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Stress-Induced Enhancement of the Startle Reflex

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The goals of the research are to study neural systems involved in the production and inhibition of fear and anxiety. Previous research has found that the acoustic startle reflex is senstive to both fear and stress. Many effects produced by fear or stress are inimicked by infusion of the peptide corticotropin releasing factor (CRF) directly into the brain. This year we have found that infusion of CRF into the brain causes a pronounced, dose-dependent enhancement of the acoustic startle reflex in rats. This excitatory effect was blocked by a CRF antagonist or by lesions of the amygdala, a brain structure known to be involved in fear and stress. The present data indicate that the amygdala is part of the neural circuitry required for CRF to elevate startle. Because startle is mediated by a well-defined neural pathway, CRF-enhanced startle is a useful behavioral assay to analyze the neural systems upon which exogenous CRF acts to produce its behavioral effects.

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Research Objectives

The goals of the research are to study neural systems involved in the production and inhibition of fear and anxiety. Fear is a natural, adaptive response to threatening stimuli which prepares the organism to cope with the provocation. However, high levels of fear or long periods of stress can lead to abnormal, maladaptive behaviors which compromise the ability of the organism to cope with its environment. A great deal of data now indicate that the amygdala, especially the central nucleus of the amygdala, is critically involved in fear and stress. Recent evidence now strongly suggests that the peptide corticotropin releasing factor (CRF) is released within the brain in response to fear or stress. Many effects produced by fear or stress are mimicked by infusion of CRF directly into the brain and several stress-related behaviors produced by natural stimuli can be blocked by direct infusion of a CRF antagonist into the brain . A major goal of the present research will evaluate the effects of infusion of CRF into the brain on the acoustic startle reflex, a simple behavioral measure known to be sensitive to fear and stress.

Accomplishments and progress

Intraventricular (icv) infusion of corticotropin-releasing factor (CRF) (0.1 - 1.0 mg) produced a pronounced, dose-dependent enhancement of the acoustic startle reflex in rats. This excitatory effect began about 20-30 min after infusion, grew steadily over the 2-hr test period, and lasted at least 6 hrs. Higher doses of CRF (10 μg) often produced marked facilitation and then inhibition of startle which oscillated repeatedly with a period of 10-20 min. CRF-enhanced startle did not result from an increase in sensitization produced by repetition of the startle stimulus or from a blockade of habituation. Peripheral injections of the autonomic ganglionic blockers hexamethonium (10 mg/kg) or chlorisondamine (3 mg/kg) slightly attenuated the magnitude of CRF-enhanced startle, suggesting a partial role of peripheral sympathetic activation. Intraventricular infusion of the CRF antagonist a-helical CRF_{9-41}(ahCRF - 25 or 50 μg) blocked CRF-enhanced startle when infused 5 min prior to CRF, indicating a central site of action. CRF-enhanced startle also was reversed when ahCRF was given 90 min after infusion of CRF. This suggests that exogenously applied CRF remains in the brain for a very long time after administration or that CRF given exogenously initiates a process that results in a long-lasting activation of endogenous CRF.

Intracisternal infusion of CRF (0.1 - 1.0 mg) increased startle with a time course and magnitude similar to that produced by icv CRF, unlike intrathecal infusion which produced a small, more rapid enhancement of startle. While lesions of the paraventricular nucleus of the

hypothalamus had no effect on icv CRF-enhanced startle, bilateral lesions of the central nucleus of the amygdala significantly attenuated the excitatory effect of icv CRF, but had no effect on intrathecal CRF-enhanced startle. Even though lesions of the amygdala blocked icv CRF-enhanced startle, local infusion of CRF into the amygdala did not significantly elevate startle. The present data indicate that the amygdala is part of the neural circuitry required for icv CRF to elevate startle, but does not appear to be the primary receptor area where CRF acts. The involvement of the amygdala in icv CRF-enhanced startle is consistent with the hypothesis that both the amygdala and CRF are critically involved in fear and stress. Because the startle reflex is elevated by both conditioned and unconditioned fear, these data lend further support to the idea that CRF infusion produces a behavioral state that resembles fear or anxiety. Because startle is mediated by a well-defined neural pathway, CRF-enhanced startle may provide a useful behavioral assay to analyze the neural systems upon which exogenous CRF acts to produce its behavioral effects.

Working conclusions and future studies:

These data indicate that the amygdala is ultimately involved in CRF enhanced startle but does not appear to be the primary site of action where icv-CRF initiates its action. Because a large number of studies have now found that icv-CRF produces a constellation of behavioral effects that resemble fear and stress, it is important to continue to determine the primary site where CRF acts after icv administration to produce these effects. If this site(s) could be found, then this would provide an anatomical anchor with which to begin to map out the neural circuitry involved in CRF induced fear and anxiety, which presumably will have relevance to a more general anatomical substrate of fear and anxiety. Using the startle reflex as a marker, further studies will evaluate the role of various anatomical structures in CRF enhanced startle. Initially, we will pick structures which a) have CRF receptors and are close to the ventricular surface and b) project to the amygdala. These will include the septal area and the bed nucleus of the stria terminalis. These areas will first be lesioned and then animals will be implanted with icv cannulas. After recovery, lesioned animals will be tested to see if icv CRF still elevates startle in these animals. If specific lesions prevent icv-CRF enhanced startle, then future studies will locally infuse CRF into these areas to see if CRF will elevate startle at lower doses and with a faster onset than that produced by icv CRF.

Because CRF markedly increases startle, it is possible that CRF may be released during a state of fear and thereby lead to an elevation of the startle reflex. We now have a great deal of data that startle is elevated by both conditioned and unconditioned fear (fear-potentiated startle). If these effects are mediated by a release of CRF, than icv or local infusion of the CRF antagonist ahCRF might block these effects, as suggested by a recent report (Swerdlow et al., 1989). Hence, a second goal of the upcoming year will be to test whether fear-potentiated startle will be blocked by icv infusion of ahCRF.

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Papers delivered relevant to this work:

1. Neural Systems Involved in Fear and Anxiety. Course in Neurobiology, Laboratory of Marine Biology, Woods Hole, Mass., June 27, 1990.

2. The Relative Roles of the Amygdala vs. the Hippocampus in Fear and Anxiety. NIMH Conference on Emotion, Mautauk Point, Long Island, New York, Sept. 22, 1990.

3. Neural Systems Involved in Fear and Anxiety. Department of Anatomy, University of Connecticut, Farminington, Ct., Oct. 10, 1990.

4. Neural Systems Involved in Fear and Anxiety. Special Invited Lecture, Society for Psychophysiology, Boston, Mass., Oct. 19, 1990.

5. Fear-potentiated startle in humans during anticipatory anxiety. With C. Grillon, R. Ameli, S.W. Woods, & K. Merikangas, Society for Neuroscience, St. Louis, MO., Oct. 29, 1990.

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13. The Role of NMDA Receptors in the Amygdala in Conditioned Fear. Invited symposium speaker, Conference on Learning and Memory, Park City, Utah, Dec. 14, 1991.

14. Neural Systems Involved in Fear and Anxiety, Department of Psychology, University of Florida, Gainsville, Florida, March 8, 1991.

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