stimuli in an age-specific way. Their endocrine and behavioral responses reflect the potential threat of the situation at a given age. The aim of the present study was to determine whether corticotropin-releasing factor (CRF) is involved in the endocrine and behavioral responses to threat and their developmental changes in young rats. Preweaning 14-day-old and postweaning 26-day-old rats were exposed to two age-specific threats, cat odor and an adult male rat. The acute behavioral response was determined during exposure. After exposure, the time courses of the corticosterone response and of CRF expression in the paraventricular nucleus of the hypothalamus (PVN) and in extrahypothalamic areas were assessed. Preweaning rats became immobile when exposed to cat odor or the male rat, whereas postweaning rats became immobile to cat odor only. Male exposure increased serum corticosterone levels in 14-day-old rats, but cat odor failed to increase levels at either age. Exposure induced elevation of CRF mRNA levels in the PVN that paralleled changes in corticosterone levels. CRF may thus play a role in endocrine regulation and its developmental changes during early life. Neither cat odor nor the adult male altered CRF mRNA levels in the bed nucleus of the stria terminalis (BNST) or the amygdala, but both stimuli increased levels in the hippocampus. Hippocampal CRF mRNA expression levels did not parallel cat odor or male-induced immobility, indicating that CRF is not involved in this response in young rats but may be involved in aspects of learning and memory.

Keywords: Immobility; Fear; Predation; HPA axis; Glucocorticoid

Introduction

Interest in the neural processes that govern behavioral changes in early life has increased considerably over the last decade. Basic as well as clinical research has demonstrated that the brain undergoes fundamental changes in early development and that such changes may contribute to age-specific alterations in behavior (Spear, 2000). The succession of changes in the neural organization allows the growing organism to adapt to rapidly changing environments (Oppenheim, 1981; Stamps, 2003). Ontogenetic plasticity, however, renders an individual vulnerable to aversive experience and increases the risk for psychopathologies later in life (Sánchez et al., 2001; Steinberg and Avenevoli, 2000). For example, traumatic experience may shape the development of neural systems that mediate fear- and anxiety-related behaviors and is associated with the development of anxiety disorders such as posttraumatic stress disorder (Heim and Nemeroff, 2001). Knowledge of the neural processes underlying ontogenetic changes in behavior is crucial in our understanding of the extent and limits of developmental plasticity.

In young rats, behavioral responses to threatening stimuli that are ecologically relevant change during the first weeks

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of life. When exposed to an unfamiliar adult male rat, preweaning rats become immobile (freeze), whereas around weaning and after weaning, rats approach the male (Hepper, 1986; Takahashi, 1992; Wiedenmayer and Barr, 1998). In contrast, rats become immobile throughout ontogeny when exposed to cat cues, although responsivity increases with age (Bronstein and Hirsch, 1976; Hubbard et al., 2004; Wiedenmayer and Barr, 2001b). Endocrine responses to threat undergo changes in early life as well. Various stressors, such as exposure to cold, to ether vapor, or restraint, increase corticosterone levels in an age-specific way (Dent et al., 2000a; Walker et al., 1991; Yi and Baram, 1994). For example, a saline injection increased corticosterone levels only in 18-day-old but not in younger rats (Dent et al., 2000b).

Behavioral and endocrine responsivity to threat and their ontogenetic changes may be mediated by the neuropeptide corticotropin-releasing factor (CRF). CRF plays a critical role as neuromodulator in both the fear and stress pathway. CRF was originally identified as a releasing factor that activates the hypothalamus–pituitary–adrenal (HPA) stress axis (Vale et al., 1981). Upon aversive stimulation, CRF is secreted from cells in the paraventricular nucleus of the hypothalamus (PVN) and induces, via a cascade of neuroendocrine events, the release of glucocorticoids from the adrenal glands (Herman and Cullinan, 1997). CRF acts also as a neurotransmitter in brain pathways outside the hypothalamus that mediate fear- and anxiety-related behaviors. Aversive stimulation induced the expression of CRF mRNA (Hsu et al., 1998; Kalin et al., 1994; Makino et al., 1999) and the release of CRF (Cook, 2002; Merali et al., 1998; Merlo Pich et al., 1995) in brain areas of the fear pathway (LeDoux, 2000) such as the amygdala and the bed nucleus of the stria terminalis (BNST). When CRF antagonists or CRF antisense were centrally infused either into the ventricular system or directly into the amygdala or locus ceruleus of rats submitted to aversive stimulation, responses such as freezing, anxiety-like behaviors in the elevated plus-maze, and withdrawal into a shelter were attenuated or blocked (Heinrichs et al., 1992; Kalin et al., 1988; Skutella et al., 1994; Smagin et al., 1996; Swiergiel et al., 1992, 1993; Takahashi et al., 1989).

Consistent with a dual role of CRF, aversive stimuli that activated CRF in the amygdala induced CRF expression in the PVN as well (Hsu et al., 1998; Kalin et al., 1994). When CRF was blocked centrally, both HPA axis activity and fear-related behaviors were attenuated (Skutella et al., 1994). Mice that lack the CRF receptor 1 in the amygdala and other extrahypothalamic areas but not in the PVN showed reduced anxiety-like behaviors, but their basal HPA activity was unaffected (Müller et al., 2003).

In young animals, CRF has been implicated in developmental aspects of HPA axis regulation (for reviews, see Brunson et al., 2001; Dallman, 2000; Levine, 2001). Only a few studies have demonstrated that CRF is involved in behavioral responses to aversive stimulation. CRF and CRF antagonists modulated ultrasonic vocalizations in preweaning rat pups that were separated from dam and nest (Harvey and Hennessy, 1995; Insel and Harbaugh, 1989; Kehne et al., 2000). However, little is known about the role of CRF in other anxiety- and fear-like behaviors in the infant.

The aim of the present study was to examine if developmental changes of threat-induced CRF activation parallel changes in the endocrine and behavioral response in young rats. CRF expression levels were assessed in the PVN and in extrahypothalamic areas of the fear pathway in preweaning 14-day-old and postweaning 26-day-old rats exposed to cat odor or an adult male rat. We hypothesized that in preweaning rats, both male and cat cues induce immobility, corticosterone secretion, and CRF expression, whereas in postweaning rats, only cat cues are effective.

Materials and methods

Animals

Long-Evans hooded rats were housed under standard laboratory conditions in a colony room with a 12-h light–dark cycle with light onset at 6:00 AM. When the females were pregnant, the males were removed from the breeding cages. Cages were monitored daily in the morning and evening for the presence of newborn pups, and the date of birth was considered as day 0. On postnatal day 23, the mother was removed, and littermates were kept together in the same cage. Because standard laboratory cages are too small for litters of 10 animals kept together up to an age of 26 days, rats were housed in larger cages (55 × 38 × 21 cm). A sexually experienced unrelated adult male was housed in the same colony room. Treatments were according to the guidelines of the Institutional Animal Care and Use Committee.

Little is known about sex differences in endocrine and behavioral responses and in CRF expression in young rats. No gender effect was found in male-induced immobility in 14- and 21-day-old rats (Wiedenmayer and Barr, 1998). The studies quoted in this paper that assessed corticosterone and CRF mRNA levels used in most cases mixed litters. However, sex differences were never reported or discussed. In the present study, only male rats were used.

Testing procedures

On the day of testing, on postnatal days 14 and 26, rats of a litter were assigned to four groups: (1) unexposed control, (2) control-exposed, (3) cat odor-exposed, and (4) male-exposed. First, one rat was taken from the home cage and decapitated within a minute. This animal represented the unexposed control that provided basal measurements of corticosterone and CRF (see below). Then one of the groups 2–4 was removed from the home cage and placed in the testing cage. Each group consisted of a small huddle of three rats to decrease isolation-induced stress (Hennessy and