ELSEVIER

Brain Research 640 (1994) 105–112

BRAIN RESEARCH

Research Report

Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus

Shinya Makino, Phillip W. Gold, Jay Schulkin *

Behavioral Neuroscience Unit, Clinical Neuroendocrinology Branch, National Institute of Mental Health, Bldg. 10, #3S-231, 9000 Rockville Pike, Bethesda, MD 20892, USA

(Accepted 9 November 1993)

Abstract

Using in situ hybridization histochemistry, we report differential expression of corticotropin-releasing hormone (CRH) mRNA in the central nucleus of the amygdala (CEA) and the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN) following systemic treatment with corticosterone (CORT) in adrenally-intact rats. Both injection of low (1 mg/kg/day) and high (5 mg/day) CORT reduced CRH mRNA expression in the PVN in a dose-dependent manner, although it returned to normal at the low dose by 14 days. By contrast, the high dose of CORT increased CRH mRNA transiently in the CEA at 4 days, although the low dose of CORT decreased it at 14 days. In a second experiment, we implanted a slowly-releasing CORT pellet for 2 weeks (200 mg, 60 day release) subcutaneously. This treatment produced an elevation of CRH mRNA in the CEA both at 1 and 2 weeks, whereas CRH mRNA in the PVN was decreased to a large extent as seen in the high CORT group of the first experiment. These results suggest that glucocorticoids can facilitate CRH mRNA expression in the CEA, a site implicated in anxiety and fear, while restraining the hypothalamic-pituitary-adrenal axis as indicated by the reduction in CRH mRNA in the PVN.

Key words: Corticosterone; Corticotropin-releasing hormone; Amygdala; Paraventricular nucleus; mRNA

1. Introduction

The central nucleus of the amygdala (CEA) is part of the neural circuit underlying the expression of fear and anxiety [2,7,9,13,20]. It is also a large source of extra-hypothalamic corticotropin-releasing hormone (CRH) [24,33]. Moreover, a growing body of functional work demonstrates that ablation of the CEA abolishes many of the behavioral effects of centrally delivered CRH (e.g. conditioned startle, fear and anxiety), while lesions of the paraventricular nucleus (PVN) produce no such effects [7,21]. This functional work is consistent with the anatomical demonstration of perhaps at least two CRH systems in the brain; one tied to the regulation of the hypothalamic-pituitary-adrenal (HPA) axis and the other to the above behavioral functions.

It has been well established that elevated glucocorticoids decrease CRH in the parvocellular region of the PVN of the hypothalamus. Decreasing CRH counterregulates the activated HPA axis that typically results from stress [3,6,25]. In contrast, the effects of glucocorticoids on extra-hypothalamic CRH are not well understood [1,12,27,37].

Recently, there have been reports of differential effects of corticosterone (CORT) on CRH mRNA in hypothalamic and extra-hypothalamic brain regions [16,22,34]. Swanson and Simmons [34] demonstrated that adrenalectomized rats given systemic high doses of CORT treatment for one week increased CRH mRNA in the intermediate region of the CEA, and reduced CRH mRNA in the medial part of the parvocellular PVN. Moreover, adrenalectomy reduces CRH in the CEA and increases it in the parvocellular PVN (Palkovits et al., personal communication). These findings are of importance, for they suggest that glucocorti-

* Corresponding author.

SSDI 0006-8993(93)E1526-9

coids may have diverse effects on two functionally different CRH systems.

We therefore extended the observation of Swanson and Simmons [34] by examining whether CORT would affect similarly CRH mRNA expression in the CEA and PVN of adrenally-intact rats by using in Situ hybridization histochemistry (as adrenalectomy also affects plasma levels of epinephrine and adrenal steroids other than CORT). In doing so, we varied the duration and dose over which rats were exposed to the CORT. We found that, in adrenally-intact rats, CORT also had differential effects on the expression of CRH mRNA in the parvocellular region of the PVN and the CEA.

2. Materials and methods

2.1. Experiment 1

Male Sprague–Dawley rats weighing 180–220 g (Taconic Farms, Germantown, NY) were housed four per cage at 24°C in a humidity controlled room. They were on a 12-h light/dark cycle (Lights on at 06.00 h and off at 18.00 h). Purina chow and water were available ad libitum throughout the experiment.

Rats were randomly separated into 3 groups and were injected subcutaneously once daily in the morning with either vehicle (sesame oil) or CORT (low; 1 mg/kg/day, high; 5 mg/rat/day, dissolved in the sesame oil, Sigma Chemicals Co., St. Louis, MO). The dose of CORT was determined by previous reports [12,31]. Groups of rats received the injection for 2, 4, 8 or 14 days ($n = 8$ in each dose of group at each time point), and were sacrificed 24 h after the last injection. Control rats were also sacrificed at each of the time points in which an experimental group was sacrificed. Control rats in each time point did not show any significant differences in all parameters, so their data were pooled (total $n = 20$).

2.2. Experiment 2

Another set of male Sprague–Dawley rats weighing 230–280 g were used for a CORT implant study. Slowly-releasing pellets containing 200 mg of CORT or placebo pellet (60 day release, Innovative Research of America, Toledo, OH) were implanted subcutaneously in adrenally-intact rats. Rats were sacrificed at 1 and 2 weeks after the CORT implantation ($n = 7$ at each time point). Control rats which were sacrificed at each time point were pooled (total $n = 12$).

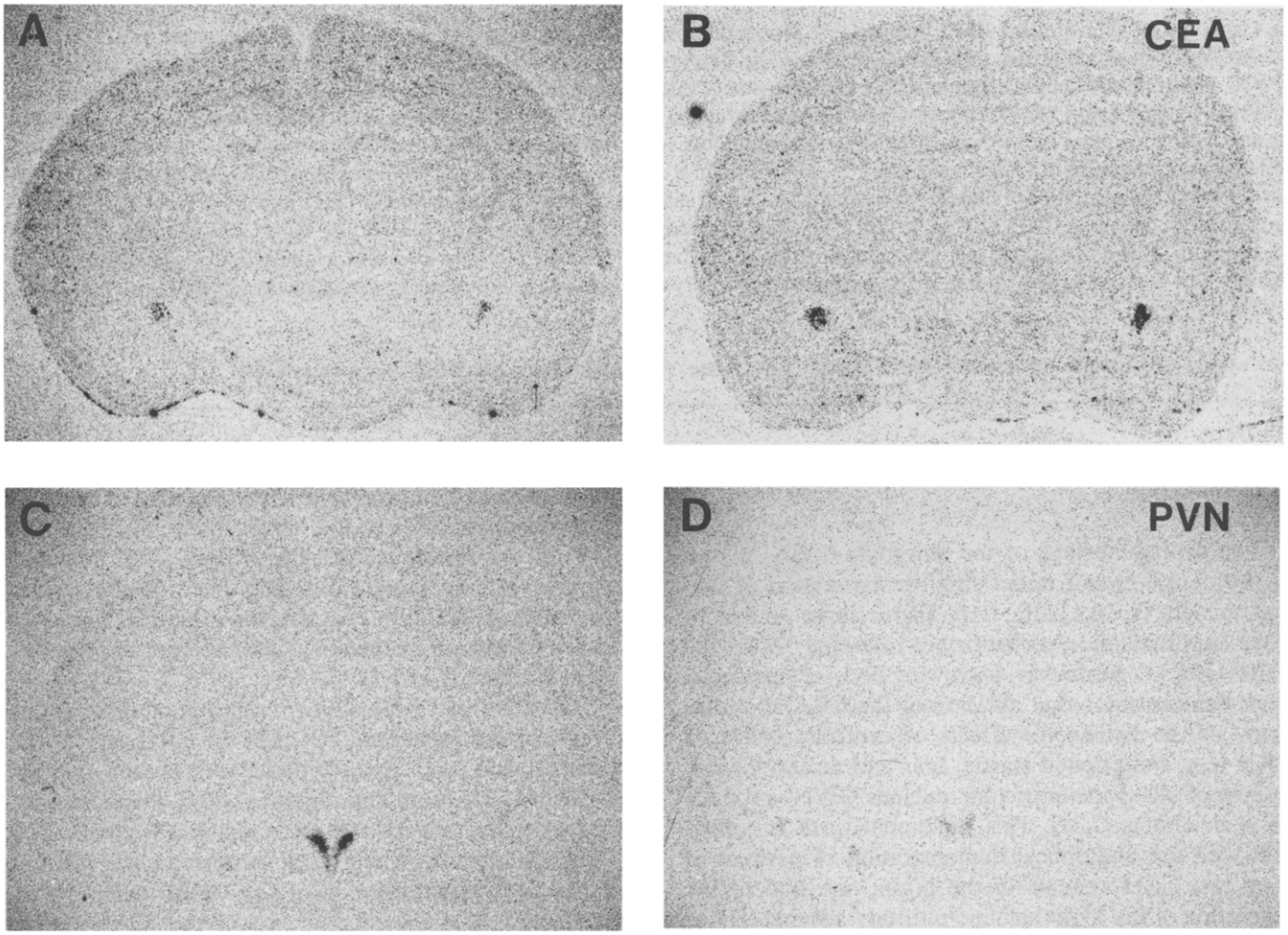

Fig. 1. Autoradiographs depict the effects of corticosterone (CORT) injection on CRH mRNA levels in the central nucleus of the amygdala (CEA) (A and B) and the paraventricular nucleus (PVN) (C and D) of control (A, C) and high CORT-treated (B, D; at 4 days) rats. Black silver grains mark the location of hybridized probe and accumulation of grains are seen in the CEA and the PVN, which is adapted from Paxinos and Watson [29]. (Magnification ×6.5).

For both experiments, the rats were decapitated between 09.00 h and 11.00 h, corresponding to 3–5 h after lights-on, and their brains were quickly removed and frozen by immersion in 2-methyl butane at −30°C, then stored at −70°C until sectioning the tissue on the cryostat. Frozen tissue was cut coronally in 15 μm thick sections. The sections were thaw-mounted and air dried on gelatin-coated slides, and were stored at −70°C prior to in situ hybridization histochemistry.

2.3. In situ Hybridization

In situ hybridization was performed in two brain regions; the parvocellular region of the PVN and the CEA. The sections of the CEA were taken at 2.6–2.8 mm posterior from the bregma (0.6–0.8 mm posterior from one of the last sections of the PVN). We observed that this was the densest CRH mRNA containing region in the amygdala (unpublished observations). The sections of the PVN were determined by staining with thionin at the anterior and posterior ends of the nucleus.

The hybridization procedures were carried out as previously described [36]. In brief, sections were fixed in 4% formaldehyde, subsequently treated with 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% saline (pH 8.0) over a 10 min period to reduce non-specific hybridization of the probe. Then the sections were dehydrated in increasing concentrations of ethanol, and delipidated with chloroform for 5 min, rinsed in ethanol and air dried. We used a synthetic 48-based oligodeoxyribonucleotide probe directed against rat CRH, bases encoding amino acids 496–543 [17]. The probe was labeled with [α-^{35}S]dATP (> 1,000 Ci/mmol, DuPont/NEN) using terminal deoxynucleotidyl transferase (25 units/μl, Boehringer-Mannheim Biochemicals, Indianapolis, IN) and tailing buffer (Bethesda Research Laboratory, Bethesda, MD). Sections were hybridized overnight at 37°C with 5×10^5 cpm of labeled probe per section. Then non-specifically hybridized probe was removed through washing the sections in four 15 min rinses of 2×SSC (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7.2) containing 50% formamide at 40°C, followed by two 30 min rinses of 1×SSC at 25°C.

2.4. Analysis and quantification

For analysis of CRH mRNA, the slides and ^{14}C-standards of known radioactivity (American Radiochemicals Inc., St. Louis, MO) were placed in X-ray cassettes, apposed to ^{35}S-sensitive film (Hyperfilm-βMax, Amersham) for 6 days and 21 days for CRH in the PVN and CEA, respectively. Films were then developed (D19, Kodak) for 5 min at 20°C. The amount of probe hybridized in the PVN or CEA was measured as regional optical densities of autoradiographic film images with a computerized image analysis system composed of a light box, a solid state video camera, and Macintosh II-based IMAGE software developed by Wayne Rasband, Research Service Branch, National Institute of Mental Health. Optical densities for each region were obtained in 2 consecutive sections per rat. In cases in which we had obvious laterality in the intensity of the image in the CEA, we adopted the density on the dark side. Values were converted to disintegrations per minute per milligram (dpm/mg) of rat brain tissue using a standard curve generated by ^{14}C standards which had been matched with [α-^{35}S]dATP-impregnated rat brain paste standards. The average value for each rat was used to calculate

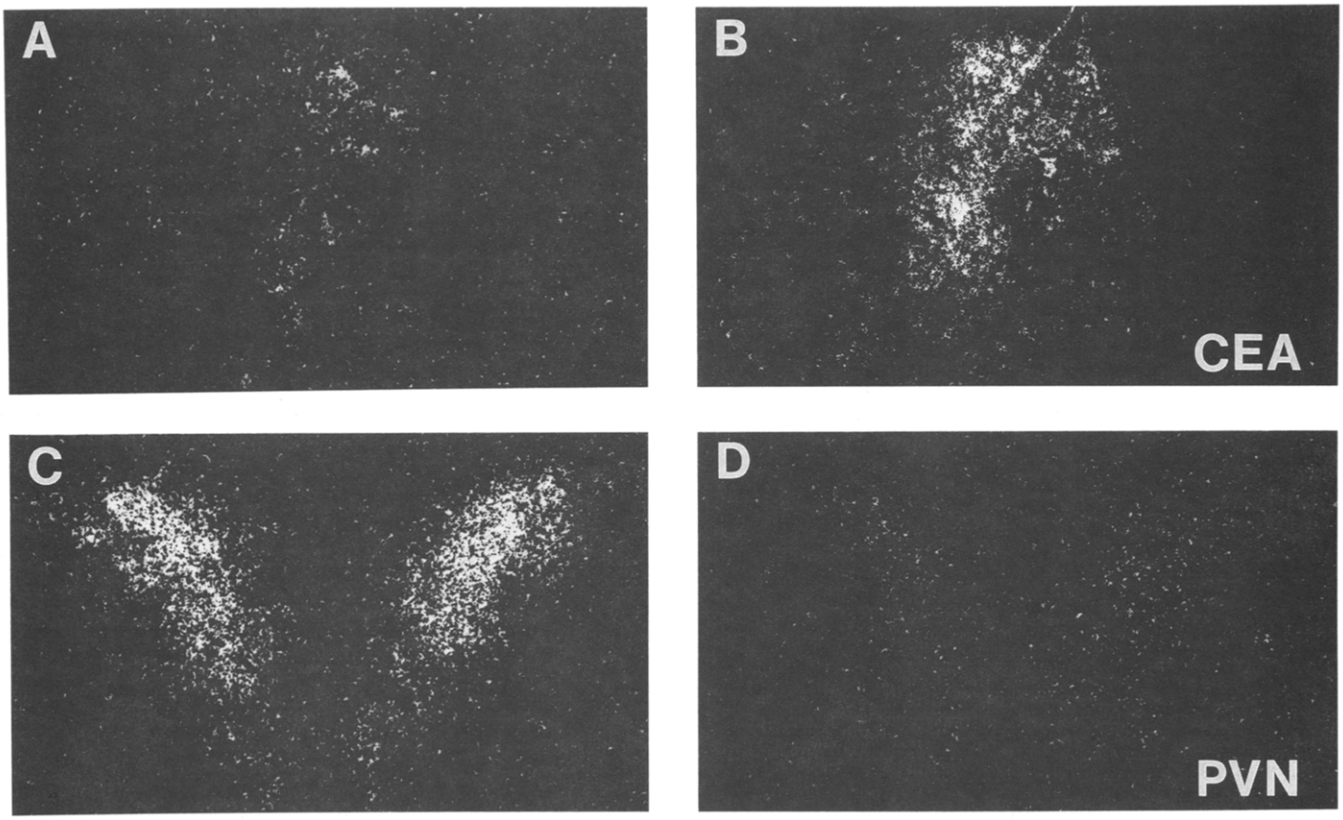

Fig. 2. Effects of corticosterone (CORT) injection on CRH mRNA levels in the central nucleus of the amygdala (CEA) and the paraventricular nucleus (PVN). Darkfield photomicrographs show the autoradiographic distribution of CRH mRNA in the CEA (A and B) and in the PVN (C and D) of control (A, C) and high CORT-treated (B, D; at 4 days) rats. Autoradiographic silver grains appear white. High CORT treatment increased CRH mRNA in the CEA, whereas it was decreased in the PVN. (Magnification ×70).

group means. Statistical significance between the control and experimental groups was determined by one-way ANOVA followed by Fisher's protected least significant difference (PLSD) test.

2.5. Hormonal Assays and organ weights

At the time of decapitation, trunk blood was collected on ice, centrifuged and stored at −70°C until assay. Plasma corticosterone and ACTH were measured by commercially available radioimmunoassay kit (ICN biomedicals Inc. Cleveland, OH). The intra-assay coefficient of variance was <10%. In addition, adrenal glands and thymus were removed and weighed as changes in adrenal and thymus weight are important biological responses as cumulative evidences of CORT actions [6,25,32]. These data were also evaluated statistically by one-way ANOVA followed by Fisher's PLSD test.

3. Results

3.1. Experiment 1

Effect of CORT on CRH mRNA levels in the CEA

Figs. 1 and 2 show our best representative sections in which the high dose of CORT increased CRH mRNA in the CEA at 4 days (B compared with A). Fig. 1 also reveals the localization of CRH mRNA in the CEA (A and B, which is adapted from Paxinos and Watson [29]). The high dose of CORT increased CRH mRNA in the CEA significantly at 4 days ($P < 0.05$, Fig. 3). The low dose of CORT decreased CRH mRNA in the CEA significantly at 14 days ($P < 0.05$, Fig. 3).

Effect of CORT on CRH mRNA levels in the PVN

Our best representative sections are again shown in Figs. 1 and 2 (high dose of CORT injection decreased CRH mRNA in the PVN at 4 days; D compared with C). The high dose of CORT decreased CRH mRNA in the PVN at all time points ($P < 0.001$, Fig. 4). The low dose of CORT resulted in significant decreases in CRH mRNA in the PVN at 2, 4 and 8 days, and then tended to return to the control levels at 14 days (at 4 days; $P < 0.01$, Fig. 4). These results support previous reports demonstrating glucocorticoid negative feedback effects on hypothalamic CRH mRNA levels [1,18,19,34].

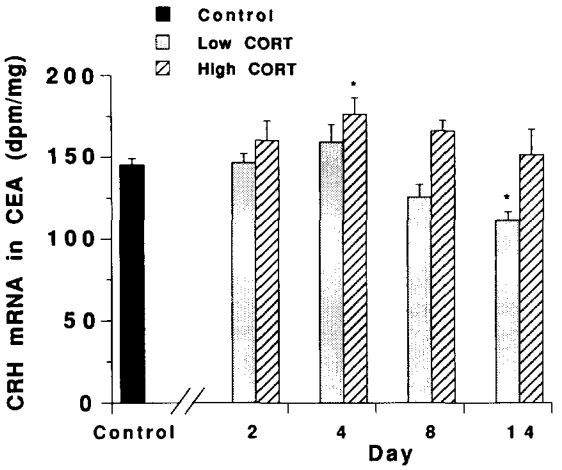

Fig. 3. CRH mRNA hybridization levels in the central nucleus of the amygdala induced by high (5 mg/day) or low (1 mg/kg/day) dose of corticosterone (CORT) injection. Control rats ($n = 20$) were obtained from the pool of rats sacrificed at the same time points as high ($n = 8$ in each time point) and low ($n = 8$ in each time point) CORT group. Values are means ± S.E.M. * $P < 0.05$ vs. control.

Effect of CORT on plasma CORT and ACTH levels

In the high dose group, plasma CORT levels were significantly higher than in the control group at all time points ($P < 0.001$), even though it decreased gradually. Plasma ACTH was lower than in the control group at all time points, but it was not statistically significant (Table 1). In the low dose group, plasma CORT level gradually increased, and at the later time period it was significantly higher than the control group ($P < 0.01$, at 14 days). The low dose replacement did not affect plasma ACTH levels (Table 1).

Effect of CORT on adrenal and thymus weight

In the low dose group, the thymus weight decreased slightly after 4 days ($P < 0.05$), but adrenal weight did not change. The high dose of CORT induced massive

Table 1
Effect of corticosterone (CORT) administration on plasma CORT and ACTH levels in rats

Group		Day 2	Day 4	Day 8	Day 14
Plasma CORT (ng/ml)					
Control	21.2 ± 6.1				
Low CORT		40.0 ± 13.3	59.0 ± 16.4	74.3 ± 19.2 *	81.6 ± 32.6 **
High CORT		175.2 ± 24.1 #	141.3 ± 19.1 #	112.8 ± 12.0 #	113.4 ± 12.1 #
Plasma ACTH (pg/ml)					
Control	25.5 ± 4.7				
Low CORT		20.6 ± 2.7	22.9 ± 5.8	37.4 ± 13.3	34.6 ± 14.8
High CORT		18.6 ± 10.4	19.2 ± 5.0	13.8 ± 2.7	7.7 ± 2.5

Rats were subcutaneously injected once daily in the morning with either sesame oil (control group; $n = 20$), low dose of CORT (1 mg/kg/day, low CORT group; $n = 8$ at each time point) or high dose of CORT (5 mg/day, high CORT group; $n = 8$ at each time point). Blood was collected 24 h after the last injection.

Values are means ± S.E.M. * $P < 0.05$ vs. control. ** $P < 0.01$ vs. control. # $P < 0.001$ vs. control.

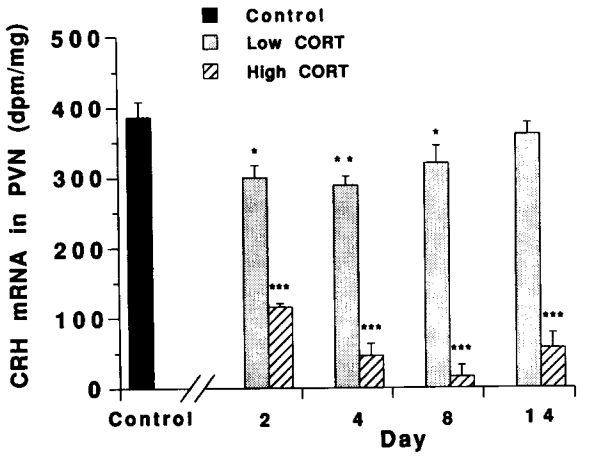

Fig. 4. CRH mRNA hybridization levels in the paraventricular nucleus induced by high or low dose of corticosterone (CORT) administration. Control rats ($n = 20$) were obtained from the pool of rats sacrificed at the same time points as the high ($n = 8$ in each time point) and low ($n = 8$ in each time point) CORT groups. Values are mean ± S.E.M. * $P < 0.05$ vs. control. ** $P < 0.01$ vs. control. *** $P < 0.001$ vs. control.

atrophy of both adrenal gland ($P < 0.05$) and thymus ($P < 0.001$) at all time points (Table 2).

3.2. Experiment 2

Effect of CORT on CRH mRNA levels in the CEA

Fig. 5 shows our best representative sections in which the CORT pellet increased CRH mRNA in the CEA (A: control, B: 1 week, C: 2 weeks). The CORT pellet increased CRH mRNA in the CEA at both 1 week ($P < 0.01$) and 2 weeks ($P < 0.001$, Fig. 6). This indicates that the CORT pellet produced a continuous elevation on CRH mRNA in the CEA compared with a transient increase by the high CORT injection. The pellet implantation induces a constant blood hormonal level, while the surges and subsequent declines are associated with the injection [6,23]. Continuous occupancy of glucocorticoid receptors by a constant high level of CORT may cause the sustained elevation of CRH mRNA in the CEA. Alternatively, in the CORT injection study, higher doses of CORT may be required to produce the continuous elevation of CRH mRNA in the CEA.

Effect of CORT on CRH mRNA levels in the PVN

Our best representative sections are shown in Fig. 5. The CORT pellet decreased CRH mRNA in the PVN (D: control, E: 1 week, F: 2 weeks). At 1 week, we could not detect any CRH mRNA expression. At 2 weeks, CRH mRNA was slightly expressed in the PVN, but it was also significantly lower than control group ($P < 0.001$, Fig. 7).

Effect of CORT on plasma CORT and ACTH levels

Plasma CORT levels were significantly higher than the control group both at 1 and 2 weeks ($P < 0.001$). Plasma ACTH levels were significantly lower than the control group at 2 weeks ($P < 0.01$, Table 3).

Effect of CORT on adrenal and thymus weight

The adrenal gland and thymus shrank both at 1 and 2 weeks ($P < 0.001$, Table 3).

4. Discussion

We have demonstrated that CORT has differential effects on CRH mRNA levels in the CEA versus the parvocellular PVN in adrenally-intact rats. In the CORT injection study, at the high dose there is a modest and transient increase in CRH mRNA, while at the low dose there is a decrease in CRH in the CEA at the late period. The CORT pellet study produced a modest but continuous increase in CRH mRNA in the CEA. By contrast, in the parvocellular PVN there is a significant reduction of CRH mRNA following both CORT injection and by implanting CORT. These results provide further evidence that glucocorticoids can have differential effects on target tissue; in some con-

Table 2
Effect of corticosterone (CORT) administration on adrenal and thymus weight in rats

Group		Day 2	Day 4	Day 8	Day 14
Adrenal weight (mg)					
Control	37.1 ± 0.9				
Low CORT		34.9 ± 1.2	35.8 ± 0.6	35.5 ± 0.8	37.1 ± 2.1
High CORT		28.1 ± 0.6 *	26.2 ± 1.0 *	22.2 ± 0.3 *	20.0 ± 0.8 *
Thymus weight (mg/100 g b.w.)					
Control	205.7 ± 10.0				
Low CORT		192.4 ± 28.1	172.7 ± 11.1 *	170.7 ± 3.5 *	168.2 ± 5.7 *
High CORT		102.0 ± 6.9 **	53.6 ± 3.4 **	34.0 ± 1.7 **	22.4 ± 2.1 **

Rats were subcutaneously injected once daily in the morning with either sesami oil (control group; $n = 20$), low dose of CORT (1 mg/kg/day, low CORT group; $n = 8$ at each time point) or high dose of CORT (5 mg/day, high CORT group; $n = 8$ at each time point). Values are means ± S.E.M. * $P < 0.05$ vs. control. ** $P < 0.001$ vs. control.

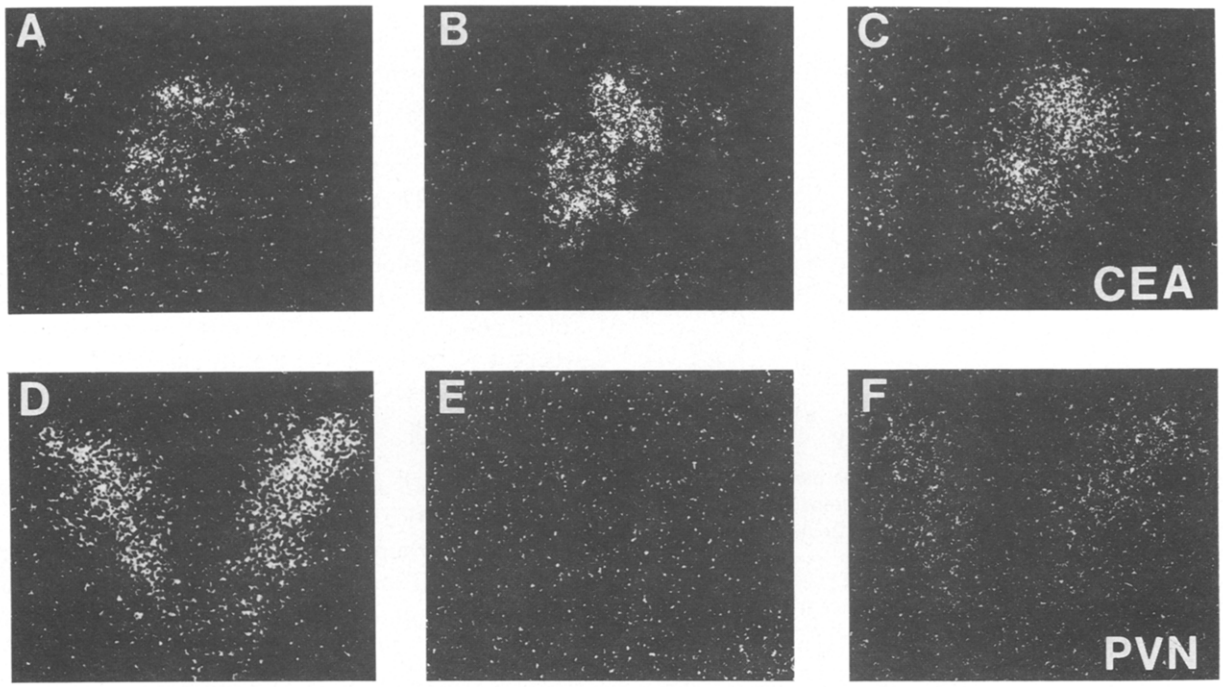

Fig. 5. Effects of corticosterone (CORT) pellet implantation on CRH mRNA levels in the central nucleus of the amygdala (CEA) and the paraventricular nucleus (PVN). Darkfield photomicrographs show the autoradiographic distribution of CRH mRNA in the CEA (A,B,C) and in the PVN (D,E,F) of control (A,D), 1 week (B,E) and 2 weeks (C,F). Autoradiographic silver grains appear white. CORT pellet (200 mg) implantation produced a continuous elevation of CRH mRNA in the CEA, whereas it was decreased in the PVN. (original magnification, ×70).

texts decreasing gene transcription, and increasing it in others. Glucocorticoid receptors are co-localized in CRH cells in the CEA [5,14] as well as in the PVN [4], suggesting that CORT might directly affect CRH gene transcription in the CEA or the PVN.

Our results, however, showed that the changes in CRH mRNA in the CEA were smaller than in the PVN at the same dose of CORT. This indicates that the CEA and the PVN may have different thresholds in response to CORT, which is consistent with the findings of Swanson and Simmons [35]. Since there is less CRH mRNA in the CEA than in the PVN, small changes may have functional significance.

It is unclear why the low dose of CORT injection decreased CRH mRNA in the CEA and why CRH mRNA in the PVN returned to normal at 14 days. It would appear that, even in the same area, CORT may

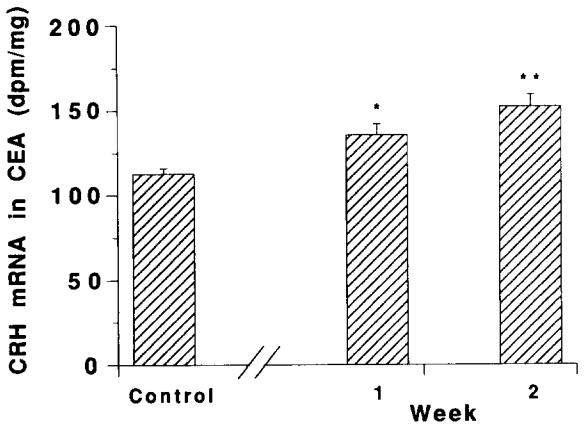

Fig. 6. CRH mRNA hybridization levels in the central nucleus of the amygdala induced by CORT pellet implantation over 2 weeks. Control rats ($n = 12$) were obtained from the pool of rats sacrificed at the same time points as the experimental groups ($n = 7$ in each time point). Values are means ± S.E.M. * $P < 0.01$ vs. control. ** $P < 0.001$ vs. control.

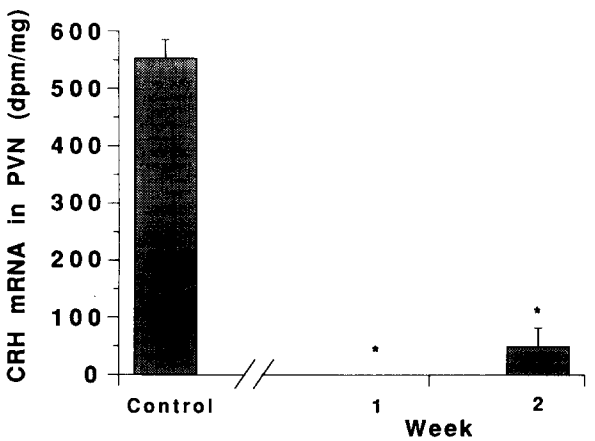

Fig. 7. CRH mRNA hybridization levels in the paraventricular nucleus induced by CORT pellet (200 mg) implantation. Control rats ($n = 12$) were obtained from the pool of rats sacrificed at the same time points as experimental group ($n = 7$ in each time point). Values are mean ± S.E.M. * $P < 0.001$ vs. control.

Table 3
Effect of corticosterone (CORT) implantation on Plasma CORT and ACTH levels in rats

	control	CORT	
		1 week	2 weeks
Plasma CORT (ng/ml)	37.7±7.5	296.2±24.9 **	255.4±34.8 **
Plasma ACTH (pg/ml)	48.7±8.8	27.2±10.3	16.0± 1.3 *
Adrenal weight (mg)	45.9±1.1	25.6± 1.4 **	20.8± 1.2 **
Thymus weight (mg/100 g b.w.)	124.0±4.3	15.0± 0.5 **	13.9± 2.3 **

Rats were subcutaneously implanted with slowly-releasing pellets containing 200 mg of CORT. Rats were sacrificed 1 week ($n = 7$) or 2 weeks ($n = 7$) later. Control rats were sacrificed at the same time and then data were pooled ($n = 12$). Values are means ± S.E.M. * $P < 0.01$ vs. control. ** $P < 0.001$ vs. control.

have a differential effect on CRH gene transcription at different doses, or different durations of exposure. However, this remains to be clarified.

Our results may add to the growing body of evidence that suggests that the CEA is functionally tied to CRH neuronal activation following stress. In fact, CRH in addition to norepinephrine is activated in the CEA following immobilization-induced stress, a condition in which CORT is elevated [28,30]. We also found that repeated immobilization stress increased CRH mRNA in the CEA (unpublished observations). The exogenous CORT elevation may mimic part of the stress response [12,31]. Although plasma CORT levels in this study may be higher than in chronically stressed rats, our results raise the possibility that, in stress, CORT could elevate CRH neuronal activity in the CEA, instead of counterregulating or restraining it. In a variety of experimentally-induced stressful conditions, other factors (e.g. catecholamines [28] or transcription factors [15]) may enhance CORT effects on CRH neuronal activation in the CEA. However, the actual effect of endogenous CORT in stress on CRH gene expression in the CEA remains to be elucidated.

Since there is a growing body of evidence that the CEA and CRH are related to arousal and anxiety [7,8,20,21,35] which can be characteristic features of melancholic depression [11], CORT, by amplifying the CRH signal in the CEA, may be increasing the sense of adversity that is known to occur following central administration of CRH [8]. This is consistent with clinical observations that depressed patients often show hypercortisolism with high levels of CRH in the cerebrospinal fluid (CSF) [10,26]. Although the origins of CRH in the CSF is not well known in depressed individuals, our results indicate that the CEA may be at least partly responsible for this.

Acknowledgements. We thank our collegues for their suggestions, and we specially thank Dr. Mark A. Smith (Biological Psychiatry Branch, NIMH) for his generous advice and support.

5. References

[1] Beyer, H.S., Matta, S.G. and Sharp, B.M., Regulation of the messenger ribonucleic acid for corticotropin-releasing factor in the paraventricular nucleus and other brain sites of the rat, *Endocrinology*, 123 (1988) 2117–2123.

[2] Blanchard, D.C. and Blanchard, R.J., Innate and conditioned reactions to threat in rats with amygdaloid lesions, *J. Comp. Physiol. Psychol.*, 81 (1972) 281–290.

[3] Chrousos, G.P. and Gold, P.W., The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis, *J. Am. Med. Assoc.*, 267 (1992) 1244–1252.

[4] Cintra, A., Fuxe, K., Harfstrand, A., Agnati, L.F., Wikstrom, A.-C., Vale, W. and Gustafsson, J.-A., Evidence for the presence of glucocorticoid receptor immunoreactivity in corticotropin-releasing factor and in growth hormone releasing factor immunoreactive neurones of the rat di- and telencephalon, *Neurosci. Lett.*, 76 (1987) 269–274.

[5] Cintra, A., Fuxe, K., Solfrini, V., Agnati, L.F., Tinner, B., Wikstrom, A.-C., Staines, W., Okret, S. and Gustafsson, J.-A., Central peptidergic neurons as targets for glucocorticoid action: evidence for the presence of glucocorticoid immunoreactivity in various types of classes of peptidergic neurons, *J. Steroid Biochem. Mol. Biol.*, 40 (1991) 93–103.

[6] Dallman, M.F., Akana, S.F., Cascio, C.S., Darlington, D.N., Jacobson, L. and Levin, N., Regulation of ACTH secretion: variations on a theme of B, *Recent Progress in Hormone Res.*, 43 (1987) 113–173.

[7] Davis, M., The Role of the Amygdala in Fear and Anxeity, *Annu. Rev. Neurosci.*, 15 (1992) 353–375.

[8] Dunn, A.J. and Berridge, C.W., Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress response?, *Brain Res. Rev.*, 15 (1990) 71–100.

[9] Gallagher, M., Graham, P.W. and Holland, P.C., The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior, *J. Neurosci.*, 10 (1990) 1906–1911.

[10] Gold, P.W., Chrousos, G.P., Kellner, C.H., Post, R.M., Roy, A., Avgerinos, P.C., Schulte, H.M., Oldfield, E.H. and Loriaux, D.L., Psychiatric implications of basic and clinical studies with corticotropin-releasing factor, *Am. J. Psychiatry*, 141 (1984) 619–627.

[11] Gold, P.W., Goodwin, F.K. and Chrousos, G.P., Clinical and biochemical manifestations of depression; relation to the neurobiology of stress, *N. Engl. J. Med.*, 319 (1988) 348–353, 413–420.

[12] Hauger, R.L., Millian, M.A., Catt, K.J. and Aguilera, G., Differential regulation of brain and pituitary corticotropin-releasing factor receptors by corticosterone, *Endocrinology*, 120 (1987) 1527–1533.

[13] Helmstetter, F., The amygdala is essential for the expression of conditioned hypoalgesia, *Behav. Neurosci.*, 106 (1992) 518–528.

[14] Honkaniemi, J., Pelto-Huikko, M., Rechard, L., Isola, J., Lammi, A., Fuxe, K., Gustafsson, J.A., Wikstrom, A.C. and Hokfelt, T., Colocalization of peptide and glucocorticoid receptor immunoreactivities in rat central amygdaloid nucleus, *Neuroendocrinology*, 55 (1992) 451–459.

[15] Honkaniemi, J., Fuxe, K., Rechardt, L., Koistinaho, J., Isola, J., Gustafsson, J., Okret, S. and Pelto-Huikko, M., Colocalization of Fos- and glucocorticoid receptor-like immunoreactivities in the rat amygdaloid complex after immobilization stress, *J. Neuroendocrinol.*, 4 (1992) 547–555.

[16] Imaki, T., Nahan, J., Rivier, C., Sawchenko, P.E. and Vale, W., Differential regulation of corticotropin-releasing factor mRNA in rat brain regions by glucocorticoids and stress, *J. Neurosci.*, 11 (1991) 585–599.

[17] Jingami, H., Mizuno, N., Takahashi, H., Shibahara, S., Furutani,

Y., Imura, H. and Numa, S., Cloning and sequence analysis of cDNA for rat corticotropin-releasing factor precursor, *FEBS Lett.*, 191 (1985) 63–66.

[18] Jingami, H., Matsukura, S., Numa, S. and Imura, H., Effect of adrenalectomy and dexamethasone administration on the level of prepro-corticotropin-releasing factor messenger ribonucleic acid (mRNA) in the hypothalamus and adrenocorticotropin/β-lipotropin precursor mRNA in the pituitary in rats, *Endocrinology*, 117 (1985) 1314–1320.

[19] Kovacs, K.J. and Mezey, E., Dexamethasone inhibits corticotropin-releasing factor gene expression in the rat paraventricular nucleus, *Neuroendocrinology*, 46 (1987) 365–368.

[20] LeDoux, J.E., Emotion and the amygdala. In Aggleton, J.P. (Ed.), *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*, Wiley-Liss, Inc., New York, 1992, pp. 339–351.

[21] Liang, K.C., Melia, K.R., Campeau, S., Falls, W.A., Miserendino, M.J.D. and Davis, M., Lesions of central nucleus of the amygdala, but not the paraventricular nucleus of the hypothalamus, block the excitatory effects of corticotropin-releasing factor on the acoustic startle reflex, *J. Neurosci.*, 12 (1992) 2313–2320.

[22] Makino, S., Gold, P.W. and Schulkin, J., Corticosterone effects on CRH mRNA in the parvocellular PVN and the central nucleus of the amygdala, *Soc. Neurosci. Abstr.*, (1993).

[23] Meyer, J.S., Micco, D.J., Stephenson, B.S., Krey, L.C. and McEwen, B.S., Subcutaneous implantation method for chronic glucocorticoid replacement therapy, *Physiol. Behav.*, 22 (1979) 867–870.

[24] Moga, M.M. and Gray, T.S., Evidence for corticotropin-releasing factor, neurotensin, and somatostatin in the neural pathway from the central nucleus of the amygdala to the parabrachial nucleus, *J. Comp. Neurol.*, 241 (1985) 275–284.

[25] Munck, A., Guyre, P.M. and Holbrook, N.J., Physiological Functions of Glucocorticoids in Stress and their Relation to Pharmacological Actions, *Endocrine Rev.*, 5 (1984) 25–44.

[26] Nemeroff, C.B., Widerlov, E., Bissette, G., Walleus, H., Karlsson, I., Kilts, C.D., Vale, W. and Loosen, P.T., Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients, *Science*, 226 (1984) 1342–1344.

[27] Owens, M.J., Bartolome, J., Schanberg, S.M. and Nemeroff, C.B., Corticotropin-releasing factor concentrations exhibit an apparent diurnal rhythm in hypothalamic and extrahypothalamic brain regions: differential sensitivity to corticosterone, *Neuroendocrinology*, 52 (1990) 626–631.

[28] Pacak, K., Palkovits, M., Kvetnansky, R., Fukuhara, K., Armando, I., Kopin, I.J. and Goldstein, D.S., Effects of single or repeated immobilization on release of norepinephrine and its metabolites in the central nucleus of the amygdala in conscious rats, *Neuroendocrinology*, 57 (1993) 626–633.

[29] Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic press, Orlando, 1986.

[30] Pich, E.,M., Goob, G.F., Sattler, S.C., Menaghi, F., Helig, M., Heinrichs, S.C. Vale, W. and Weiss, F., Stress-Induced Release of Corticotropin-Releasing Factor in the Amygdala measured by in vivo dialysis, *Soc. Neurosci. Abstr.*, 1992.

[31] Sapolsky, R.M., Krey, L.C. and McEwen, B.S., Stress down-regulates corticosterone receptors in a site-specific manner in the brain, *Endocrinology*, 114 (1984) 287–292.

[32] Smith, P.E., Hypophysectomy and a replacement therapy in the rat, *Am. J. Anat.*, 45 (1930) 205–273.

[33] Swanson, L.W., Sawchenko, P.E., Rivier, J. and Vale, W.W., Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study, *Neuroendocrinology*, 36 (1983) 165–186.

[34] Swanson, L.W. and Simmons, D.M., Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: A hybridization histochemical study in the rat, *J. Comp. Neurol.*, 285 (1989) 413–435.

[35] Swerdlew, N.R., Britton, K.T. and Koob, G.F., Potentiation of acoustic startle by corticotropin-releasing factor and by fear are both reversed by alpha-helical CRF, *Neuropsychopharmacology*, 2 (1989) 285–292.

[36] Young, S., In situ hybridization histochemical detection of neuropeptide mRNA using DNA and RNA probes, *Methods Enzymol.*, 168 (1989) 702–710.

[37] Wynn, P.C., Hauger, R.L., Holmes, M.C., Millian, M.A., Catt, K.J. and Aguilera, G., Brain and pituitary receptors for corticotropin-releasing factor: localization and differential regulation after adrenalectomy, *Peptides*, 5 (1984) 1077–1084.