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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 940228	3. REPORT TYPE AND DATES COVERED ~~Interim~~ Technical 01Feb93-31Jan94 Annual

4. TITLE AND SUBTITLE	5. FUNDING NUMBERS
Cerebral Neurochemical Mechanisms in Stress and Anxiety	Grant F49620-93-1-0125 DEF 61102F 2312 BS

6. AUTHOR(S)
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9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSORING/MONITORING AGENCY REPORT NUMBER
Air Force Office of Scientific Research (AFMC) Bolling Air Force Base, DC 20332-6448 Dr. Haddad	2312/BS

94-18977

DTIC ELECTE JUN 21 1994 S B

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT	12b. DISTRIBUTION CODE
Approved for public release; distribution unlimited.	Approved for public release. Distribution: Unlimited

94 6

13. ABSTRACT (Maximum 200 words)
Investigations are concerned with the cerebral mechanisms involved in stress. Current experiments focused on the locus coeruleus noradrenergic (LC-NE) system. *In vivo* microdialysis studies showed that both hemodynamic stress induced by nitroprusside, and electric footshock increased the apparent release of norepinephrine (NE) in the hypothalamus and prefrontal cortex. The potential role of corticotropin-releasing factor (CRF) in the activation of the LC-NE system was investigated. CRF infused into the LC, but not in surrounding brain structures (such as the parabrachial nucleus), increased the apparent synaptic release of cortical NE. This effect was largely unilateral, and appears to involve CRF-receptors. We have performed preliminary studies using the new technique of *in vivo* voltammetry. These studies have confirmed the increased appearance of extracellular NE following nitroprusside infusion. The superior time resolution of this technique indicated that the NE response to nitroprusside was short-lived. The classic benzodiazepine anxiolytic, chlordiazepoxide (CDP), appeared to diminish the NE response to footshock and may also affect basal NE release.

Behavioral studies indicated that activation of NE system with idazoxan almost completely inhibited stress-induced ultrasonic vocalization, with relatively small changes in stress-induced freezing. We failed to find any consistent effects of 6-hydroxydopamine-induced lesions of the dorsal noradrenergic bundle, although vocalization was slightly potentiated.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Stress Anxiety Norepinephrine Microdialysis Benzodiazepine Voltammetry Behavior	5 + 39 Enclosures
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	

NSN 7540-01-280-5500

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CEREBRAL NEUROCHEMICAL MECHANISMS IN STRESS AND ANXIETY

First Annual Technical Report - February 1, 1993 - January 31, 1994

A. Several experiments using *in vivo* microdialysis in rats were completed:

1. Sodium nitroprusside infusions activate cortical and hypothalamic noradrenergic systems in rats.

To demonstrate the suitability of *in vivo* microdialysis for studies of cerebral neurochemical mechanisms in stress, we examined the norepinephrine (NE) response to acute hypotension induced by intravenous infusion of sodium nitroprusside. Nitroprusside is known from electrophysiological data to activate the locus coeruleus (LC) noradrenergic neurons. In anesthetized rats such treatment enhanced the apparent release of NE in two LC projection fields, namely the medial prefrontal cortex (PFM) and medial hypothalamus. The increase in extracellular NE in hypothalamus (67%) was greater than that in the prefrontal cortex (41%). The increase in extracellular NE in both regions outlasted the decrease in blood pressure. It is concluded that microdialysis is sensitive enough to monitor physiologically induced changes in the activity of the cerebral NE system. The observed activation of the cortical NE system reflects activation of the LC-NE neurons and may be responsible for the behavioral responses in stress. The hypothalamic NE system is activated as a part of a larger system responsible for the regulation of blood pressure, and its activation may reflect an autonomic component of the organismic response to stress. The data were published in *Neuroscience Research Communications* and a poster was presented at the 1993 Annual Meeting of the Society for Neuroscience (copies attached).

2. Corticotropin-releasing factor administered into the locus coeruleus but not the parabrachial nucleus stimulates norepinephrine release in the prefrontal cortex.

Previous studies indicated that intracerebroventricular application of corticotropin-releasing factor (CRF) activated noradrenergic neurons in the brainstem LC and NE metabolism in several brain regions. To assess whether CRF has direct effects on LC noradrenergic neurons, CRF was infused into the LC and concentrations of NE and its metabolites were measured in microdialysates collected from the PFM. Infusion of 100 ng of CRF into the LC significantly increased dialysate concentrations of NE and of its catabolite, MHPG, in the ipsilateral PFM, whereas no significant changes were observed following infusion of artificial CSF. No response

was observed when the infusions of CRF occurred outside of the LC, including those in the parabrachial nucleus. Although CRF administered into the LC slightly increased dialysate concentrations of NE in the contralateral PFM, this effect was not statistically significant. The effect of CRF injected into the LC on dialysate NE was prevented by combination with a ten-fold excess of the CRF antagonist, alpha-helical CRF_{9-41}, indicating some specificity in the response. These results are consistent with anatomical and electrophysiological evidence suggesting that CRF may directly activate noradrenergic neurons in or close to the LC. Submitted for publication in *Brain Research Bulletin* and to be presented at the 1994 Annual Meeting of the Society for Neuroscience (copies attached).

3. Chlordiazepoxide attenuates overflow of extracellular NE in prefrontal cortex caused by CRF infused into LC.

In the previous experiment, we demonstrated that in anesthetized rats administration of CRF into LC increased the concentration of NE in microdialysates collected from the prefrontal cortex. Those results were replicated in the current experiment in which we tested the effect of a benzodiazepine on the noradrenergic response. Preliminary results indicated that the response could be attenuated by pretreatment with peripherally administered chlordiazepoxide (CDP) (Figure 1 attached).

4. Effects of repeated footshock on overflow of hypothalamic norepinephrine in freely moving rats.

Footshock augments the release of catecholamines in the hypothalamus. This study examined the effects of repeated footshock on release of hypothalamic NE. Rats were subjected to two 20-minute sessions of footshock (60×0.1-0.2 mA) separated by 100 min and the NE concentrations were measured in microdialysates collected over subsequent 20 min periods. After the first footshock session the concentration of NE in the dialysate samples was augmented by 64% ($p < 0.01$) in comparison with baseline. After the second footshock session the increase reached 313% of baseline ($p < 0.05$) of the baseline preceding the first stress session, and 251% ($p < 0.05$) of the value immediately preceding the second footshock session. It is concluded that the NE response to footshock may be potentiated by a previous period of footshock.

A manuscript is in preparation and a figure is enclosed (Figure 2).

5. Effects of chlordiazepoxide on stress-induced overflow of cerebral norepinephrine.

Synaptic release of cerebral catecholamines is increased during stress. Benzodiazepines, such as CDP are the most commonly used anxiolytic drugs. The effects of the benzodiazepines on stress-related release of catecholamines have been controversial. We examined the effect of CDP pretreatment on the footshock-induced release of cerebral NE. Freely moving rats were implanted with microdialysis probes in the medial hypothalamus and the medial prefrontal cortex (PFM). Footshock (60 x 0.1-0.2 mA shocks in 20 min) significantly increased microdialysate concentrations of NE in the first sample collected after initiation of footshock. In the hypothalamus, dialysate NE was augmented by 50% of baseline, and in the PFM by 143%. CDP administration (5 mg/kg ip) had no statistically significant effects on the basal dialysate concentrations of NE, although there was a tendency towards a reduction. CDP administered one sample before the footshock attenuated the dialysate concentrations of NE in footshock animals. These results suggested that footshock increased the synaptic release of NE in the cortex and hypothalamus, and that the response could be attenuated by CDP. The experimental design used could not distinguish whether CDP altered the input to LC noradrenergic neurons, or whether the benzodiazepines exerted a direct effect on the noradrenergic neurons. Experiments involving local cortical administration of CDP are in progress.

A manuscript is in preparation and a poster will be presented at the 1994 Society for Neuroscience Meeting (abstract enclosed).

6. In *vivo* voltammetry: Effects of sodium nitroprusside and idazoxan on catecholaminergic activity

Experiments like these described in Section 1 above but using in *vivo* voltammetry were initiated. With recording electrode in the PFM we observed that intravenous infusion of sodium nitroprusside triggered a short-lasting (< 200 sec) increase in an oxidation current with characteristics like those of NE. Local administration of idazoxan produced a similar result, suggesting that the recorded oxidation current was related to release of NE. The voltammetric experiments confirm the results of the microdialysis study and suggest that nitroprusside activates the noradrenergic system for a rather short period of time.

Examples of two voltammetrograms are enclosed (Figures 3 and 4).

B. Behavioral studies using several animal models of anxiety were initiated:

7. Effects of lesions of the dorsal noradrenergic ascending bundle (DNAB) on shock-induced freezing and vocalization in rats.

Adult rats received bilateral infusions of 6-OHDA into the dorsal noradrenergic ascending bundle (DNAB) that resulted in almost complete depletion of the cortical and hippocampal NE. The following behavioral patterns were observed after the lesions: 1. Air-puff-induced freezing and ultrasonic vocalization (nonpainful); 2. Electric footshock-induced freezing and vocalization (painful); 3. Conditioned freezing and vocalization (nonpainful); 4. Exploratory behavior in elevated plus-maze; 5 Withdrawal behavior and exploration in an open field provided with a refuge. The above behavioral patterns are sensitive to a variety of anxiolytic and anxiogenic compounds that may interact with the brain noradrenergic system. However, we did not find any significant effects of the DNAB lesions on the observed behaviors. Ultrasonic vocalization was potentiated in the DNAB rats but not to an extent that would suggest any significant role of the ascending NE system in this response. The study will be repeated.

A poster was presented at the 1993 Annual Meeting of the Society for Neuroscience (copy attached).

8. Effects of idazoxan on stress-induced freezing and ultrasonic vocalization.

The effects of idazoxan (1.25, 2.5 and 5 mg/kg) on stress-induced freezing and ultrasonic vocalization were studied. Preliminary results indicated that activation of NE system by idazoxan resulted in almost complete inhibition of stress-induced vocalization, and relatively small changes in stress-induced freezing. Although unexpected, the results agreed with our previous experiments in which it had been demonstrated that depletion of the dorsal noradrenergic system with a neurotoxin DSP-4 dramatically enhanced stress-induced vocalization.

Publications

1. Smagin, G. A., Swiergiel A. H., Dunn A. J. Sodium nitroprusside infusions activate cortical and hypothalamic noradrenergic systems in rats. *Neuroscience Research Communications* 14: 85-91 (1994).

2. Smagin, G. A., Swiergiel A. H., Dunn A. J. Corticotropin-releasing factor administered into the locus coeruleus but not the parabrachial nucleus stimulates norepinephrine release in the prefrontal cortex. *Brain Research Bulletin* (submitted for publication).

3. Smagin, G. A., Swiergiel A. H., Brown R. D., Dunn A. J. Activation of norepinephrine release in cortex and hypothalamus by nitroprusside infusions. *Society for Neuroscience Abstracts* 19: 1451 (1993).

4. Swiergiel A. H., Dunn A. J. Effects of lesions of the dorsal noradrenergic ascending bundle on shock-induced freezing and vocalization in rats. *Society for Neuroscience Abstracts* 19: 1244 (1993).

5. Smagin, G. A., Swiergiel A. H., Guerin G,. Dunn A. J. Corticotropin-releasing factor infused into the locus coeruleus increases norepinephrine release in medial prefrontal cortex. *Society for Neuroscience Abstracts* (1994).

6. Swiergiel A. H., Wei, Z., Li, Y., Dunn A. J. 1994. Effects of chlordiazepoxide (CDP) on footshock-induced overflow of cerebral norepinephrine. *Society for Neuroscience Abstracts* (1994).
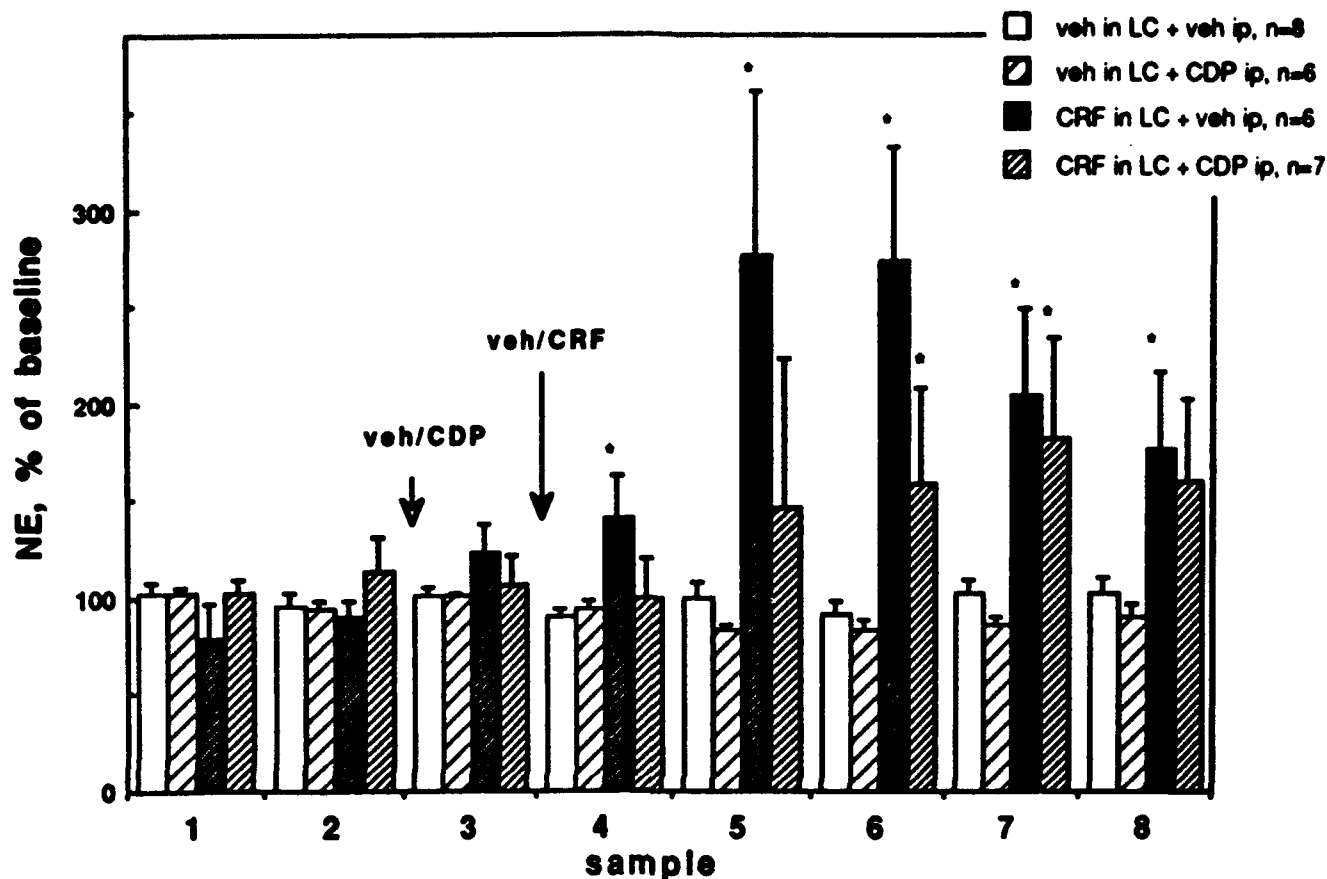
Figure 1. Efffects of chlordiazepoxide (CDP, 5 mg/kg ip,) on release of NE in frontal cortex caused by injection of corticotropin-releasing factor (CRF, 100 ng) into the locus coeruleus.

The mean value of the control samples (open bars) preceding the first treatment was used to define the baseline (I.e. 100%). The remaining pre-, and post-treatment values were then expressed and analyzed as percentages of this baseline value.

*) Different from baseline, p < 0.05.
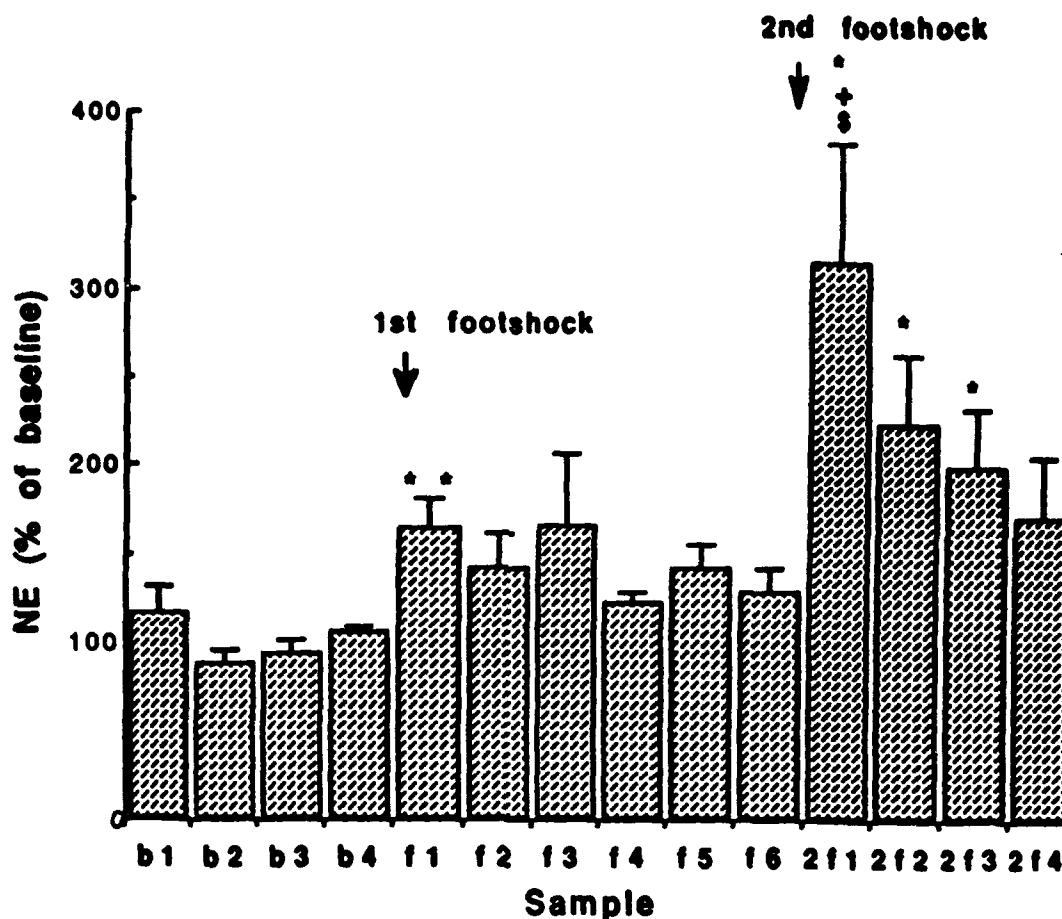
Figure 2. Effects of repeated footshock on concentration of NE in microdialysates collected from the paraventricular nucleus of the hypothalamus.

The mean value of the four samples immediately preceding the first footshock session was used to define the baseline (i.e. 100%, average of baseline samples b1 - b4).

The pre-, and post-shock values were then expressed and analyzed as percentages of this baseline value.

*; ** Different from baseline (average of samples b1-b4); $p < 0.05$; 0.01, respectively;

+ Different from sample f6; $p < 0.05$;

$ Different from sample f1; $p < 0.05$.

Figure 3. Effect of intravenous infusion of sodium nitroprusside on NE-like oxidation current recorded from a single carbon fiber electrode place in the prefrontal medial cortex of a rat. The infusion began at time 0 sec and lasted for 600 sec. Oxidation current began to increase at 260 sec, reached a peak at 325 sec, and returned to baseline at 455 sec.

Figure 4. Effect of local microinfusion of idazoxan ($1\mu M/2\mu l/min$) on NE-like oxidation current recorded from a single carbon fiber electrode place in the prefrontal medial cortex of a rat. Fused silica microtubing used to make the infusion was glued alongside the recording electrode. The infusion began at time 0 sec and lasted for 35 min. Oxidation current began to increase at 400 sec, reached a plateau at 500 sec, and began to return to baseline at 2100 sec (end of infusion).

FIGURE 3.

RELEASE #1
AMPLITUDE   2.267
SIGNAL/NOISE   0.11492
RED/OX RATIO   0.17
HYSTERESIS   0 Min 0 Secs
RISE TIME   5 Min 32 Secs
T 1/2   6 Min 36 Secs
TIME COURSE   0 Min 0 Secs

17: 44: 45

NOREPINEPHRINE-LIKE OXIDATION CURRENT

INFUSION OF SODIUM NITROPRUSSIDE (30μg/30μl/min)

TIME (Seconds)

MICROMOLAR CONC.

2.00
1.50
1.00
0.50
0.00

0   65   130   195   260   325   390   455   520   585

RAT 3VT-2

NAME

FIGURE 4.

RELEASE #2
AMPLITUDE   13.061
SIGNAL/NOISE   0.14069
RED/OX RATIO   0.98
HYSTERESIS   0 Min 0 Secs
RISE TIME   9 Min 6 Secs
T 1/2   68 Min 44 Secs
TIME COURSE   0 Min 0 Secs

14: 24: 28

NOREPINEPHRINE-LIKE OXIDATION CURRENT

INTRACORTICAL INFUSION OF IDAZOXAN

MICROMOLAR CONC.

TIME (Seconds)

NAME

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1st topic title: Stress
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2nd theme title: Neurotransmitters   theme letter: D

2nd topic title: Catecholamines
topic number: 61

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EFFECTS OF CHLORDIAZEPOXIDE ON FOOTSHOCK-INDUCED OVERFLOW OF CEREBRAL NOREPINEPHRINE.
Artur H. Swiergiel*, Zhongyou Wei, Yaohui Li and Adrian J. Dunn. Department of Pharmacology, Louisiana State Univ. Med. Ctr., Shreveport, LA 71130-3932.

Synaptic release of cerebral catecholamines is increased during stress. The effects of benzodiazepines on the stress-related release of catecholamines has been varied. We have used *in vivo* microdialysis to examine the effect of chlordiazepoxide (CDP) pretreatment on the footshock-induced release of norepinephrine (NE). Freely moving rats were implanted with microdialysis probes in the medial hypothalamus and the medial prefrontal cortex (PFM). Footshock (60 x 0.1-0.2 mA shocks over 20 min) significantly increased microdialysate concentrations of NE in the first sample collected. The subsequent two samples showed small elevations that were not statistically significant. In both the hypothalamus and the PFM dialysates, NE was significantly augmented over the prefootshock baseline. CDP administration (5 mg/kg ip) had no statistically significant effects on the basal dialysate concentrations of NE, although there was a tendency towards a reduction. CDP administered before the footshock significantly attenuated the dialysate concentrations of NE.

These results suggest that footshock increases the synaptic release of NE in the cortex and hypothalamus, and that this response is attenuated by CDP. The experimental design used cannot determine whether systemic CDP alters the input to LC noradrenergic neurons, or whether benzodiazepines exert a direct effect on the noradrenergic neurons.

Supported by grants from the Air Force of Scientific Research.

KEY WORDS: (see instructions p. 4)

1. Benzodiazepine
2.
3.
4.

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2nd theme title: Neural Basis of Behavior _____ theme letter: I

2nd topic title: Monoamines and Behavior _____ topic number: 129

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None

ACTIVATION OF NOREPINEPHRINE RELEASE IN CORTEX AND HYPOTHALAMUS BY NITROPRUSSIDE INFUSIONS.

Gennady N. Smagin, Artur H. Swiergiel, R. Don Brown*, Adrian J. Dunn. Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Intravenous infusion of sodium nitroprusside (NP) decreases blood pressure and results in a hemodynamic stress that accelerates the firing rate of noradrenergic neurons in the locus coeruleus (LC). We tested whether NP increased the release of norepinephrine (NE) in the medial prefrontal cortex (PFM) and medial hypothalamus (MHT) as measured by microdialysis and HPLC. Rats were anesthetized with pentobarbital and urethane and microdialysis probes were inserted into the PFM and/or the MHT. NP infused into the femoral vein at a dose of 10 μg/30 μl/min for 15 min rapidly decreased blood pressure by 48%. During NP infusion the concentrations of NE in the cortical and hypothalamic microdialysates were elevated by 41% ($p < .001$) and 67% ($p < .05$) of the pre-infusion value, respectively. After infusion, PFM NE rebounded to 127% (ns) and MHT NE to 133% ($p < .05$). MHT MHPG increased by 51% ($p < .05$) but PFM MHPG and concentrations of DOPAC, 5-HIAA and HVA from PFM or MHT were not significantly affected by the infusion.

We conclude that hemodynamic stress activates NE neurons projecting to the PFM and MHT. We presume that the increase in cortical NE release is due to activation of the LC and may reflect a behavioral aspect of the stress. The hypothalamic response may be involved in the autonomic regulation of blood pressure.

KEY WORDS: (see instructions p. 4)

1. Locus Coeruleus
2. Sodium Nitroprusside
3. Hypotension
4. _____

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1st topic title Monoamines and Behavior topic number: 129

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EFFECTS OF LESIONS OF THE DORSAL NORADRENERGIC ASCENDING BUNDLE ON SHOCK-INDUCED FREEZING AND VOCALIZATION IN RATS.

Artur H. Swiergiel* and Adrian J. Dunn.
Dept. of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Previously, we reported that lesioning of the noradrenergic (NE) neurons in the locus coeruleus (LC) with the neurotoxin DSP-4 potentiated the duration of footshock-induced freezing and increased ultrasonic vocalization. Conditioned freezing and vocalization were not affected. Because peripherally administered DSP-4 lesions both ascending and descending LC projections, we studied 6-OHDA lesions of the dorsal noradrenergic ascending bundle. Footshock-induced freezing and vocalization and conditioned freezing and vocalization were observed 96 and 120 hr, respectively, after the lesions, and in another group of rats 6 weeks after the lesions. There were no significant changes in behavioral patterns in rats observed shortly after the lesions. However, 6 weeks after the lesions, footshock-induced vocalization was potentiated by 292%. The lesions did not alter the responses evoked by re-exposure to the cages in which footshock was administered. Thus vocalization was affected by painful stimuli (footshock), but not by nonpainful (conditioned) stimuli. The LC-NE system is considered to play an important role in mediating behavioral responses observed in animal models of fear and anxiety. These results suggest that LC-NE system may play a role in stress induced vocalization, perhaps by modifying sensitivity to pain during footshock.

KEY WORDS: (see instructions p. 4)

1. Locus Coeruleus
2. Anxiety
3. Fear
4.

Society for Neuroscience member's signature   A.H. Swiergiel   (318) 674-7869
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1st topic title: Neuropeptides and Behavior topic number 29

2nd theme title: Neural Basis of Behavior theme letter: I
2nd topic title: Stress   topic number: 126

Special Requests (e.g., projection/video requirements)

CORTICOTROPIN-RELEASING FACTOR INFUSED INTO THE LOCUS COERULEUS INCREASES NOREPINEPHRINE RELEASE IN MEDIAL PREFRONTAL CORTEX. Gennady N. Smagin, Artur H. Swiergiel, Glenn Guerin* and Adrian J. Dunn. Dept Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Previous studies have indicated that intracerebroventricular administration of corticotropin-releasing factor (CRF) increases the firing rate of noradrenergic neurons in the brain stem locus coeruleus (LC) and metabolites of norepinephrine (NE) metabolism in terminal regions in the forebrain, as well as extracellular concentrations of NE. To assess the possibility of direct effects of CRF in the LC, 100 ng of CRF was infused into the LC and concentrations of NE and metabolites were measured in microdialysates collected from ipsilateral or contralateral medial prefrontal cortex (PFM) with a 22 min sampling interval. Infusion of CRF significantly increased concentrations of NE in dialysates from the ipsilateral PFM in the first three postinfusion samples (26, 82 and 54% of baseline, respectively). No changes were observed with infusions of aCSF, or when CRF infusions occurred outside the LC - in the parabrachial nucleus, the tegmental area, the fourth ventricle or the cerebellum. Although dialysate concentrations of NE were slightly increased in the contralateral PFM, this effect was not statistically significant. The effect of CRF on NE release was prevented by a simultaneous infusion into the LC of 1 μg of the CRF antagonist, alpha-helical CRF_{9-41}. These results are consistent with anatomical and electrophysiological evidence suggesting that CRF may directly activate noradrenergic neurons in or close to the LC, affecting cortical NE secretion and behavior.

Supported by grants from NINDS and the Air Force AFOSR.

KEY WORDS: (see instructions p. 4)

1. Microdialysis
2. Parabrachial Nucleus
3.
4.

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Telephone number

SODIUM NITROPRUSSIDE INFUSIONS ACTIVATE CORTICAL AND HYPOTHALAMIC NORADRENERGIC SYSTEMS IN RATS

Gennady N. Smagin, Artur H. Swiergiel and Adrian J. Dunn

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SUMMARY

Sodium nitroprusside (NP) infusions cause hypotension and accelerate the firing rate of locus coeruleus (LC) noradrenergic neurons. NP was infused into anesthetized rats implanted with microdialysis probes in the medial prefrontal cortex and medial hypothalamus. The infusion decreased blood pressure by 42%. During the 15 min infusion, the norepinephrine (NE) concentration in the cortical dialysate was elevated by 41%, and in the hypothalamic dialysate by 67%. After infusion, the cortical dialysate NE concentration promptly returned to the pre-infusion value, whereas the hypothalamic concentration remained elevated for 45 min. Dialysate concentrations of dopamine and its catabolites, and of the serotonin catabolite, 5-HIAA, were not significantly altered, suggesting a selective response of NE neurons. Increased release of NE in the cortex probably reflects activation of LC-NE neurons. However, because the hypothalamus receives only a small input from the LC, the larger changes in this region suggest the activation of other brain stem sources of noradrenergic projections.

KEY WORDS: cortex; hypothalamus; microdialysis; nitroprusside;
              norepinephrine; locus coeruleus

INTRODUCTION

Intravenous infusions of sodium nitroprusside (NP) produce vasodilation and an acute decrease in blood pressure. Using a voltammetric method Bhaskaran and Freed (2) observed that the resulting hemodynamic stress altered the activity of locus coeruleus (LC) neurons. It has recently been demonstrated that the fall in blood

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pressure produced by NP infusions increases the rate of spontaneous electrical discharge of the LC noradrenergic neurons (LC-NE) in both conscious and anesthetized rats (4, 17, 20). As the largest noradrenergic center projecting to the forebrain, the LC may mediate behavioral and autonomic responses in stress (12). Because the LC is the main source of norepinephrine (NE) innervation of the cortex (18), LC activation is likely to be reflected in changes in cortical noradrenergic activity. However, it is not known whether an increase in the rate of firing of the LC-NE neurons caused by a physiological stressor alters NE release in the terminal fields.

We studied whether hemodynamic stress that is known to activate LC-NE neurons alters extracellular concentrations of catecholamines in the medial frontal cortex and medial hypothalamus as determined by *in vivo* microdialysis.

MATERIALS AND METHODS

Animals and surgery. Male Sprague-Dawley rats purchased from Harlan Inc., Indianapolis, and weighing 300-350 g at the time of the experiment were used. The rats were anesthetized with sodium pentobarbital (25 mg/kg i.p.) immediately followed by urethane dissolved in distilled water (800 mg/kg i.p.). Anesthesia usually lasted for more than 12 hours. On rare occasions, after 4-5 hours, some rats that appeared to become vigilant received an additional 12 mg/kg of pentobarbital. The animals were placed in a stereotaxic apparatus with the incisor bar raised + 5.0 mm. A microdialysis probe was lowered into the medial prefrontal cortex using the following coordinates: AP: + 3.6 mm; L ± 1.6 mm; V: 5.0 mm below skull surface, tilted medially at an angle of 15°. A second probe was inserted into the medial hypothalamus in the approximate region of the paraventricular nucleus (PVN) at: AP: +0.2 mm; L ± 0.6 mm; V: 10 mm below skull surface. A catheter connected to a blood pressure transducer was inserted into the femoral artery and a second catheter connected to a syringe filled with either physiological saline or NP was inserted into the femoral vein.

Microdialysis and HPLC catecholamine assays. Concentric design microdialysis probes were constructed in our laboratory from an outer 26-ga stainless steel tubing and an inner fused silica tubing. The uninterrupted silica tubing was long enough to reach the collection vials and its dead volume did not exceed 3 µL. The dialysis membrane tube 250 µm in diameter with a cut-off molecular weight of 6,000 (Spectra/Por® Fiber) was glued to the outer steel tubing and sealed at the tip. The active length of the dialysis membrane was 4.0 mm for the cortical probes and 3.0 mm for the hypothalamic ones. The probes were perfused with artificial cerebrospinal fluid (aCSF: 1.2 mM CaCl₂, 1.2 mM Na₂HPO₄, 0.3 mM NaH₂PO₄, 3.4 mM KCl, 140 mM NaCl, adjusted to pH 7.2) at a flow rate of 2.2 µl/min. Microdialysate samples were collected every 15 min into microvials containing 50 µl of 0.1 M HClO₄ and 1.25 mg of NMDA (N-methyldopamine) as internal standard. The samples were analysed for NE, MHPG (3-methoxy,4-hydroxyphenylethyleneglycol), DA (dopamine), DOPAC (3,4-dihydroxyphenylacetic acid), HVA (homovanillic acid) and 5-HIAA (5-hydroxy-

indoleacetic acid) using the HPLC system previously described (11). Relative *in vitro* recovery of compounds by microdialysis probes was determined as described previously and exceeded 10% for all analysed compounds (11). Because the diffusion of neurotransmitters from within brain tissue is likely to be different from that in saline, we did not correct our *in vivo* data for these *in vitro* recoveries.

Experimental procedure. After insertion, the probes were perfused for 2 h and the microdialysate discarded. Five pretreatment samples were then collected. Sodium NP was then infused into the femoral vein at a dose of 10 µg in 30 µl/min for 15 min (a total dose of 150 µg) and the microdialysate samples were collected for the next two hours. Mean arterial blood pressure was recorded throughout the whole experiment. Preliminary experiments indicated that control infusions of physiological saline had no effect whatsoever on the observed parameters. Therefore, all changes observed during and after the NP infusions were related to the pre-infusion values and not to the control physiological saline infusions.

Data analysis. Only data obtained from animals with the microdialysis probes placed within the medial prefrontal cortex or the medial hypothalamus, close to the PVN, were included. The mean value of the three samples immediately preceding the NP infusion defined the baseline value (i.e. 100%). The pre- and post-infusion values were then expressed and analysed as percentages of the baseline value. Analysis of variance was used to determine whether there were significant changes in dialysate concentrations of compounds over time. Dunnett's *t*-tests were used *post-hoc* to determine the statistical significance of any changes in relation to the baseline value.

RESULTS

Prior to the NP infusion, the rats maintained stable mean arterial blood pressure and heart rate (Fig. 1A). After initiation of the infusion the blood pressure decreased to 58% of the pre-infusion value (p < 0.001) by the second minute and remained at that level throughout NP infusion. When infusion was terminated, there was a brief overshoot to 120% of the pre-infusion blood pressure, followed by a slow return to the pre-infusion pressure.

Dialysis of medial prefrontal cortex.

Analysis of variance revealed that the infusion of sodium nitroprusside significantly altered dialysate concentrations of NE (F(9,54) = 5.0, p < 0.001). During the infusion the NE concentration in the dialysate increased to 141 ± 13% (p < 0.001) of the pre-infusion value (Fig. 1B). At the end of the infusion, the NE concentration returned to a value not significantly different from the pre-infusion value. Concentrations of MHPG, DOPAC, HVA and 5-HIAA in the microdialysate samples collected during the infusions were 120 ± 20, 114 ± 10, 107 ± 10 and 129 ± 20%, respectively, of the pre-infusion values (not significantly different from baseline).
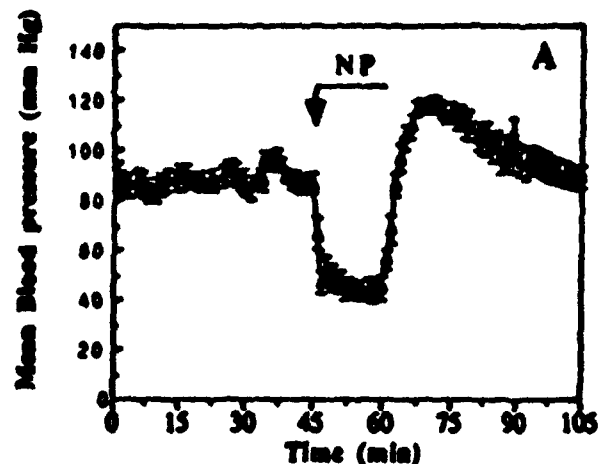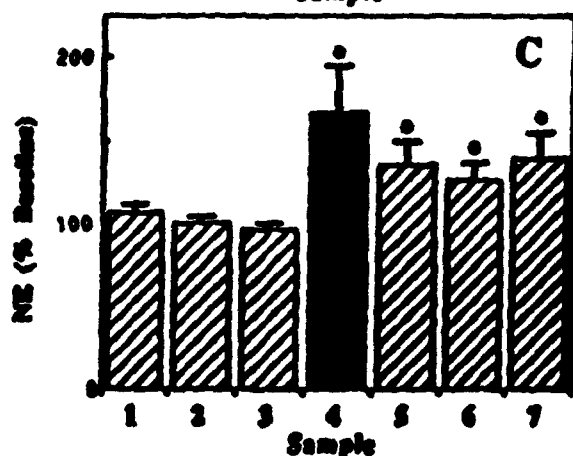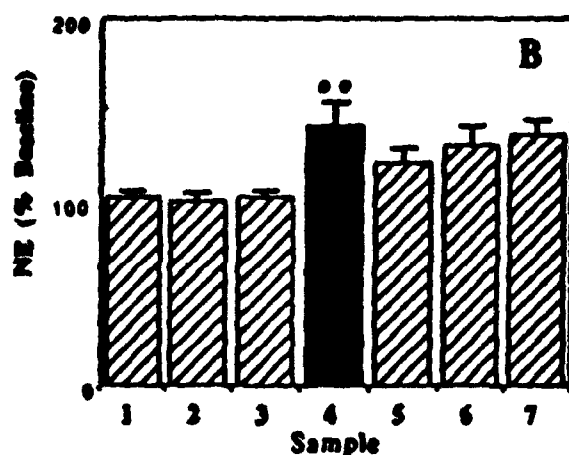
Fig. 1.
Effect of i.v. infusion of sodium nitroprusside (10 μg/30 μl/min for 15 min) on:

A. Mean arterial blood pressure (mm Hg); n=10;

B. Concentration of NE in microdialysate samples collected from medial prefrontal cortex; n=10;

C. Concentration of NE in microdialysate samples from hypothalamus; n=8.

Data are presented as the mean ± SEM of the percentage change from the baseline. The mean values of NE in the three samples preceding sodium nitroprusside infusion were taken as 100 %.
Beginning and duration of the sodium nitroprusside infusion is indicated by an arrow. Solid bars represent samples collected during the infusion. Sampling time was 15 min.
**P<0.001; *P<0.01; (ANOVA followed by Dunnett's t-test).

Dialysis of medial hypothalamus.

Infusion of NP significantly altered the concentrations of NE in the hypothalamic microdialysates ($F_{(7,42)}$ = 3.5, $p < 0.01$). The infusion elevated the concentration of NE to 167 ± 28% ($p < 0.01$) of the pre-infusion value (Fig. 1C). When the infusion was terminated, concentrations of NE decreased, but remained above the pre-infusion level ($p < 0.01$) for the next 3 sampling periods (45 min), after which they were not significantly different from baseline. Hypothalamic MHPG increased to 151 ± 27% ($p < 0.05$). Concentrations of DOPAC, HVA and 5-HIAA in dialysates collected during the infusions were 95 ± 10, 130 ± 19 and 124 ± 14%, respectively, of the pre-infusion values (not significantly different from baseline).

DISCUSSION

These results demonstrate that intravenous infusion of sodium nitroprusside activates noradrenergic systems in the medial prefrontal cortex and the medial hypothalamus. Increased dialysate concentrations of NE indicate elevated extracellular concentrations of NE which most probably derive from increased release. The finding that the increased concentrations of NE were accompanied by elevated concentrations of MHPG also suggests an increased NE release (9). Concentrations of DA and its catabolites, and the serotonin catabolite, 5-HIAA were not significantly altered, suggesting that the effect of NP was selective for noradrenergic neurons.

There are good reasons to believe that the observed noradrenergic responses are mediated, at least in part, via LC noradrenergic neurons. The LC is the sole source of NE innervation in the frontal cortex (18). It has been demonstrated that electrical stimulation of LC activates cortical noradrenergic systems (10). The hypothalamus receives noradrenergic projections from A1, A2 and A6 brain stem areas. The major innervation is from the A2 cell group (15), so the changes we observed most likely result from activation of this region. However, LC neurons receive cardiovascular-related afferent information (5) and project to cardiovascular regulatory areas of hypothalamus. Zhou, Zou and Ku (22) showed that injection of glutamate into the LC evoked a pressor response. This pressor response decreased after a brain transection caudal to the PVN, but was unchanged when the transection was rostral to the PVN. The LC-pressor response could also be attenuated by preinjection of phentolamine, propranolol, or atropine into the rostral ventrolateral medulla. These results suggest that the LC-pressor response is mediated by the medulla, and by the PVN. Moreover, it has been observed that electrical stimulation of LC increases release of NE in the hypothalamus (8). LC may thus provide a small, but perhaps functionally important, part of the NE hypothalamic innervation (14). This innervation may contribute to the

noradrenergic response observed in the present experiment. Changes in the electrochemical activity of the LC neurons have been observed in response to NP- and phenylephrine-induced alterations in blood pressure (2, 16). It has also been observed that experimentally induced changes in blood pressure were associated with changes in the firing rate of the LC neurons (6, 20). Most important, Valentino and co-workers (2, 20) demonstrated that injections of NP identical to those used in the present experiment consistently increased discharge rate of LC neurons.

What is the functional significance of the observed changes? It is possible that activation of the cortical noradrenergic system reflects arousal commonly observed during stress. In conscious rats, arousal correlates well with LC activation (7) and this would support the possibility that the LC mediates behavioral responses during stress. It has also been reported that LC activation elicits electroencephalographic (EEG) signs of arousal recorded from the frontal cortex in anesthetized rats (1, 20). These findings suggest that LC activation may play some role in maintaining arousal during cardiovascular challenge.

The LC innervates brain structures that are involved in the control of cardiovascular function. These include the cardiovasculatory regulatory areas of the hypothalamus and brainstem (the nucleus of the solitary tract, the nucleus ambiguus, the dorsal motor nuclei of the vagus) (see 15, 16). Several studies have shown that changes in blood pressure are associated with changes in the activity of the LC, suggesting that the LC may function as a sensory nucleus responding to hemodynamic changes (19). An intriguing possibility is that the prefrontal cortex participates in the regulation of cardiovascular function. Zbrozyna and Westwood (21) demonstrated that stimulation of the prefrontal cortex inhibited conditioned increases in blood pressure. The authors suggested that the prefrontal cortex plays an essential role in suppression of cardiovascular and behavioral changes activated by fear or aggression.

There are no reports that acute hypotension results in arousal, and in human subjects, administration of clonidine, resulting in a decrease in arterial pressure and heart rate, produces a significant reduction in anxiety (3). It has also been reported that the onset of LC activation by NP was temporally correlated with changes in EEG activity of the frontal cortex (20). The present data suggest a very similar time course for release of NE from cortical terminals, suggesting a close relationship between the electrical activity of LC-NE neurons, NE release, and the EEG changes. It is not possible to know whether the observed activation of the hypothalamic noradrenergic system is mediated by the rather minor contribution from the LC, by the ventral noradrenergic bundle originating mainly from the A1 and A2 cell groups, or by an intrinsic hypothalamic system participating in the maintenance of blood pressure homeostasis.

We conclude that experimentally induced hypotension causes increases in the concentration of NE in dialysates from the medial prefrontal cortex and medial hypothalamus, most probably reflecting increased NE release. This activation of the cortical noradrenergic system reflects activation of the LC-NE neurons and may be responsible for the behavioral responses in stress. The hypothalamic NE system is activated as a part of a larger system responsible for the regulation of blood pressure, and its activation may reflect an autonomic component of the organismic response to stress.

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Corticotropin-Releasing Factor Administered into the Locus Coeruleus but not the Parabrachial Nucleus Stimulates Norepinephrine Release in the Prefrontal Cortex.

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Running title: CRF and Locus coeruleus

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ABSTRACT

SMAGIN, G. N., A. H. SWIERGIEL AND A. J. DUNN. *Corticotropin-releasing factor administered into the locus coeruleus but not the parabrachial nucleus stimulates norepinephrine release in the prefrontal cortex.* BRAIN RES BULL xxx 1994. - Previous studies have indicated that intracerebroventricular application of corticotropin-releasing factor (CRF) activates noradrenergic neurons in the brain stem locus coeruleus (LC) and norepinephrine (NE) metabolism in several brain regions. To assess whether CRF has direct effects on LC noradrenergic neurons, CRF was infused into the LC and concentrations of NE and its metabolites were measured in microdialysates collected from the medial prefrontal cortex (PFM). Infusion of 100 ng of CRF into the LC significantly increased dialysate concentrations of NE and of its catabolite MHPG in the ipsilateral PFM, whereas no significant changes were observed following infusion of artificial CSF. No response was observed when the infusions of CRF occurred outside of the LC, including those in the parabrachial nucleus. Although CRF administered into the LC slightly increased dialysate concentrations of NE in the contralateral PFM, this effect was not statistically significant. The effect of CRF injected into the LC on dialysate NE was prevented by combination with a ten-fold excess of the CRF antagonist, alpha-helical CRF_{9-41}, indicating some specificity in the response. These results are consistent with anatomical and electrophysiological evidence suggesting that CRF may directly activate noradrenergic neurons in or close to the LC.

Keywords: norepinephrine   microdialysis   prefrontal cortex
corticotropin-releasing factor   locus coeruleus   parabrachial nucleus

INTRODUCTION

Many studies have demonstrated increased norepinephrine (NE) metabolism associated with stressful treatments (12,27). This increased metabolism has been attributed to increased synaptic release of NE, consistent with *in vivo* microdialysis studies that have indicated increased extracellular concentrations of NE associated with stress (3,6,21,24). Similar increases in NE metabolism have been observed following intracerebroventricular (icv) administration of corticotropin-releasing factor (CRF: (1,7,11,13,17). This has also been supported by microdialysis studies in which icv CRF increased dialysate concentrations of NE collected from the medial prefrontal cortex and the hypothalamus (14,15).

Because the noradrenergic innervation of the frontal cortex originates from the brain stem locus coeruleus (LC) the changes in NE metabolism likely result from increased activity of the LC neurons. Electrophysiological studies have indicated that LC noradrenergic neurons are activated during stress (2). Such activation also occurs following icv administration of CRF (29). Immunohistochemical studies have indicated a close apposition between CRF-containing neurons and noradrenergic neurons in the LC (25,30). Consistent with a functional significance for this anatomical arrangement, it has been reported that local application of CRF in the region of the LC also activates the noradrenergic neurons electrophysiologically (29).

In a recent study we observed that intravenous infusion of sodium nitroprusside augmented concentrations of NE in dialysates collected from the medial prefrontal cortex and medial hypothalamus (26). Nitroprusside is known to increase the rate of discharge of LC noradrenergic neurons, presumably because of the consequent acute hypotension (32). A relationship between CRF and noradrenergic neurons may be functional during hypotension because

application of the CRF antagonist, alpha-helical CRF$_{9-41}$ (ahCRF), directly into the LC prevented the increase in LC firing due to nitroprusside (31). ahCRF injected into the LC also prevented the increase in tyrosine hydroxylase activity caused by a paradigm involving intermittent foot shock and noise (18).

In the present study we examined the hypothesis that CRF-receptors located in or close to the LC or the parabrachial nucleus (PBN) mediate changes in the activity of LC noradrenergic neurons. CRF was infused directly into the LC or the PBN and the concentrations of NE and its catabolites in microdialysates from the medial prefrontal cortex were determined.

MATERIALS AND METHODS

Materials

Corticotropin-releasing factor (CRF) and the CRF antagonist, alpha-helical CRF$_{9-41}$ (ahCRF) were gifts from Dr. Jean Rivier (Peptide Laboratory, The Salk Institute, San Diego, Ca).

Animals

Male Sprague-Dawley rats purchased from Harlan Sprague Dawley (Houston, TX). weighed between 250 and 350 g at the time of experiment. For at least one week prior to an experiment the rats were housed singly in plastic cages with ad libitum access to Purina rat chow and tap water. A 12 h light:12 h dark cycle with light on at 07:30 was maintained.

Stereotaxic surgery and infusion procedure

Rats were anesthetized with sodium pentobarbital (25 mg/kg ip) followed by urethane (dissolved in water, 0.8 g/kg ip). They were placed in a stereotaxic frame with the incisor bar raised 5.0 mm. Tips of microdialysis probes were placed in the medial prefrontal cortex (PFM) using the following coordinates: AP = +3.6 mm from bregma; L = ±1.7 mm with the arm tilted 15° towards the

midline, and DV = 5.0 mm below the skull surface. A hole was drilled in the skull above the LC for later infusions. An injector was made from 33 ga fused silica tubing (OD 0.175mm: Polymicro Technologies, Phoenix, AZ) inserted into 27 ga stainless steel tubing and attached to an arm of the stereotaxic apparatus. The fused silica tubing protruded 2 mm from the stainless steel tubing and was connected at its other end to a 5 µl Hamilton syringe driven by an infusion pump (Razel Scientific Instruments, Stamford, CT). The injector tip was aimed at the dorsal aspect of the LC using the following coordinates: AP = -2.0 mm from the interaural line; L = ±1.1 mm from the midline; and DV = 8.0 mm below the skull surface. For the PBN, the same coordinates were used, except that L was ±2.0 mm. These procedures were approved by the Louisiana State University Medical Center Animal Care Committee and conform to National Institutes of Health guidelines.

Microdialysis and HPLC catecholamine assays.

Concentric design microdialysis probes were constructed from an outer 26-ga stainless steel tubing and an inner fused silica tubing. The uninterrupted silica tubing was sufficiently long to reach the collection vials and its dead volume did not exceed 3 µl. The dialysis membrane tube, 250 µm in diameter with a cut-off molecular weight of 6,000 (Spectra/Por® Fiber: Spectron Medical, Los Angeles, CA), was glued to the outer steel tubing and sealed at the tip. The active length of the dialysis membrane was 4.0 mm. The probes were perfused with artificial cerebrospinal fluid (aCSF: 1.2 mM $CaCl_2$, 1.2 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, 3.4 mM KCl, 140 mM NaCl adjusted to pH 7.2) at a flow rate of 2.25 µl/min using a CMA/100 Microinjection pump. Microdialysate samples were collected by a CMA/140 Microfraction collector every 22 min directly into microvials containing 50 µl of 0.1 M $HClO_4$ and 800 pg of N-methyldopamine (NMDA) as internal standard.

The samples were analyzed for NE, 3-methoxy,4-hydroxyphenylethyleneglycol (MHPG), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) using an HPLC system consisted of a Waters M-6000A Pump, and WISP 712 refrigerated autoinjector (Waters Associates Inc.), a Spherisorb ODS1, 25 cm 5 μm reverse phase column (Keystone Scientific, Inc., Bellefonte, PA), a Bioanalytical Systems (BAS, West Lafayette, IN), LC-4B electrochemical detector with an Antec VT-03-300 electrode (Antec BV, Leiden, The Netherlands) and LC-22A temperature controller (BAS). The working electrode was set at +0.78 V with respect to a Ag/AgCl reference electrode. The mobile phase contained 0.1 M NaH_2PO_4, 0.1 mM EDTA, 0.3 mM octanesulfonic acid and 3.5% acetonitrile, pH 3.1. The flow rate was maintained at 0.9 ml/min. Minor changes of pH, octanesulfonic acid concentration and flow rate were made to obtain optimal separations.

Relative *in vitro* recovery of compounds by microdialysis probes was determined as described previously (15) and exceeded 10% for all analyzed compounds. Because the diffusion of neurotransmitters from within brain tissue is likely to be different from *in vitro* conditions, *in vivo* data were not corrected for *in vitro* recoveries.

Experimental procedure, drugs and dosage.

After insertion, the probes were perfused for 2 h and the microdialysate discarded. Five pretreatment samples were then collected. 200 nl of aCSF, CRF (100 ng in aCSF) or CRF-ahCRF mixture (100 ng of CRF and 1 μg of ahCRF in aCSF) was injected into the LC over 2 min. Sample collection was continued during the next 2 hours (6 samples). The samples were frozen soon after collection and stored at -70°C until analyzed.

RESULTS

Effect of the CRF injection.

In the first series of animals, microdialysis probes were inserted into the right medial prefrontal cortex (PFM) and the CRF or aCSF injection was aimed at the LC on the same side. The histological analysis indicated that the injector tip was located properly within the LC in eight rats that received injections of CRF, and eight that received injections of aCSF (see example in Figure 1). Ten further animals received injections of CRF above the LC, either in the cerebellum (eight) or the fourth ventricle (two); two were in the dorsal tegmental area; and one was in the locus subcoeruleus. Figure 2A shows the changes in the concentrations of NE in the dialysate over time from animals that had injector placements in the LC. Administration of 100 ng of CRF increased the dialysate NE concentration by 26% of baseline (p < 0.05) in the first sample after injection, with a maximum increase of 82% in the second postinjection sample (p < 0.02). The NE concentration was no longer significantly different from baseline by the fourth postinjection sample, corresponding to 60-80 min after injection. Injection of aCSF into the LC did not alter the dialysate concentrations of NE at any time during the sampling period. Six of the eight animals injected with CRF exhibited increases in NE of 20% or more in the first three samples (i.e. 1 hour) following injection, whereas none of the animals receiving aCSF showed such a response.

CRF administration into the LC also significantly increased the dialysate MHPG concentration (34%; p < 0.05) immediately after injection (Figure 2B). The concentrations of the other catecholamines and metabolites measured (DOPAC, HVA and 5-HIAA) were not significantly altered (data not shown).

In animals in which histological analysis indicated that injections were outside the LC region, we did not observe any significant changes in NE or

MHPG concentrations in dialysates from PFM. Figure 3 shows the results obtained from eight animals that received injections of 100 ng CRF in which the injector tip was located in the cerebellum. Increases of 20% were observed in only one of the first three samples following injection in two of the eight rats. No such increases were observed in the animals that had injector placements in the tegmentum or locus subcoeruleus. In one rat, the injection cannula passed through the LC and its tip was located just dorsal of the facial nerve. This animal showed a large increase in dialysate NE in the first sample after CRF injection, but not in subsequent samples. Because the parabrachial nucleus (PBN) contains receptors for CRF (10) and is a major terminal region for CRF neurons from the amygdala (19), we tested animals with injection cannulae aimed at the PBN. Figure 3 also shows the results from eight animals injected with 100 ng CRF in which the injector tip was located in the parabrachial nucleus. None of these animals showed any increase in dialysate NE following CRF injections. In two animals the injector tip was located in the fourth ventricle. These animals showed small sustained increases in dialysate NE, similar to those observed in our previous study (15).

In a second series of rats, microdialysis probes were inserted bilaterally into the PFM and samples were collected simultaneously from the PFM on both sides of the brain. CRF was injected into the LC on one side only. CRF injection was followed by a significant increase in NE concentration in microdialysis samples collected from ipsilateral PFM (Figure 4), with a maximum in the first sample postinjection (45%; $p < 0.05$). CRF injection slightly increased the dialysate concentrations of NE in the contralateral PFM, but this effect was not statistically significant (Figure 4). MHPG concentrations were also elevated in dialysates from the ipsilateral, but not the contralateral PFM (data not shown).

Effect of CRF and ahCRF injection

In a third group of animals, a mixture of CRF and ahCRF was injected into the LC and microdialysis samples were collected from the ipsilateral PFM. The amount of ahCRF chosen was based on the results of Rivier (23) which indicated that approximately a 10-fold excess of ahCRF was required to prevent the pituitary responses to CRF, and on previous experiments in which it was found that 100-200 ng of the peptide injected into the LC was effective in blocking the stress-related changes in tyrosine hydroxylase (18) or shock-induced freezing behavior (28). Figure 5 shows the results of this experiment. Significant increases in dialysate NE were observed in the first four samples (31%, 56%, 30% and 34%, respectively) following CRF injection, but injection of the CRF-ahCRF mixture had no statistically significant effect on dialysate concentrations of NE or MHPG (data for MHPG not shown).

DISCUSSION

Microdialysis studies permit estimations of extracellular concentrations of NE. Such measurements reflect the net "overflow" of released neurotransmitters, i.e. the amount released, less that taken up by the releasing neuron and other cells in the region. Although such extracellular concentrations of NE are an indirect estimate of release, studies with drugs that inhibit action potentials (e.g., tetrodotoxin), Ca^{2+} chelating agents, and neurotransmitter re-uptake inhibitors indicate that dialysate concentrations of catecholamines generally reflect synaptic release (5,8). At the very least, changes in extracellular concentrations are likely to reflect changes in synaptic release.

The results of the present experiments demonstrate that CRF infused directly into the LC increases the concentration of NE and its catabolite MHPG in microdialysates collected from the ipsilateral medial prefrontal cortex. This finding extends earlier data indicating the ability of icv CRF to increase dialysate

concentrations of NE in cortex and hypothalamus (14,15). It also complements the finding that injection of CRF into the LC elevates amygdaloid concentrations of the NE catabolite, dihydroxyphenylglycol (7), and depletes hippocampal and amygdaloid concentrations of NE (9). Two findings attest to the specificity of this response: first no equivalent response was observed following infusion of artificial CSF into the LC, indicating that the increased dialysate NE was not merely a nonspecific response to the injection. Second, CRF was ineffective when it was mixed with a ten-fold excess of the CRF-receptor antagonist, alpha-helical CRF$_{9-41}$.

There are good reasons to believe that the CRF acted on receptors localized within the LC region. CRF infusions within the LC were effective, whereas none of the injection sites outside the LC was effective. It is particularly interesting that injections into the parabrachial nucleus were not effective, because this region appears to contain CRF receptors (10) and the terminals of the major descending CRF projection from the central amygdaloid nucleus (19). Moreover, CRF injected into the PBN has been shown to be behaviorally active (1,16). Thus our results suggest that the behavioral effects of CRF injected into the PBN are not mediated via LC noradrenergic neurons, which is consistent with the lack of direct connections between PBN and LC (4). Second, the dose of CRF injected into the LC was considerably more potent than the same dose injected into the lateral cerebral ventricles (15). Thirdly, an increase in dialysate NE concentration was observed in the first 22-min period following CRF administration into the LC, with a peak within 40 min, whereas, when the CRF was administered into the lateral cerebral ventricle, a small response in NE occurred in the first 22-min collection period, but the peak response did not occur until 1-1.5 h. Such a pattern is consistent with the hypothesis that icv CRF produced its effects after reaching the LC. The results suggest that the CRF

receptors located in or close to the LC can activate the noradrenergic neurons that project to the prefrontal cortex. However, our results do not indicate whether the CRF-receptors are on the noradrenergic LC neurons or on other interneurons in or close to the LC.

The possibility that CRF can act directly or indirectly on noradrenergic neurons within the LC is consistent with anatomical data indicating the presence of CRF-receptors in this region (10), and with an apparent apposition between CRF-containing terminals and LC noradrenergic neurons (25, for a full discussion, see Ref. 30). It is also consistent with electrophysiological data suggesting that local application of CRF within the LC increases the firing rate of noradrenergic cell bodies in the LC (29). Thus our results provide additional evidence that CRF terminals in the region of the LC can increase the activity of LC noradrenergic neurons.

It is well documented that exposure to stressful stimuli results in changes in noradrenergic activity in the cortex (12,27). Microdialysis studies suggest that increased NE release occurs (3,6,24). Thus a further question is whether or not CRF mediates the activation of LC noradrenergic neurons observed during stress. Valentino has shown that local injection of ahCRF into the LC can prevent the activation of LC noradrenergic neurons that normally occurs in respond to nitroprusside infusion, but this did not occur when LC neurons were activated by sciatic nerve stimulation (31,32). In unpublished data, we have failed to find any effect of icv ahCRF (25 µg) on the noradrenergic responses to footshock in mice. Although in these experiments, the ahCRF was injected icv, and not locally in the LC, this suggests that whether or not CRF mediates the noradrenergic response may depend on the form of stress, consistent with the results of Valentino (32). Behavioral results also suggest a role for CRF in the LC region in modulating stress-induced behavioral changes. Administration of CRF (100 ng) into the LC

induced defensive withdrawal in rats, and injections in this location were more potent than icv (7). Bilateral injections of CRF (50 or 100ng) into the LC immediately after training enhanced retention one day later (9). Moreover, bilateral injections of ahCRF into the LC (100 ng per side) were able to attenuate shock-induced freezing (28), and defensive withdrawal (1.25 µg per side) in naive animals (Gorman & Dunn, unpublished observations).

The observed laterality of the noradrenergic response in the cortex supports the conclusion of a discrete, rather than a diffuse, site of action for CRF in the brain stem. It is interesting that there appeared to be some response in the contralateral PFM, even though this effect was not statistically significant in our experiments. Such a response could be due to the small percentage of noradrenergic fibers that decussate on their way to the cortex (20), or it could imply that there are local connections within the brain stem, which may serve to coordinate the activations of the two loci coerulei.

In summary, our results suggest that local application of CRF in the region of the brain stem locus coeruleus increases synaptic release of NE in the cortex. This result is consistent with anatomical, electrophysiological and behavioral data suggesting a functional interaction between CRF-containing terminals in the brain stem and noradrenergic neurons in the LC.

Acknowledgements

Figure Captions

Figure 1. A cresyl violet-stained coronal section of the brain at the level of the locus coeruleus. There is damage at the dorsolateral aspect of the LC indicating the location of the tip of the injection cannula (arrow).

Figure 2. The effect of aCSF (open bars) or 100 ng CRF (solid bars) ipsilateral injection into the LC on the concentrations of NE (A) and MHPG (B) in dialysates from PFM. The first three samples before injection (indicated with an arrow) defined the baseline for each animal. Each sample represents 20 min collection interval. Each group contained 8 animals. Data are presented as the mean ± SEM of the percentage change from baseline.

* Significantly different from the baseline ($p < 0.05$, ANOVA followed by Student's t test).

Figure 3. Effect of CRF injection (100 ng) on NE concentrations in microdialysates from PFM. Animals were from the same group as in the experiment of Figure 2, but histological analysis indicated that the injector tip was located outside the LC. Upper figure shows the results from eight animals in which the injection was aimed at the PBN. The lower figure indicates the results from eight animals in which the injector tip was located in the cerebellum.

Fig.4. Effect of CRF injection (100 ng) into LC on NE concentrations in dialysates collected from PFM ipsilateral (solid bars) and contralateral (striped bars) to the LC injection. Procedures as in the experiment of Figure 2. *Significantly different from the baseline ($p < 0.05$, ANOVA followed by Student's t-test).

Fig.5. Effect of ipsilateral injection of CRF (dark bars) or CRF and ahCRF mixture (lined bars) on NE concentrations in dialysate samples collected from PFM. Injection time indicated by the arrow. The CRF injected animals (n=16) are the combined data from the animals shown in figures 2 and 4. Seven rats were injected with the CRF-ahCRF mixture. *Significantly different from the baseline ($p < 0.05$, ANOVA followed by Student's t-test).

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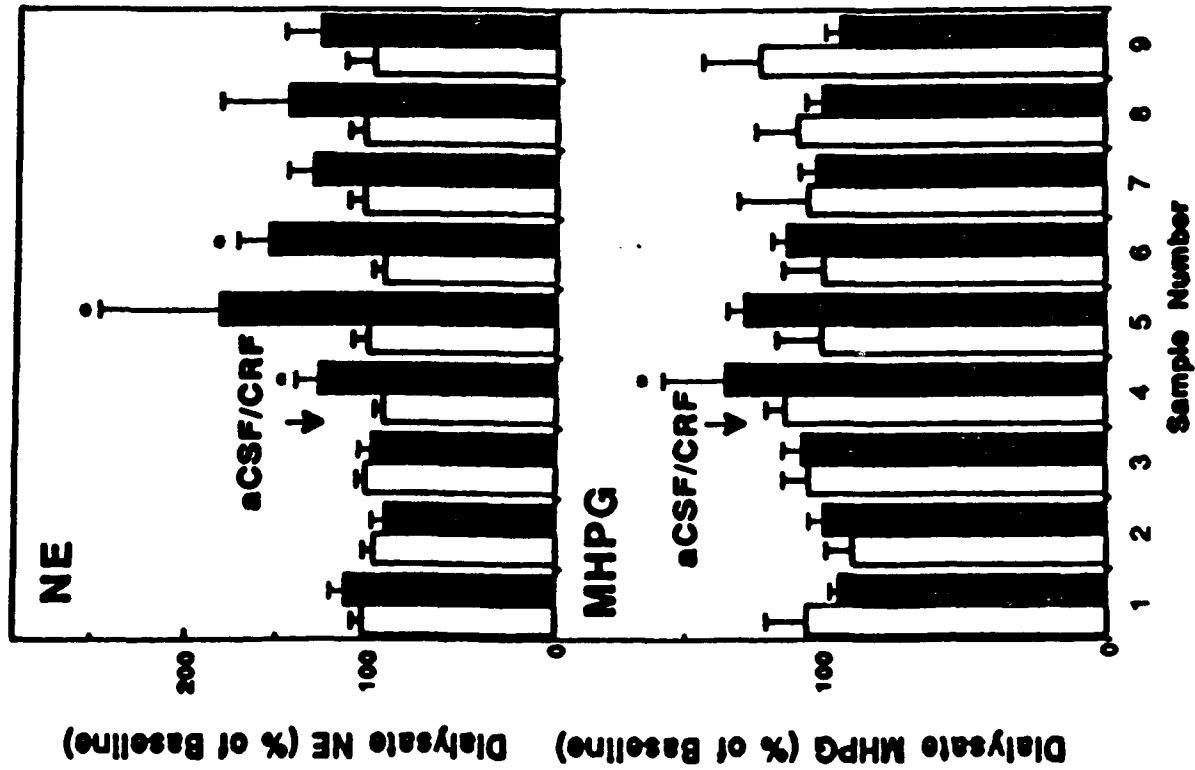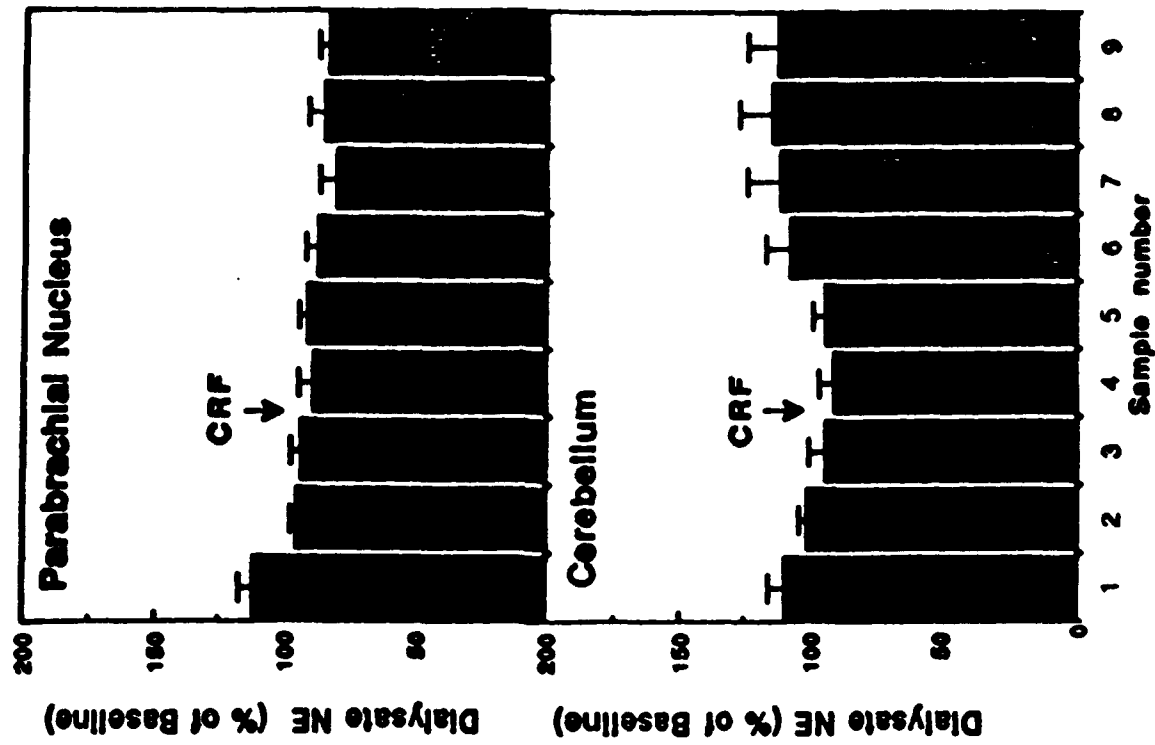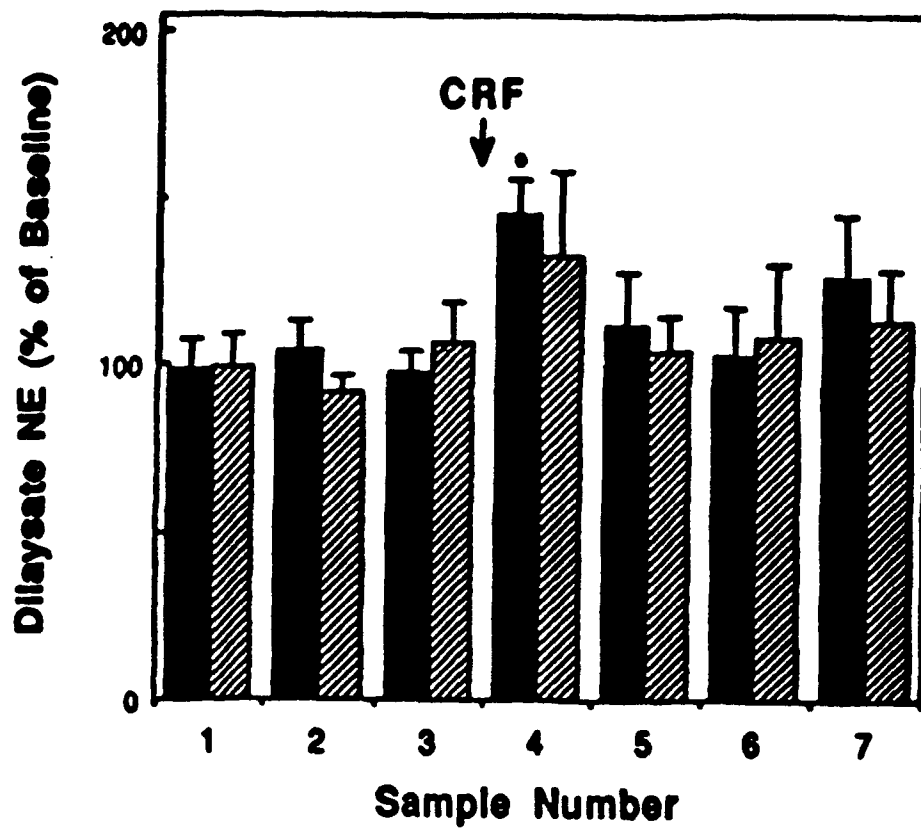
Figure 1

Figure 3

Figure 2

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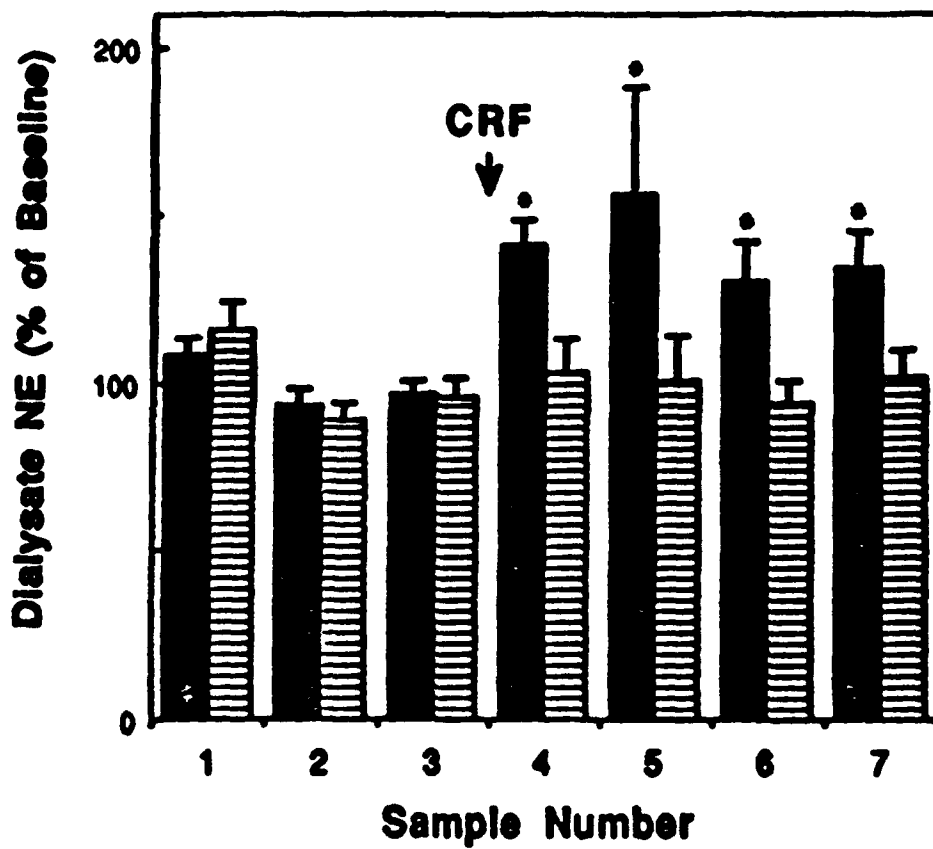

Figure 5