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Co-Investigator: Louis van de Kar

Project Title: Amygdalo-hypothalamic Pathways Mediate Stress-induced ACTH Release

Abstract

The research goals of this project are to determine the anatomical pathways and neurotransmitters that participate in the neuroendocrine response to stressors. We have focused our study on the amygdala which is composed of a heterogeneous group of nuclei and is part of the limbic system. Studies are designed to determine which corticotropin releasing cells of the hypothalamus are innervated by the amygdala, to identify the neurotransmitters that influence corticotropin releasing cells, and to determine the role of the amygdala in release of ACTH and related "stress" hormones to different psychological and physical stressors. Studies have been completed that identify the anatomical connection between the amygdala and ACTH releasing cells of the hypothalamus. The results show that the central nucleus of the amygdala and the bed nucleus of the *stria terminalis are directly linked to cells which* control anterior pituitary release of ACTH and thus can modulate the release of corticosterone release from the adrenal glands. Studies have also been completed that demonstrate that destruction of cells in the central amygdala blunt the plasma increases corticosterone and renin which normally increase in response to stress. In addition, studies have demonstrated that central injections of thyrotropin releasing hormone (TRF) cause the release of ACTH, probably mediated through excitation of corticotropin releasing factor neurons of the hypothalamus. The ACTH releasing properties of TRF have not been reported previously and implicate an important role for TRF as a neurotransmitter controlling the release of CRF and the cascade of events that lead to corticosterone release during the stress response. Thus, these studies provide data demonstrating a direct connection from the amygdala to the hypothalamus, its functional importance and identify a potentially important neurotransmitter that can activate the hypothalamic pituitary response to stress.

Introduction

The most reliable indication that humans or other mammalian species have been stressed is a substantial measurable increase in plasma corticosterone levels. The final common brain pathway that mediates this response requires activation of hypothalamic cells which stimulate the release of ACTH from the anterior pituitary gland. The cells within the hypothalamus that modulate the release of ACTH have been relatively well characterized by their chemical content, morphology and projection to the pituitary portal system (Antoni, 1986; Makara et al., 1981; Swanson et al., 1983). The essential pathways and the neurotransmitters that influence hypothalamic ACTH-releasing cells need to be more clearly defined.

The limbic system has been characterized as an important modulator of psychological responses to stress. The limbic system functions as intermediate structure between regions of the brain that mediate sensory perceptions and neuroendocrine output. Damage to various structures within the limbic system reduces the amount of corticosterone released into the blood (Allen and Allen, 1974; Beaulieu et al., 1986; Beaulieu et al., 1987). This effect is thought to be because of interruption of both direct and indirect pathways to hypothalamic cells which increase the release of ACTH and ultimately corticosterone from the adrenal glands. Studies in this proposal have focussed on one part of the limbic system, the amygdala. Numerous previous studies have demonstrated that the amygdala is a particularly important in the mediation of neuroendocrine as well as cardiovascular responses to stress producing stimuli.

Methods

Anatomical Studies. The animal model studied in the present experiments is the rat. Anatomical tracing methods involve injection of a lectin, *Phaseolus vulgaris* leucoagglutinin lectin, into the amygdala where a discrete collection of cell bodies will "take up" the tracer and transport it down their axons. The lectin can be identified using standard immunohistochemical techniques using a reaction that labels cell bodies and their terminals brown. Initial experiments involved examining the distribution of the amygdaloid terminals within the regions of the hypothalamus (i.e., the paraventricular nucleus) that contained ACTH

releasing cells (CRF, vasopressin and oxytocin cells). The location of the terminals were correlated with the previously identified location of CRF, vasopressin and oxytocin cells. Recently, we developed a technique whereby the location of amygdaloid terminals are identified using the brown immunoperoxidase reaction and the ACTH releasing cells are marked using a blue glucose immunoreaction. This now allows to confirm that amygdaloid terminals directly contact chemically and functionally defined cells within the hypothalamus. The number of cells contacted by amygdaloid terminals and the releasing factor-hormone that they express can be determined.

Behavioral Studies. These experiments examine the effects of immobilization stress and "psychological" stress using the "conditioned emotional response" (CER) paradigm. The cell bodies within the amygdala are chemically destroyed using ibotenic acid. Immobilization stress is placing the animal within a plastic tube restraining device for 20 min following which blood samples are collected. The CER paradigm involves placing the animal within a chamber for 10 min after which a mild shock is delivered for 10 sec. This is done on three consecutive days. On the fourth day the animals are placed in the chamber for 10 min without shock and the blood samples are collected. Control animals are placed in the same chamber, but are never subjected to footshock. The plasma corticosterone, prolactin and renin levels of the animals are compared to non-lesioned and lesioned control animals. Corticosterone and renin are measured in Dr. van de Kar's laboratory. Prolactin levels are measured by Dr. C.L. Bethea, Department of Physiology, Oregon Regional Primate Center.

Results and Discussion

Anatomical Studies. Several studies have been completed. **First,** a study that characterizes the anatomical organization of the pathway from the amygdala to the paraventricular nucleus of the hypothalamus was recently published in Neuroendocrinology. The results of this study demonstrate that the amygdala directly innervates the posterior and anterior parvocellular regions of the paraventricular hypothalamic nucleus. The central amygdaloid nucleus projects to the posterior parvocellular subregions of the paraventricular nucleus where there are CRF cells that can influence pituitary release. **Second,** studies examining

which types of hypothalamic cells (i.e., corticotropin releasing factor, vasopressin and oxytocin) are innervated by the amygdala are being prepared for publication. The results demonstrate that the central amygdaloid axon terminals directly contact parvocellular CRF, vasopressin and oxytocin cells. Magnocellular CRF, vasopressin and oxytocin cells were not strongly innervated by the amygdala. Thus, this amygdalo-hypothalamic pathway is selectively linked to cells that have established ACTH releasing properties. This data is now being prepared for publication. **Third**, tracer has been injected within bed nucleus of the stria terminalis, a structure that is considered an extension of the amygdala. The bed nucleus of the stria terminals is strongly interconnected with the central amygdaloid nucleus. The results suggest that this region projects massively into the paraventricular nucleus of the hypothalamus. The bed nucleus of the stria terminalis heavily innervates both parvocellular and magnocellular parts of the paraventricular nucleus. Corticotropin releasing factor, vasopressin and oxytocin cells are innervated. Thus, the bed nucleus can affect both anterior and posterior pituitary function. The results of the anatomical studies are summarized in Figure 1.

Behavioral and Physiological Studies. **First**, experiments on the effects of amygdaloid lesions on neuroendocrine responses to psychological (i.e., immobilization or CER) stress are continuing in Dr. Van De Kar's laboratory. We are have completed studies on the effects of amygdaloid lesions on corticosterone, renin and prolactin responses to immobilization stress. Enclosed is bar graphs illustrating the effects of amygdaloid lesions on plasma corticosterone (Fig. 2), renin (Fig. 3) and prolactin (Fig. 4) levels in response to immobilization stress. The results can be summarized as follows: 1) Bilateral lesions of the central amygdaloid nucleus attenuates the corticosterone response to immobilization stress; 2) Lesions lateral and dorsal to the central amygdaloid nucleus have no effect upon this corticosterone response; 3) Unilateral lesions of the central amygdaloid nucleus cause a slight, but not significant, reduction in the corticosterone response to immobilization stress. Renin and prolactin levels were not affected by unilateral or bilateral lesions of the amygdala. **Second**, experiments on the effects of amygdaloid lesions on neuroendocrine response to CER stress are nearing

completion. This data has not been plotted graphically yet. However, lesions in the central amygdala, but not lateral or dorsal to it, attenuate the corticosterone response to CER stress. In addition, central amygdaloid nucleus lesions block plasma renin increases normally observed in the CER paradigm. We have not received the plasma prolactin data yet. Thus, the amygdala is involved in mediation of the renin response to stress in the CER paradigm, but not in response to immobilization stress. The immobilization paradigm is both a physically and psychologically stressful for the animal. The CER paradigm is primarily a psychological stressor. These preliminary results are interesting because it demonstrate that the amygdala is differentially participates in stress hormone expression as a function of the type of stressor. **Third,** studies have been conducted in collaboration with Marvin R. Brown, Dept. Medicine, University of California at San Diego. Thyrotropin releasing hormone (TRF) was injected into lateral ventricle of rats to see if TRF would increase plasma levels of ACTH. The results demonstrates that TRF increases plasma levels of ACTH. The TRF-induced ACTH response was attenuated by iv administration of the CRF antagonist alpha-helical CRF^{9-41}. Intravenous administration of CRF antisera totally blocked the TRF-induced ACTH release. Neither treatment blocked the increase of catecholamine levels in plasma that is was observed after TRF administration into brain. The results of the study suggest that thyrotropin releasing factor acts within the brain to stimulate CRF cells which in turn stimulate pituitary release of ACTH. This study was recently published in Endocrinology. Future studies will be conducted to determine if the amygdala is one of the sites of action for TRF-induced ACTH release.

Future Directions

The primary objectives of Year 3 are **1)** to determine which "neurotransmitter" candidates are contained with the cells that directly innervate hypothalamic CRF cells. **2)** to continue and complete studies on the effects of neurotoxic lesions of the amygdala and the bed nucleus of the stria terminalis on plasma release of corticosterone, prolactin and renin to stress. In addition, plasma levels of vasopressin and oxytocin will be measured.

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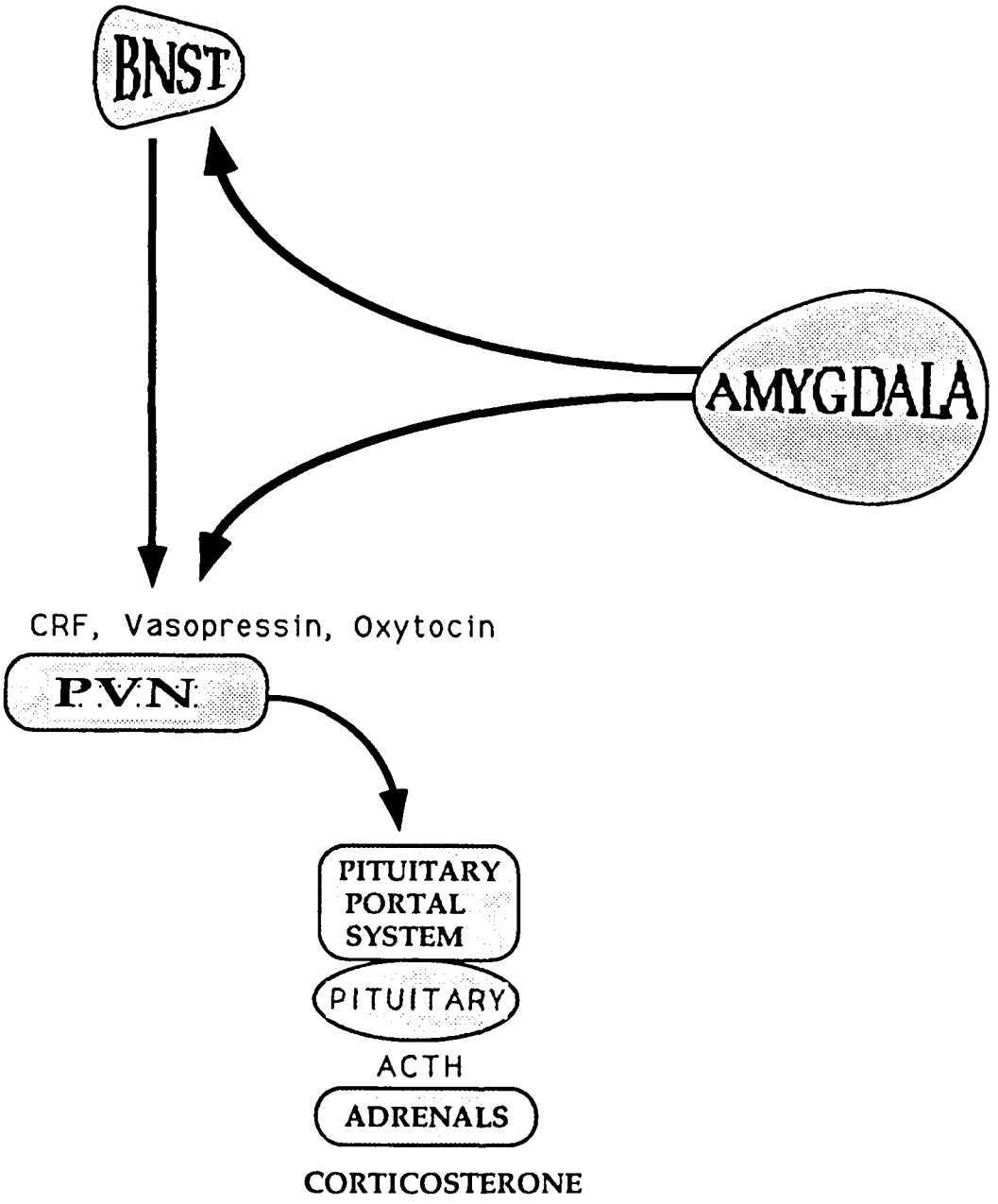
BNST
AMYGDALA
CRF, Vasopressin, Oxytocin
P.V.N.
PITUITARY PORTAL SYSTEM
PITUITARY
ACTH
ADRENALS
CORTICOSTERONE

Figure 1

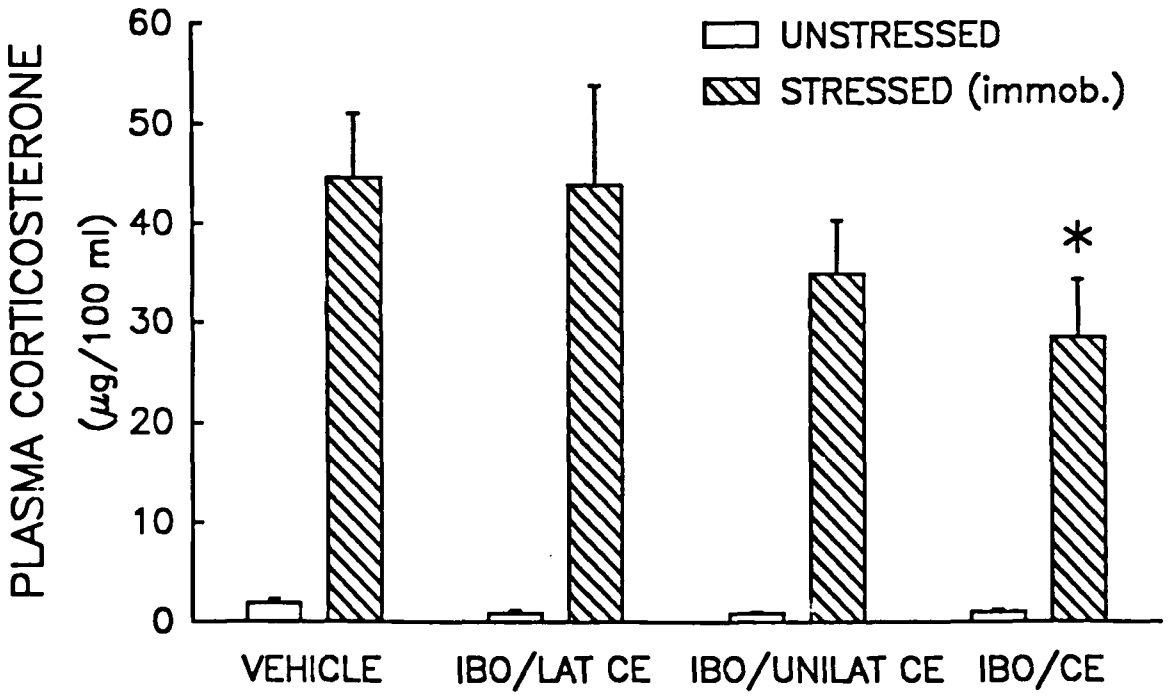
PLASMA CORTICOSTERONE
(μg/100 ml)
60
50
40
30
20
10
0
UNSTRESSED
STRESSED (immob.)
*
VEHICLE
IBO/LAT CE
IBO/UNILAT CE
IBO/CE

Figure 2

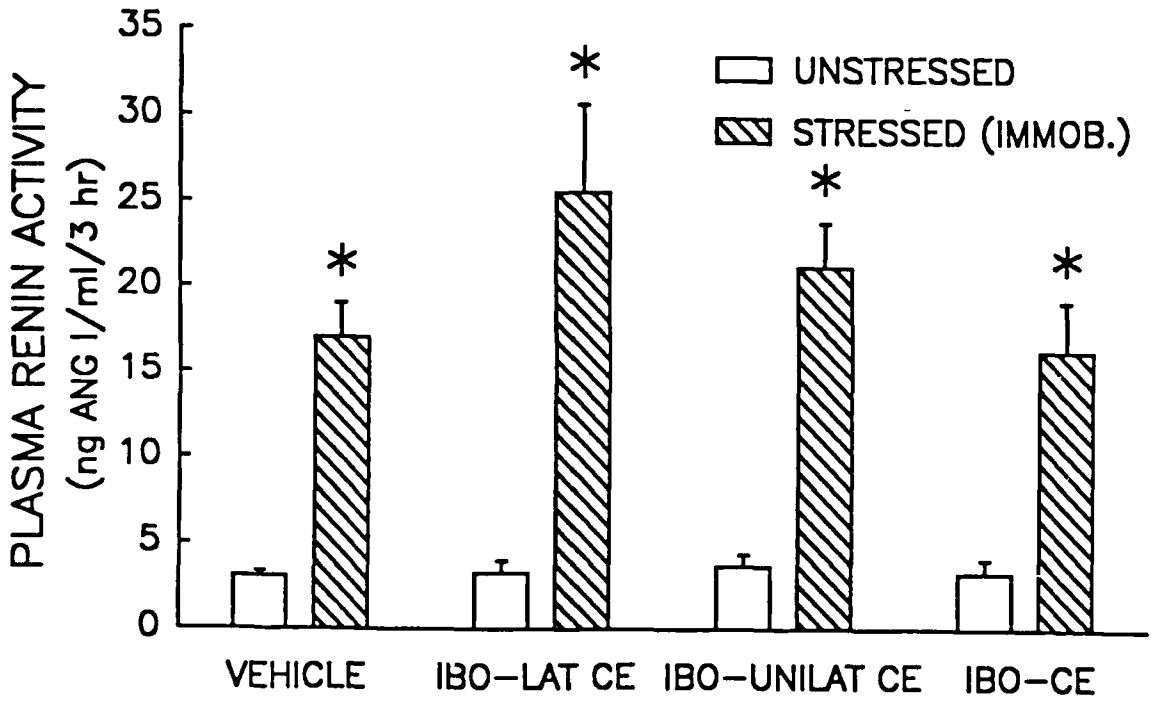
PLASMA RENIN ACTIVITY
(ng ANG I/ml/3 hr)
35
30
25
20
15
10
5
0
UNSTRESSED
STRESSED (IMMOB.)
*
*
*
*
VEHICLE
IBO–LAT CE
IBO–UNILAT CE
IBO–CE

Figure 3

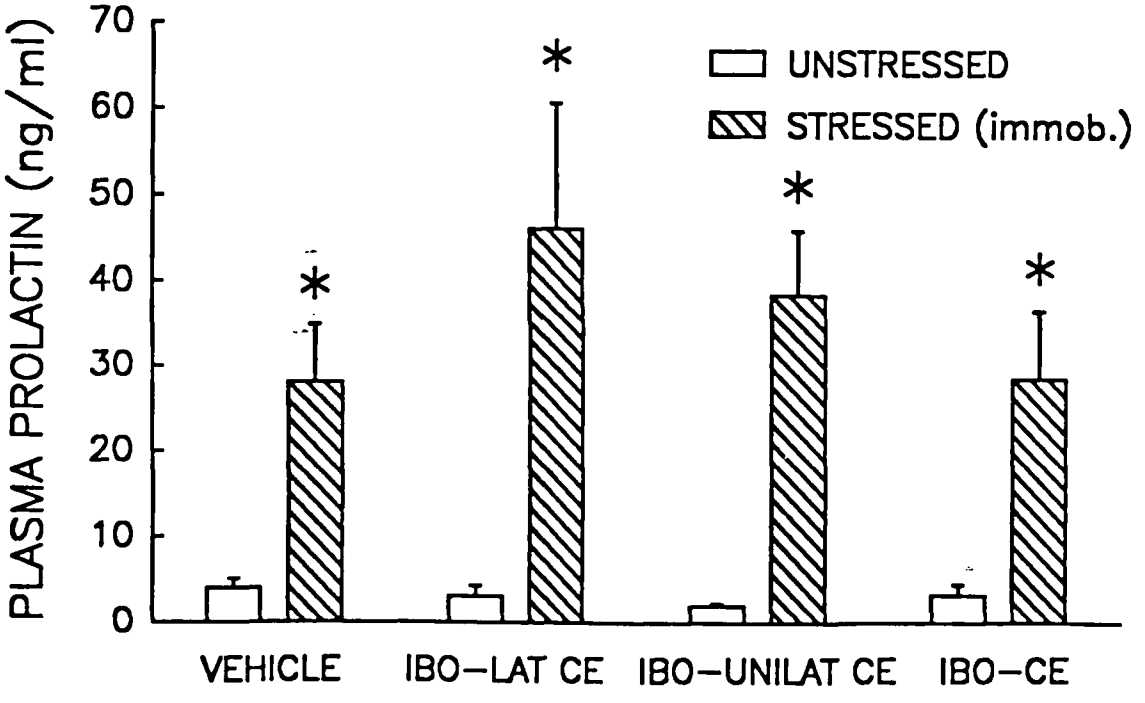
PLASMA PROLACTIN (ng/ml)
70
60
50
40
30
20
10
0
UNSTRESSED
STRESSED (immob.)
*
*
*
*
VEHICLE
IBO–LAT CE
IBO–UNILAT CE
IBO–CE

Figure 4

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