Region-specific regulation of neuropeptide mRNAs in rat limbic forebrain neurones by aldosterone and corticosterone

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1. We have determined in adrenalectomized male rats the effects of clamping plasma corticosterone and aldosterone at various concentrations on corticotropin-releasing hormone (CRH), neurotensin/neuromedin N (NT/N) and proenkephalin (pENK) mRNAs in the hypothalamus and amygdala using semi-quantitative *in situ* hybridization.

2. Corticosterone differentially regulated the levels of CRH and NT/N but not pENK mRNA. These effects were cell specific. CRH mRNA was reduced in the hypothalamic paraventricular nucleus (PVH), but increased in the central nucleus of the amygdala and bed nuclei of the stria terminalis. NT/N mRNA was never seen in the PVH, whereas levels increased in the central nucleus of the amygdala, but were unaffected in the lateral hypothalamic area. In those regions expressing pENK mRNA, levels were unaffected in all treatment groups.

3. CRH mRNA in both the central nucleus of the amygdala and PVH, and NT/N mRNA in the central nucleus of the amygdala were most sensitive to plasma corticosterone concentrations of less than 120 ng ml^{-1}, i.e. those seen away from the peak of the diurnal rhythm. In adrenalectomized animals CRH mRNA in both the central nucleus of the amygdala and PVH could be set at levels usually seen in intact animals by the same plasma concentration of corticosterone.

4. The levels of CRH mRNA in the PVH and the central nucleus of the amygdala were closely correlated, while CRH and NT/N mRNA levels were similarly correlated in the central nucleus of the amygdala suggesting the existence of a common regulatory mechanism. The ED_{50} of their responses to corticosterone and correlations with thymus weight suggested the operation of glucocorticoid (type II) receptor mechanisms.

5. In the absence of corticosterone, aldosterone increased CRH and NT/N mRNA accumulation in the central nucleus of the amygdala, and increased CRH but not NT/N mRNA accumulation in the PVH. Aldosterone also blunted the dose–response effects of corticosterone on CRH and NT/N mRNA levels in the central nucleus of the amygdala, but not in the PVH.

6. These results suggest that, in intact animals, adrenal steroids play a major role in maintaining the levels of neuropeptide mRNAs in the PVH, bed nuclei of the stria terminalis and central nucleus of the amygdala. The results underscore the importance of cell-specific mechanisms operating to regulate the expression of neuropeptide genes in different cell types in response to diverse physiological conditions.

Steroid hormones provide important signals for modulating the functions of many cell types throughout the brain. The adrenal cortex secretes two groups of hormones: glucocorticoids, which influence a broad range of neural functions (for review see de Kloet, Oitzl & Joels, 1993); and mineralocorticoids (sometimes potentiated by glucocorticoids) that can modulate sodium appetite for review see Schulkin, 1992). In the rat hypothalamus and anterior pituitary gland one of the best studied functions of corticosterone is its inhibition of adrenalectomy-induced adrenocorticotrophic hormone (ACTH) secretion (Keller-Wood & Dallman, 1984; Levin, Shinsako & Dallman, 1988). This effect is accompanied by a reduction in corticotropin-releasing hormone (CRH) mRNA and peptide in neurosecretory neurones in the paraventricular nucleus of the hypothalamus (PVH; Young, Mezey &

Siegal, 1986; Swanson & Simmons, 1989). CRH can also be found in neurones located in an interactive and interconnected network of cell groups that organizes the homeostatic response of the animal to a variety of stressors. These include the supraoptic nucleus (Lightman & Young, 1987; Watts, 1992*b*), lateral hypothalamic area (Watts, 1992*a*), bed nuclei of the stria terminalis (BST; Ju, Swanson & Simerly, 1989) particularly the oval (BSTov) and fusiform (BSTfus) subdivisions, and lateral part of the central nucleus of the amygdala (CEAl; Watts, 1992*a*). How neurones in this network function is determined in part by how they interpret and integrate the hormonal and neural signals generated by a particular stimulus. In response to these signals, neurones can modify their efferent signalling capacity, a singularly cell-specific attribute determined by how a cell controls the expression of its own genome. Fluctuating adrenocortical steroid levels occur throughout the day in unstimulated animals (Dallman, Akana, Cascio, Darlington, Jacobson & Levin, 1987) and after many stressors (Dallman *et al.* 1987), and are thus in a position to regulate a wide range of neuronal signalling events.

In many neurones, co-expressed neuropeptides constitute an important component of this signalling capacity. Thus in colchicine-treated animals, CRH, neurotensin and enkephalin are colocalized in many neurosecretory neurones in the dorsal medial parvicellular part of the PVH (PVHmpd; see Watts & Sanchez-Watts, 1995, for references); similarly all three peptides are found in the CEAl (Moga & Gray, 1985), and BSTov (Moga, Saper & Gray, 1989; Shimada, Inagaki, Kubota, Ogawa, Shibasaki & Takagi, 1989). Although these studies have provided the morphological basis for a significant role of peptides in neural function, high doses of colchicine have limited use for investigating the kinetics of neuropeptide function in more physiological models because of the accompanying pituitary and adrenal activation (Berkenbosch & Tilders, 1987). By estimating relative amounts of mRNA, semi-quantitative *in situ* hybridization offers an alternative approach for investigating neuropeptide function. Thus, alterations in neuropeptide mRNA levels in these cell groups are seen after a variety of homeostatic perturbations (Harbuz & Lightman, 1989; Lightman & Young, 1989; Watts & Swanson, 1989; Imaki, Nahan, Rivier, Sawchenko & Vale, 1991; Watts, 1991, 1992*a*,*b*; Watts & Sanchez-Watts, 1995). With regard to colocalized neuropeptides, we have recently shown that extracellular dehydration will increase the incidence of colocalization of mRNAs encoding CRH, neurotensin and enkephalin in neurones of the PVHmpd (Watts & Sanchez-Watts, 1995) suggesting that PVHmpd neurones modify their neuropeptide content in the face of a selective physiological challenge. Considered together, these results suggest that the stimulus-specific regulation of neuropeptide mRNAs plays a central role in modulating the function of neural circuits, and that adrenal corticosteroids may operate in this context. In hypothalamic and amygdalar neurones that express multiple neuropeptides, studies have focused on corticosterone's effects on CRH rather than on other neuropeptide mRNAs (e.g. Kovács & Mezey, 1987; Beyer, Matta & Sharp, 1988; Makino, Gold & Schulkin, 1994). Swanson & Simmons (1989) did investigate how corticosterone affected the accumulation of other neuropeptide mRNAs in the PVHmpd, but not in other regions. Most of these studies have relied upon adrenalectomy followed by corticosterone replacement, but since adrenalectomy also removes mineralocorticoids, any interaction between them and corticosterone has not been examined.

Using *in situ* hybridization in male adrenalectomized rats with corticosterone and aldosterone replacement we have investigated how these steroids, both separately and together, might regulate CRH, neurotensin/neuromedin N (NT/N; the precursor of neurotensin) and proenkephalin (pENK) mRNAs in the PVHmpd, the BST, CEAl and the lateral hypothalamic area. We addressed four points. (1) Can corticosterone regulate the levels of mRNA encoding CRH, NT/N and pENK in these four cell groups? (2) Do their gene regulatory mechanisms differ in their sensitivity to corticosterone? (3) Can aldosterone alone modify neuropeptide mRNA levels? And (4) does aldosterone affect the subsequent response of neuropeptide mRNAs to corticosterone?

Some results were presented at the Society for Neuroscience (Watts & Sanchez-Watts, 1993).

METHODS

Animals

Adult male Sprague–Dawley rats (280–320 g body weight (BW) at the beginning of the experiment) were maintained on a 12 h light : 12 h dark photoperiod (lights on 07.00 h) with unlimited water and rat chow available. Figure 1 shows the organization of the treatment regimens. Rats (forty animals) were purchased with bilateral adrenalectomies or sham surgery (eight animals)

List of abbreviations

BST	Bed nuclei of the stria terminalis	KPBS	Potassium phosphate buffered saline
BSTfus	Fusiform subdivision of the BST	NT/N	Neurotensin/neuromedin N
BSTov	Oval subdivision of the BST	pENK	Proenkephalin
CEA	Central nucleus of the amygdala	PVH	Hypothalamic paraventricular nucleus
CEAl	Lateral subdivision of the CEA	PVHmpd	Dorsal medial parvicellular part of the PVH
CRH	Corticotropin-releasing hormone		

performed by the vendor (Harlan Inc., San Diego, CA, USA). Upon arrival (Fig. 1; Day 1) all animals were individually caged and offered either 0·15 M sodium chloride solution (adrenalectomies) or water (intacts) to drink.

Five days after arrival (Fig. 1; Day 6) adrenalectomized rats were anaesthetized with halothane and subcutaneously implanted with a pellet containing 250 μg of d-aldosterone (Fig. 1, treatment A, four groups of five rats) or a control pellet containing no steroid (Fig. 1, treatment B, four groups of five rats). Two groups of intact rats were anaesthetized and implanted with a control pellet (Fig. 1, treatments C and D, four rats each). All steroid pellets were 21 day timed-release pellets from Innovative Research of America (IRA, Toledo, OH, USA). On the day aldosterone pellets were implanted, all rats in groups A, B and C were given the choice to drink from bottles containing 0·15 M sodium chloride solution or water. Animals in treatment D (Fig. 1) were only allowed water to drink. Starting on the third day after the implantation of the aldosterone pellet, the volume of each fluid drunk by the rats during every 24 h period throughout the experiment was recorded. The size of the aldosterone pellet was chosen based on the data of Fregly & Waters (1966) who showed that a daily dose of 100 μg (kg body weight)$^{-1}$ day^{-1} in adrenalectomized rats would maximally reduce their sodium appetite, and that a 250 μg pellet should release approximately 35 μg day^{-1} into a rat of 300 g BW. Six days (Fig. 1; day 12) after implantation of a d-aldosterone or control pellet, adrenalectomized rats in treatments A and B were briefly anaesthetized with halothane and subcutaneously implanted with either a 0, 50, 100 or 200 mg corticosterone pellet (IRA; ten rats in each group). Each intact animal in treatments C and D was again implanted with a control pellet. The delay before implanting the corticosterone pellet was to allow the reduced sodium appetite to stabilize before further steroid manipulation.

In a separate experiment, two groups of four intact rats were killed by decapitation either at 08.00 h or 18.00 h. Heparinized trunk blood was collected, and plasma separated and stored at −20 °C for plasma corticosterone determination at a later date. Plasma from these animals was used for radioimmunassay (RIA) quality controls and for comparison with values obtained from pellet-implanted animals.

Perfusion and tissue handling

Five days after the implantation of corticosterone or control pellets (Fig. 1; Day 17) all animals, along with their intact controls, were weighed and anaesthetized by an intraperitoneal injection of tribromoethanol (1 ml (100 g body weight)$^{-1}$ of a 2% solution in 0·9% saline) between 13.00 h and 16.00 h. After induction of anaesthesia, a single 1·5 ml blood sample was taken from the external jugular vein into a heparinized syringe. Blood samples were centrifuged at 4 °C, and plasma separated and stored at −20 °C for measurement of plasma corticosterone (see below). Additionally, the thymus gland of all adrenalectomized animals was dissected free from adjoining tissue and then weighed. Animals were perfused through the ascending aorta with a room temperature saline rinse followed by 500 ml of ice-cold 4% paraformaldehyde solution in 0·1 M borate buffer (pH 9·5). After perfusion the brain from each animal was removed and post-fixed for 24 h in the fixative containing 12% sucrose (w/v). Brains were frozen in powdered dry ice and immediately stored at −70 °C until sectioning at a later date. Eight series of one-in-eight, 15 μm-thick, frontal sections were cut through the hypothalamus and saved in ice-cold 0·01 M potassium phosphate buffered saline (KPBS) containing 0·25% paraformaldehyde (pH 7·4). The sections were mounted the same day on poly-L-lysine-coated gelatin-subbed slides, vacuum desiccated overnight, then post-fixed in KPBS–4% paraformaldehyde for 1 h at room temperature, rinsed for 5 × 5 min in clean KPBS, air dried, and then stored at −70 °C in air-tight containers containing silica-gel desiccant for hybridization at a later date. Serial sections were saved for thionin staining.

Determination of plasma corticosterone concentrations

Plasma corticosterone concentrations were determined in unextracted plasma samples (10 μl) using an ^{125}I-corticosterone double antibody RIA supplied in kit form from ICN Biochemicals Inc. (Costa Mesa, CA, USA). The primary antiserum was reported to have 100% cross-reactivity with corticosterone, and < 0·5% with deoxycorticosterone, < 0·05% with aldosterone and < 0·01% with other steroids (manufacturers specifications). All samples were measured in a single assay with a lower sensitivity limit of 12·5 ng ml^{-1} and an intra-assay coefficient of variation of < 8%.

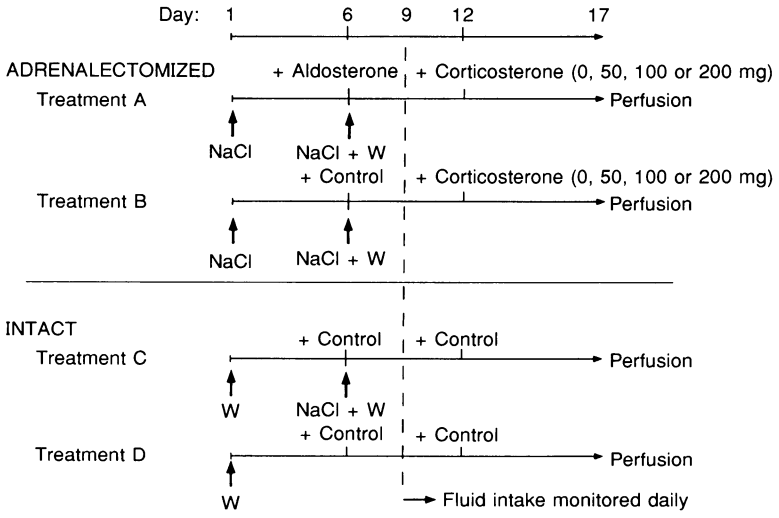

Figure 1. The organization of the treatment groups

Four groups of animals were used. Treatments A and B were adrenalectomized and implanted with either aldosterone (A) or control pellets (B) on day 6. Treatments C and D were intact and both implanted with control pellets on day 6. Treatments A, B and C were offered the choice of water (W) and 0·15 M NaCl, while treatment D was only offered water. All adrenalectomized animals (A and B) were implanted with corticosterone pellets as indicated on day 12.

In situ hybridization

Sections were hybridized with ^{35}S-UTP-labelled complementary RNA (cRNA) probes transcribed from either a 396 bp cDNA sequence coding for part of the mRNA encoding prepro (pp)-CRH, a 336 bp cDNA sequence coding for part of exon 4 of the pp-NT/N gene, or a 935 bp cDNA sequence coding for the entire coding sequence of pre-proenkephalin. Details of these probes and characterization has been given in a number of publications from this laboratory (Watts & Swanson, 1989; Watts, 1992*b*). Additional experiments (data not shown) have shown that neither RNase pretreatment followed by hybridization with antisense strand probes, nor hybridization with sense strand probes, gives any hybridization. All ^{35}S-UTP probes were synthesized using the Riboprobe Gemini System II kit (Promega Inc., Madison, WI, USA) and the appropriate RNA polymerase (Watts & Sanchez-Watts, 1995). *In situ* hybridization was performed as previously described (Watts, 1992*b*; Watts & Sanchez-Watts, 1995). Briefly, sections were pre-hybridized, hybridized for 18–20 h at 60 °C using a probe concentration of either 5×10^6 or 10^7 counts min^{-1} ml^{-1} of hybridization buffer, followed by post-hybridization with RNase treatment and room temperature washes of saline–sodium citrate (SSC) buffer from 4 × to 0·1 × SSC at 65 °C, and dehydrated in alcohols. Sections were exposed to Cronex Microvision X-ray film (Dupont, Wilmington, DE, USA) for varying periods (2–8 days), then dipped in nuclear track emulsion (Kodak NTB-2; diluted 1 : 1 with distilled water), and exposed for 5–21 days, developed and then counterstained with thionin.

Semi-quantitation of ^{35}S-UTP-cRNA hybridization

The mean grey level of labelled cells in each region was measured as previously described (Watts, 1992*a*; Watts & Sanchez-Watts, 1995). Briefly, a series of film exposures were made on Cronex Microvision X-ray film (Dupont), and image analysis performed using a Perceptics Pixel Buffer frame grabber and IPLab Spectrum software (v2.3.1e; Signal Analytics Corp., Vienna, VA, USA) mounted on a Macintosh IIfx computer. The response of the film to ^{14}C microstandards (Amersham Inc.) was linear over the mean grey levels used in this study (data not shown). The mean grey level was calculated within the anatomically selected area on a signal segmented at 3 S.D. above the mean background value. Background measurements were taken from adjacent areas showing no labelling. Preliminary experiments showed that segmentation of the image at 3 S.D. above the mean background value excluded >98% of background pixels. Finally, the mean background value was subtracted to give the mean grey level value used in subsequent calculations. For the purposes of comparison and for regression analyses, data were normalized either to the mean value found in adrenalectomized animals with no corticosterone or aldosterone, or to that of intact controls. All photomicrographs were similarly obtained using the video equipment just described, processed (minor blemish removal and contrast matching), and printed using Adobe Photoshop (Adobe Systems Inc., Mountain View, CA, USA, v2.5.1) and Illustrator (v5.0) software. The data content of the photomicrographs was not altered in any way. For clarity, dark-field photomicrographs were inverted so that image

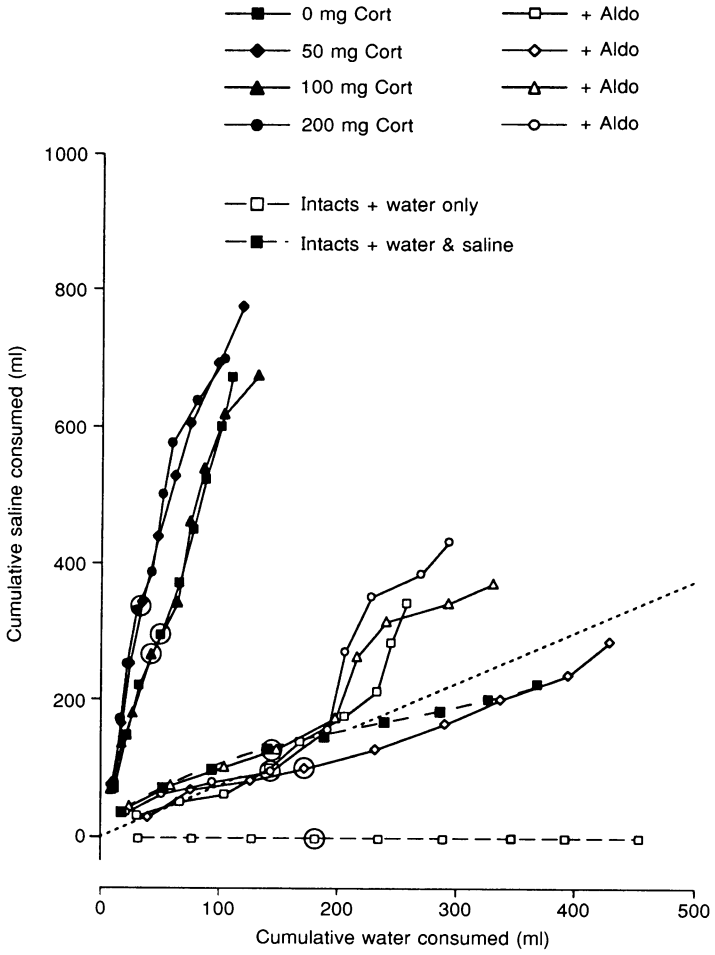

Figure 2. Cumulative daily fluid intakes from steroid-treated animals

Cumulative 24 h fluid intakes from adrenalectomized animals with no aldosterone (continuous lines and filled symbols), with aldosterone replacement (continuous lines and open symbols) or intact animals (dashed lines and square symbols). Animals were offered a choice between 0·15 M sodium chloride and water. The total of each fluid drunk in every 24 h period was recorded and cumulated throughout the experiment. The symbols represent daily means for each group; S.E.M. were omitted for clarity, and were never greater than 8% of the means. For each group, the addition of corticosterone or control pellets is represented by a circle placed around the symbols on the 4th day of measurement. The approximate sodium appetite expressed by intact animals is represented by the dotted line extending through the data.

Table 1. Mean (± S.E.M.) plasma corticosterone concentrations and thymus weights in adrenalectomized animals subcutaneously implanted with corticosterone pellets 6 days after treatment with aldosterone and 5 days before perfusion

Treatment group	*n*	Mean (± S.E.M.) plasma [corticosterone] (ng ml^{-1})	Thymus weight (mg (100 g BW)$^{-1}$)
Adrenalectomized			
No aldosterone			
Group 1 (n.d.)	(5)	n.d.	415·8 ± 16·4
Group 2 (20–50 ng ml^{-1})	(4)	35·5 ± 4·4	316·5 ± 13·9
Group 3 (51–120 ng ml^{-1})	(4)	78·7 ± 13·7	242·0 ± 26·2
Group 4 (> 120 ng ml^{-1})	(7)	176·7 ± 11·1	44·3 ± 5·8
With aldosterone			
Group 1 (n.d.)	(5)	n.d.	412·2 ± 13·6
Group 2 (20–50 ng ml^{-1})	(4)	39·9 ± 4·3	325·8 ± 13·0
Group 3 (51–120 ng ml^{-1})	(5)	97·6 ± 10·4	176·4 ± 37·4
Group 4 (> 120 ng ml^{-1})	(6)	193·9 ± 19·1	38·7 ± 4·7
Intact			
08.00 h	(4)	n.d.	
18.00 h	(4)	185·8 ± 46·2	

Data are arranged according to the 4 ranges of plasma corticosterone concentration measured at perfusion. Also shown are mean (± S.E.M.) plasma corticosterone concentrations from 2 groups of intact animals killed at 08.00 h or 18.00 h; n.d., not detectable.

white = printed black. The anatomical nomenclature of Swanson (1992) has been followed throughout.

Statistics

Statistical differences in the plasma corticosterone concentrations and neuropeptide mRNA levels within the same plasma corticosterone range were determined using an F test for unequal variances followed by Student's t test. All variances were homoscedastic. Differences in the plasma corticosterone and neuropeptide mRNA levels across groups of the same steroid treatment were determined using single factor ANOVA, followed by Fisher's least significant squares test. Single factor ANOVA followed by Dunnet's test was used to determine significant differences between intact and steroid-treated animals. Linear regression analyses were performed using the least-squares method. $P < 0{\cdot}05$ was regarded as being statistically significant for all tests. Statistical analyses were performed using Excel (v4.0; Microsoft Corp.) and Systat (v5.2; Systat Inc., Chicago, IL, USA) for the Macintosh computer.

RESULTS

Fluid intake data: effect of aldosterone and corticosterone replacement on the sodium appetite of adrenalectomized rats

The efficacy of the 250 μg d-aldosterone pellets in adrenalectomized animals was demonstrated by a marked reduction in sodium appetite manifested by a decrease in the volume of saline consumed and increased consumption of water (Fig. 2). Implantation of the 0, 100 or 200 mg corticosterone pellets transiently increased the sodium appetite in all adrenalectomized aldosterone-replaced animals, although the onset of this increase was more rapid in the animals given the higher dosage corticosterone pellets (Fig. 2). No such increase was evident in animals with 50 mg pellets. Intact animals offered the choice of saline and water also exhibited a sodium appetite with no apparent difference from that initially expressed by adrenalectomized animals implanted with d-aldosterone pellets (Fig. 2).

Plasma corticosterone levels and thymus weights in adrenalectomized animals after subcutaneous pellet implantation

Plasma corticosterone was undetectable in all adrenalectomized animals with 0 mg (control) pellets, and in intact animals killed at 08.00 h (Table 1). In animals given 50 mg corticosterone pellets plasma concentrations were approximately 40 ng ml^{-1} and approximately 185 ng ml^{-1} with 200 mg pellets. The plasma concentrations in adrenalectomized animals with 200 mg pellets were no different from those in intact animals killed at 18.00 h. In animals with corticosterone pellets of the same size, there were no significant differences in plasma corticosterone concentration between animals with and without

aldosterone replacement pellets. The efficacy of the corticosterone pellets was demonstrated by the significant correlation between thymus weight and $\log_{10}$ plasma corticosterone concentrations both in the absence ($r = 0{\cdot}967$, $F = 260{\cdot}44$, $P < 0{\cdot}0001$) and presence ($r = 0{\cdot}960$, $F = 211{\cdot}01$, $P < 0{\cdot}0001$) of aldosterone.

Analysis of the effects of adrenal steroids on neuropeptide mRNA levels

The dose–response relationships between plasma corticosterone and neuropeptide gene expression was examined in two ways. First, the correlation between neuropeptide mRNA levels and plasma corticosterone in the absence and presence of aldosterone was determined using least-squares regression analysis. Second, to explore the effects of aldosterone, animals were assigned to four groups according to their plasma corticosterone concentrations at perfusion as follows: group 1, undetectable; group 2, 20–50 ng ml^{-1}; group 3, 51–120 ng ml^{-1}; and group 4, >120 ng ml^{-1}. After this assignment, there were again no significant differences between animals with or without aldosterone replacement (Table 1).

Since there were no significant differences between the two groups of intact animals (Fig. 1, treatments C and D) in any of the data described below, values were combined for subsequent statistical analyses.

Effects of adrenal steroid replacement on mRNA levels in the PVH and supraoptic nucleus

CRH mRNA. Corticosterone decreased CRH mRNA in the PVHmpd (Figs 3 and 4). There was a highly

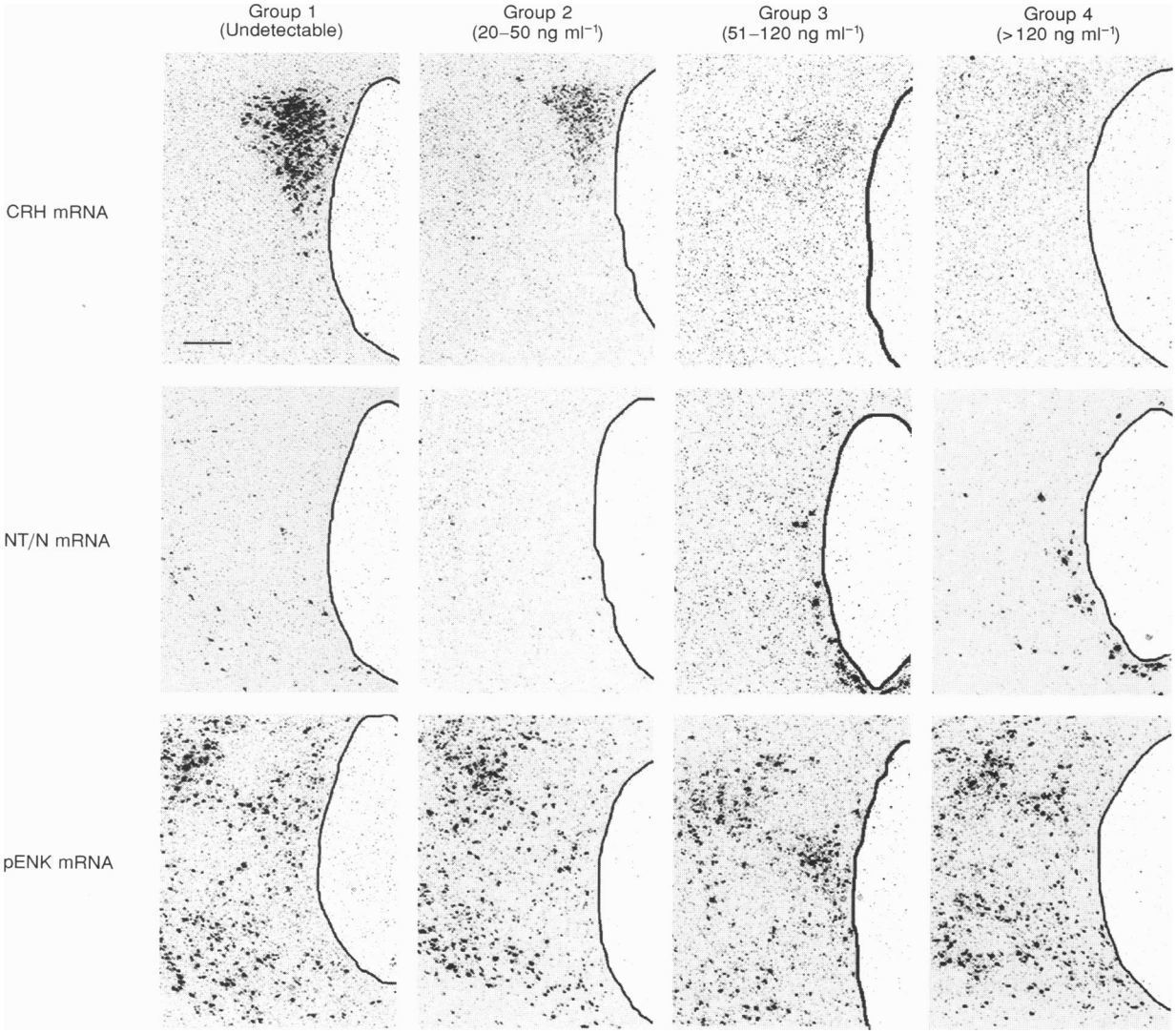

Figure 3. Peptide mRNA hybridization in the PVH

Dark-field photomicrographs of the CRH, NT/N and pENK mRNA hybridization in the PVHmpd of 4 adrenalectomized animals with CRH mean grey level values close to the mean of each of the corticosterone-only replacement groups (see Methods and Table 1 for details). For each animal the 3 sections are serial or closely adjacent. The scale bar in the first panel represents 200 μm.

Table 2. Linear regression equations

CRH mRNA	
No aldo	Aldo
PVHmpd = 164·74 − 58·75 $\log_{10}$(CORT) $r = 0{\cdot}940$, $F = 137{\cdot}03$, $P < 0{\cdot}0001$	PVHmpd = 190·31 − 71·86 $\log_{10}$(CORT) $r = 0{\cdot}949$, $F = 161{\cdot}87$, $P < 0{\cdot}0001$
CEAl = 38·63 + 56·36 $\log_{10}$(CORT) $r = 0{\cdot}778$, $F = 131{\cdot}76$, $P < 0{\cdot}0001$	CEAl = 95·42 + 36·36 $\log_{10}$(CORT) $r = 0{\cdot}660$, $F = 13{\cdot}88$, $P < 0{\cdot}002$
BSTov = 98·70 + 0·1493 CORT $r = 0{\cdot}693$, $F = 16{\cdot}62$, $P < 0{\cdot}001$	BSTov = 117·00 + 0·0018 CORT $r = 0{\cdot}071$, $F = 0{\cdot}003$, $P =$ n.s.
BSTfus = 97·56 + 0·13 CORT $r = 0{\cdot}696$, $F = 16{\cdot}95$, $P < 0{\cdot}001$	BSTfus = 110·69 + 0·13 CORT $r = 0{\cdot}071$, $F = 0{\cdot}09$, $P =$ n.s.

Equations represent the response of the mean grey level from corticotropin-releasing hormone (CRH) mRNA to plasma corticosterone, in the dorsal medial parvicellular part of the paraventricular nucleus (PVHmpd), lateral part of the central nucleus of the amygdala (CEAl), oval nucleus of the bed nucleus of the stria terminalis (BSTov), and the fusiform nucleus of the bed nucleus of the stria terminalis (BSTfus) in adrenalectomized animals without (No aldo) and with aldosterone (Aldo) replacement. n.s., not significant.

significant negative correlation between plasma corticosterone and CRH mRNA levels in the PVHmpd both with and without aldosterone (Table 2). Corticosterone both by itself and in combination with aldosterone (Fig. 4*A*), significantly reduced the levels of CRH mRNA hybridization in the PVHmpd (no aldosterone, $F = 51{\cdot}45$; with aldosterone $F = 128{\cdot}28$; both $P < 0{\cdot}0001$). Intact animals (Fig. 1; treatments C and D) had significantly lower ($P < 0{\cdot}002$) CRH mRNA levels than those seen in group 1 animals without aldosterone (Fig. 4*A*). The slopes in Fig. 4*A* suggested that CRH mRNA was most sensitive to plasma corticosterone at concentrations up to 120 ng ml^{-1}. In animals given no corticosterone replacement, aldosterone produced a small but significant ($P < 0{\cdot}05$) increase in CRH mRNA hybridization in the PVHmpd (Figs 4*A* and 5). No effects of aldosterone were seen in the presence of any dose of corticosterone (Fig. 4*A*; Table 2). The mean grey level from CRH mRNA hybridization in the PVHmpd was significantly correlated with the thymus weight ($r = 0{\cdot}923$; $F = 103{\cdot}86$; $P < 0{\cdot}0001$).

In no animal was a CRH mRNA hybridization signal detected in the supraoptic nucleus or in regions of the PVH containing magnocellular neurosecretory neurones.

Neurotensin/neuromedin N mRNA. Figure 3 shows that no NT/N mRNA was detectable in any region of the PVH or supraoptic nucleus after adrenalectomy, or treatment with aldosterone and/or corticosterone. However, as previously reported (Watts & Sanchez-Watts, 1993) corticosterone did increase NT/N mRNA in the adjacent periventricular nucleus (Fig. 3) in a dose-dependent manner.

Proenkephalin mRNA. Although pENK mRNA was found throughout the medial hypothalamus at the level of the PVH, none was detected in the dorsal part of the PVHmpd in any animal. Similarly, pENK mRNA was undetectable in the magnocellular regions of the PVH and the supraoptic nucleus after adrenalectomy or any steroid treatment.

Effects of adrenal steroid replacement on mRNA levels in the CEA

CRH mRNA. Corticosterone significantly increased CRH mRNA in the CEAl (Figs 4*B* and 6). Regression analysis showed there was a significant positive correlation between $\log_{10}$ plasma corticosterone concentrations and the CRH mRNA hybridization signal in the CEAl (Table 2). There was a similar significant relationship when the data were grouped according to plasma corticosterone concentrations (Fig. 4*B*; $F = 11{\cdot}53$; $P < 0{\cdot}0005$). The slope in Fig. 4*B* suggested that CRH mRNA was most sensitive to plasma corticosterone at concentrations up to 120 ng ml^{-1}. Aldosterone modulated the response of CRH mRNA to corticosterone as shown by the increase in the y-intercept and a reduction in the slope of the regression equation (Table 2). Inspection of the grouped data (Fig. 4*B*) showed that simultaneous administration of aldosterone blunted the effects of increasing plasma corticosterone concentrations on CRH mRNA ($F = 1{\cdot}91$; $P = 0{\cdot}169$) due to a significant increase of CRH mRNA levels in the aldosterone-treated animals (Fig. 4*B*) in corticosterone groups 1 ($P < 0{\cdot}05$) and 2 ($P < 0{\cdot}01$).

CRH mRNA levels in intact animals were not significantly different from animals in either groups 1 or 2 (Fig. 4*B*), but were significantly lower than those in

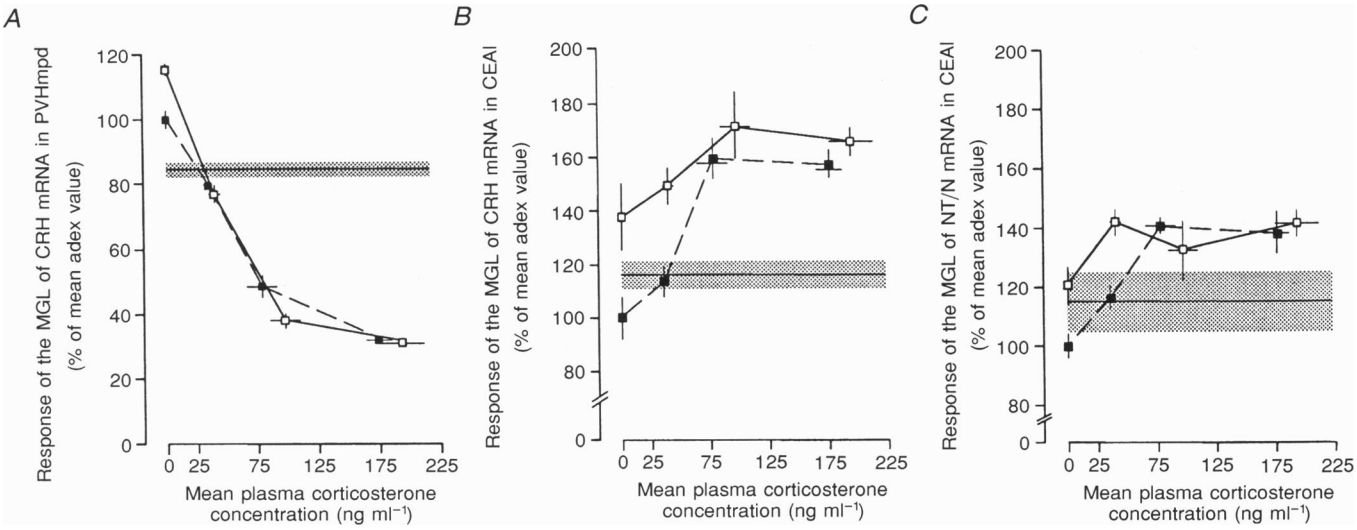

Figure 4. Dose–response relationship in the PVH or CEAl or NT/N mRNA between CRH mRNA and corticosterone

The mean (± S.E.M.) response of the mean grey level (MGL) of CRH mRNA in the PVHmpd (*A*), CEAl (*B*) or NT/N mRNA in the CEAl (*C*) to increasing plasma corticosterone in adrenalectomized (adex) animals either with (open squares, continuous line; $n = 20$) or without (filled squares, dashed line; $n = 20$) aldosterone. Animals are grouped according to the four ranges of plasma corticosterone (see Methods and Table 1 for details). Horizontal bars at each point represent the S.E.M. of the plasma corticosterone in that group. For each region the mean ± S.E.M. of the mean grey level of intact animals is represented by the horizontal shaded bar.

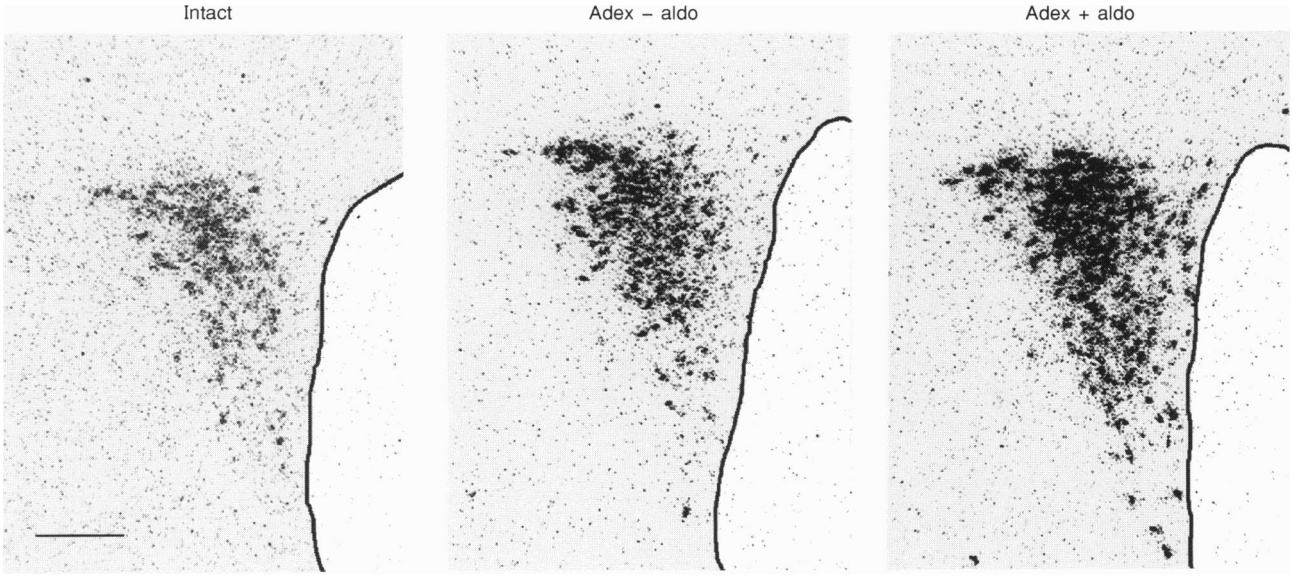

Figure 5. Effect of aldosterone on CRH mRNA hybridization in the PVH

Dark-field photomicrographs of CRH mRNA hybridization in the PVHmpd of an intact, an adrenalectomized animal with no aldosterone (Adex – aldo), and an animal with aldosterone replacement (Adex + aldo). Animals chosen had mean grey level values close to the mean of each group. Medial is to the right. The scale bar in the first panel represents 200 μm.

group 3 ($P < 0{\cdot}05$ without aldosterone; $P < 0{\cdot}0001$ with aldosterone) or group 4 ($P < 0{\cdot}01$ without aldosterone; $P < 0{\cdot}015$ with aldosterone). The mean grey level from CRH mRNA hybridization in the CEAl was significantly correlated with the thymus weight ($r = 0{\cdot}777$; $F = 27{\cdot}48$; $P < 0{\cdot}0002$).

Neurotensin/neuromedin N mRNA. Corticosterone significantly increased NT/N mRNA in the CEAl (Fig. 4*C* and 6). Regression analysis showed there was a significant positive correlation between plasma corticosterone concentrations and the NT/N mRNA hybridization signal in the CEAl ($r = 0{\cdot}65$, $F = 13{\cdot}16$, $P < 0{\cdot}002$). NT/N mRNA was again most sensitive to plasma corticosterone at concentrations up to 120 ng ml^{-1} (Fig. 4*C*) and was significantly correlated to $\log_{10}$ of the plasma corticosterone concentration ($F = 8{\cdot}87$; $P < 0{\cdot}015$). Aldosterone significantly increased the levels of NT/N mRNA in group 1 and 2 animals (Fig. 4*C*; both $P < 0{\cdot}05$), and abolished the dose-dependent effects of corticosterone (Fig. 4*C*; $F = 1{\cdot}86$; $P = 0{\cdot}177$). NT/N mRNA in the CEAl of intact animals was not significantly different from those seen in groups 1, 2 or 4 (Fig. 4*C*), but were significantly lower than those seen in group 3 ($P < 0{\cdot}05$). In the absence of aldosterone, the mean grey level from

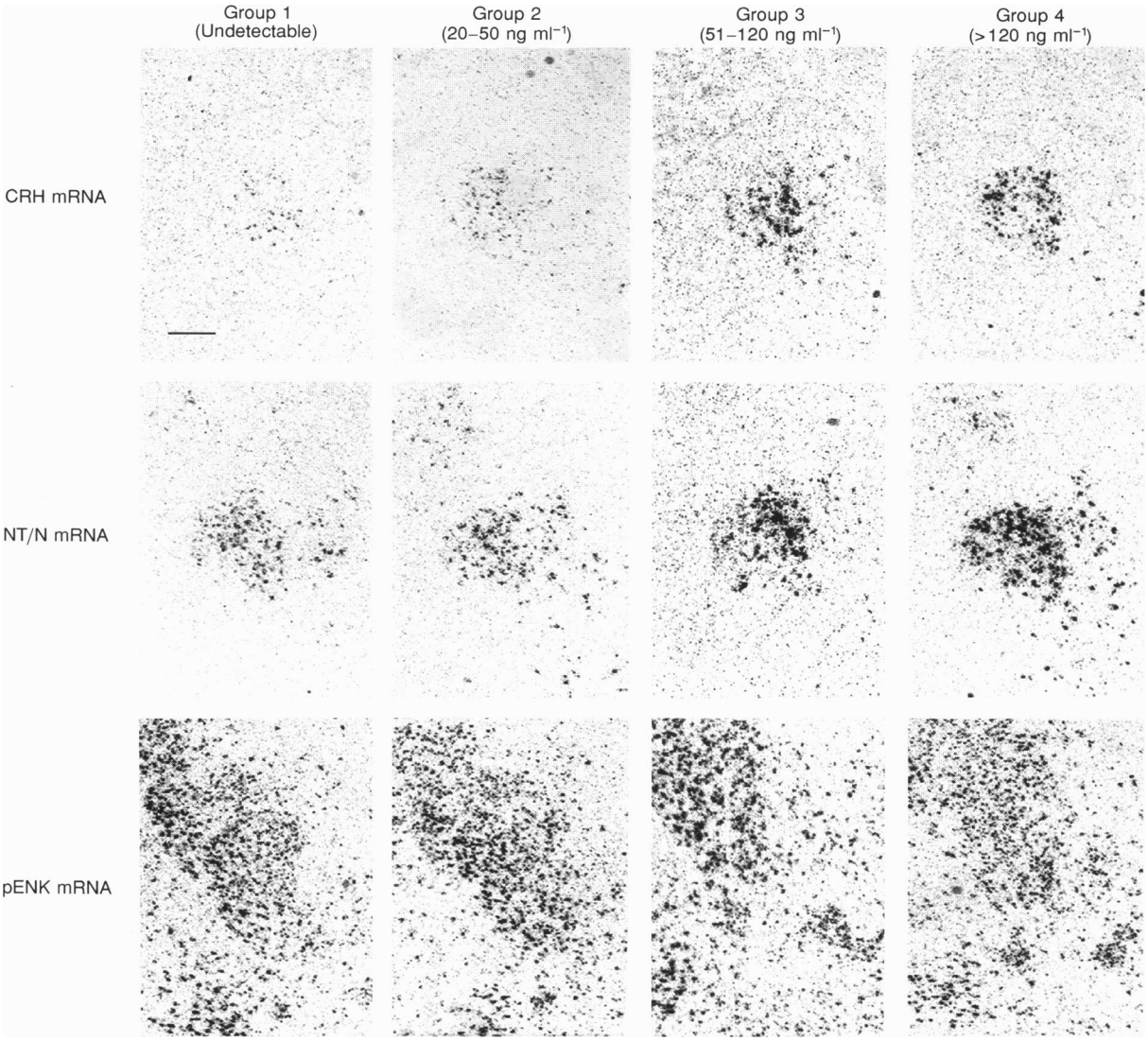

Figure 6. Peptide mRNA hybridization in the CEA

Dark-field photomicrographs of the CRH, NT/N and pENK mRNA hybridization in the CEAl of four adrenalectomized animals with CRH mean grey level values close to the mean of each of the corticosterone-only replacement groups. For each animal the three sections are serial or closely adjacent. The rostrocaudal level corresponds approximately to level 28 in the atlas of Swanson (1992). The scale bar in the first panel represents 200 μm.

NT/N mRNA hybridization in the CEAl was significantly correlated with the thymus weight ($r = 0{\cdot}563$; $F = 8{\cdot}33$; $P < 0{\cdot}01$).

Proenkephalin mRNA. pENK mRNA was widely distributed throughout the CEAl, but no steroid treatment had any apparent effects on the levels (Fig. 6). No quantitation was performed.

Co-regulation of CRH mRNA by corticosterone in the PVH and the CEA

Despite the difference in the slopes, the form of mRNA responses to corticosterone in the PVHmpd and CEAl appeared to be similar. This was evident in both regions as a decrease in sensitivity to those plasma corticosterone concentrations greater than 120 ng ml^{-1} (Fig. 4*A* and *B*). To explore this observation, we tested two related hypotheses. The first is that corticosterone regulates mRNA levels in the PVHmpd and CEAl in an associated way (hypothesis 1). This predicts that the CRH mRNA mean grey levels in the PVHmpd and CEAl must be closely correlated. It also predicts that when data from both regions are normalized with the respective mean value from adrenalectomized animals, the CRH mRNA values in the PVHmpd of intact animals should predict those in the CEAl in intact animals and vice versa. It also predicts that NT/N and CRH mRNAs in the CEAl are correlated. The second related hypothesis (hypothesis 2) is that corticosterone is a central factor in determining CRH mRNA levels in intact animals. This hypothesis emerges from the observation that mean CRH mRNA values from the CEAl and PVHmpd of intact animals are both distributed close to those of the group 2 corticosterone-only animals (Fig. 4*A* and *B*). It predicts that the same plasma corticosterone concentration must maintain CRH mRNA in both the PVHmpd and CEAl of adrenalectomized animals at levels corresponding to those found in intact animals.

Hypothesis 1 was tested in two ways. First, there was a significant correlation between the CRH mRNA values

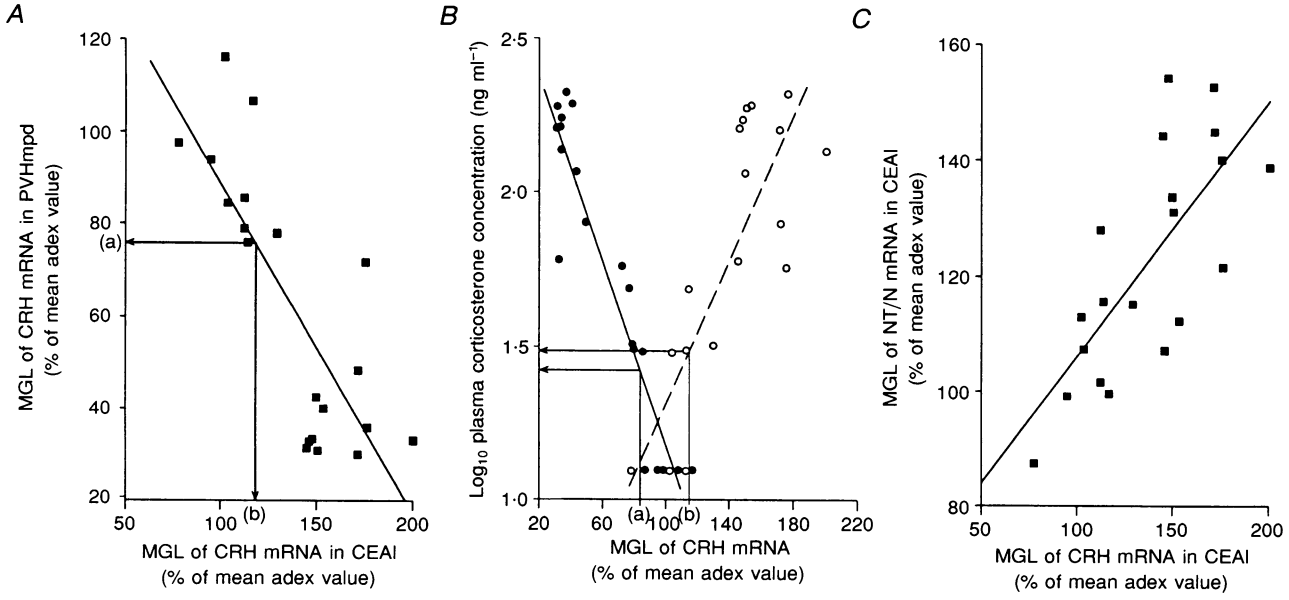

Figure 7. Regression analyses of the dose–response relationship of CRH mRNA to corticosterone

A, the response of the mean grey level (MGL) from CRH mRNA hybridization in the PVHmpd to increasing CRH mRNA in the CEAl of adrenalectomized animals (adex) with corticosterone replacement but without aldosterone ($n = 20$). (a) represents the theoretical intact mean grey level of CRH mRNA hybridization in the PVHmpd predicted from the intact values in the CEAl. (b) represents the theoretical intact mean grey level of CRH mRNA hybridization in the CEAl predicted from the intact values in the PVHmpd (see text for details). *B*, the response of $\log_{10}$ plasma corticosterone concentrations to increasing CRH mRNA in the CEAl (open symbols, dashed line), and to increasing CRH mRNA in the PVHmpd (closed symbols, continuous line), in adrenalectomized animals (adex) with corticosterone replacement without aldosterone ($n = 20$). (a) represents the mean MGL value of CRH mRNA in the PVHmpd of intact animals and the corresponding predicted $\log_{10}$ plasma corticosterone concentration; (b) represents the MGL means of CRH mRNA in the CEAl of intact animals and the corresponding predicted $\log_{10}$ plasma corticosterone concentration (see text for details). *C*, the response of the MGL from NT/N mRNA in the PVHmpd to increasing CRH mRNA in the CEAl in adrenalectomized animals (adex) with corticosterone replacement without aldosterone ($n = 20$).

from the PVHmpd and the CEAl (Fig. 7*A*; $r = 0{\cdot}72$, $F = 19{\cdot}82$, $P < 0{\cdot}0005$). Second, two least-squares regression equations were calculated from these data (Fig. 7*A*): (1) using the CRH mRNA values (expressed as a percentage of the mean adrenalectomized CRH mRNA MGL) from the CEAl as the independent variable; and (2) using the equivalent values from the PVHmpd as the independent variable. Using these two equations, there was no significant difference (unpaired *t* test) between the actual and predicted values of the intact mean grey levels when intact values of the independent variable were used to predict the intact values of the respective dependent variable: Fig. 7*A* value (a), PVHmpd as dependent variable, actual intact mean value (± S.E.M.) 84·4 ± 2·1%, predicted intact 77·1 ± 2·4% of mean adrenalectomized value, $n = 6$; Fig. 7*A* value (b), CEAl as dependent variable, actual intact mean value (± S.E.M.) 116·3 ± 5·0%, predicted intact 117·1 ± 2·1% of mean adrenalectomized value.

Hypothesis 2 predicts that in intact animals, the respective theoretical plasma corticosterone concentrations corresponding to the CRH mRNA levels in the PVHmpd and CEAl should not be significantly different, despite the opposing slopes of the response curves. To test this, two least-squares regression equations were determined using

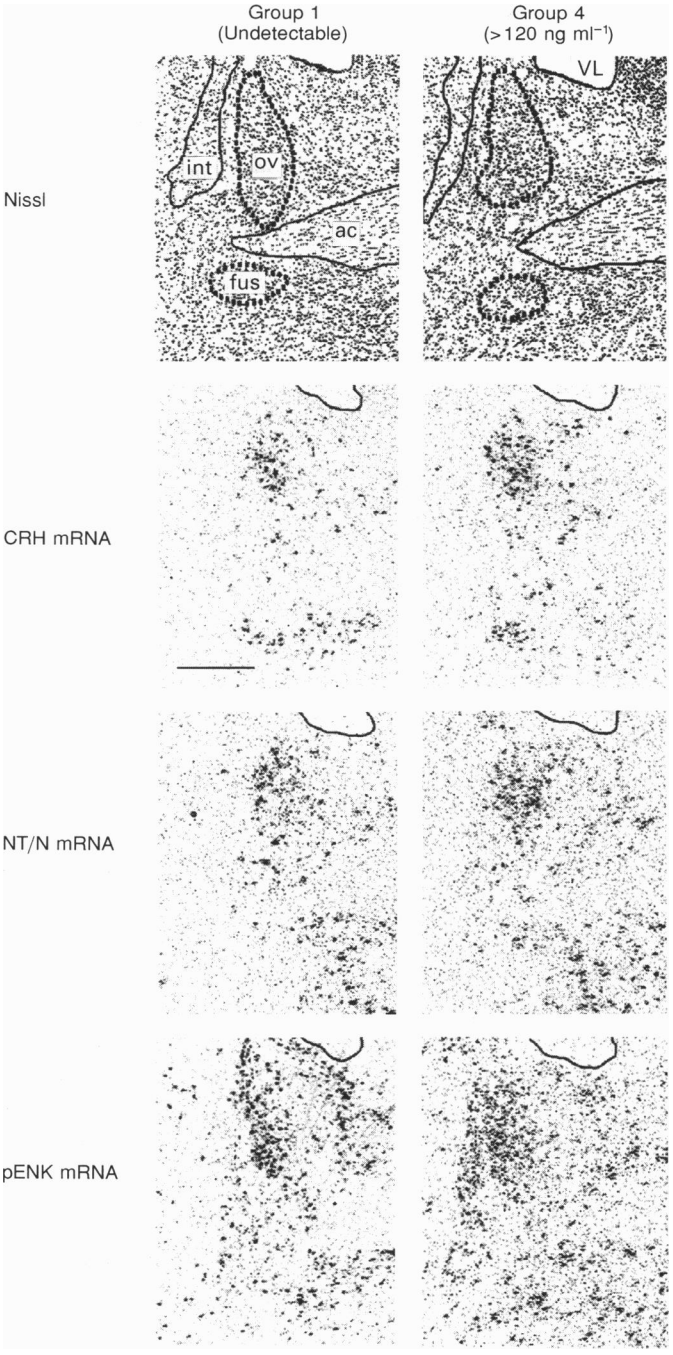

Figure 8. Peptide mRNA hybridization in the bed nuclei of the stria terminalis

Photomicrographs of the Nissl-stained section, and sections with CRH, NT/N and pENK mRNA hybridization in the BST from two adrenalectomized animals with CRH mean grey level values close to the mean of group 1 and group 4 corticosterone-only replacement groups. For each animal, the 4 sections are serial or closely adjacent. The rostrocaudal level corresponds approximately to level 19 in the atlas of Swanson (1992). Medial is to the right. ac, anterior commissure; fus, fusiform subdivision of the BST; int, internal capsule; ov, oval subdivision of the BST; VL, lateral ventricle. The scale bar in the third panel represents 500 μm. Images were recorded and processed as Fig. 4.

the $\log_{10}$ of the plasma corticosterone values as the dependent variable, and the CRH mRNA mean grey levels as the independent variables, the first equation from the CEAl and the second from the PVHmpd (Fig. 7*B*). These two regression equations were then used to predict theoretical plasma corticosterone concentrations using PVHmpd and CEAl CRH mRNA mean grey level values from each intact animal. There was no significant difference (unpaired *t* test) between the mean predicted plasma corticosterone concentration for intact values derived from the PVHmpd regression equation (Fig. 7*B*a, mean ± S.E.M., 23·9 ± 2·3 ng ml^{-1}, $n = 6$) and those derived from the CEAl regression equation (Fig. 7*B*b, mean ± S.E.M., 26·4 ± 5·1 ng ml^{-1}, $n = 6$).

Co-regulation of CRH and NT/N mRNAs by corticosterone in the CEAl. A strong correlation between CRH and NT/N mRNA levels in the CEAl ($r = 0{\cdot}740$, $F = 21{\cdot}74$, $P < 0{\cdot}0002$) was revealed by least-squares regression analysis (Fig. 7*C*).

Effects of adrenal steroid replacement on mRNA levels in the BST

CRH mRNA – the oval subdivision. Corticosterone significantly increased CRH mRNA in cells of the BSTov (Fig. 8). Although, in contrast to the effects in the PVHmpd and CEAl, they appeared to be rather insensitive (Figs 8 and 9*A*; $F = 4{\cdot}99$; $P < 0{\cdot}015$). Significant increases above the levels seen in group 1 were seen only in group 4 animals ($P < 0{\cdot}005$). This represented a 25% increase above the signal seen in the animals with no corticosterone, as compared to the 63% increase seen in the CEAl. Furthermore, the response in the BSTov appeared to be much more linear over the tested range of plasma corticosterone than in the PVHmpd or the CEAl (Fig. 9*A*). In intact animals CRH mRNA levels were not significantly different from those of groups 1, 2 or 3, but were significantly lower than those of group 4 ($P < 0{\cdot}01$).

Treatment of adrenalectomized animals with aldosterone abolished the dose-dependent effects of corticosterone (Fig. 9*A*; $F = 0{\cdot}78$; $P = 0{\cdot}522$) due to an increase in the values at low doses of corticosterone and a corresponding decrease in the overall slope (Fig. 9*A*). Figure 9*A* showed that animals with aldosterone in groups 1 and 2 had significantly higher CRH mRNA than those without aldosterone (group 1, $P < 0{\cdot}025$; group 2, $P < 0{\cdot}05$).

CRH mRNA – the fusiform subdivision. Although CRH mRNA levels were correlated with increasing plasma corticosterone concentrations (Table 2), the slope of the response suggested these cells were somewhat insensitive. This was confirmed when the data were examined as grouped data; the increases were not quite significant over the range of plasma corticosterone examined ($F = 2{\cdot}98$; $P = 0{\cdot}063$), or in the presence of aldosterone ($F = 1{\cdot}47$; $P = 0{\cdot}261$).

NT/N mRNA – the oval subdivision. Although there was a tendency for plasma corticosterone – both with and without aldosterone – to increase NT/N mRNA levels,

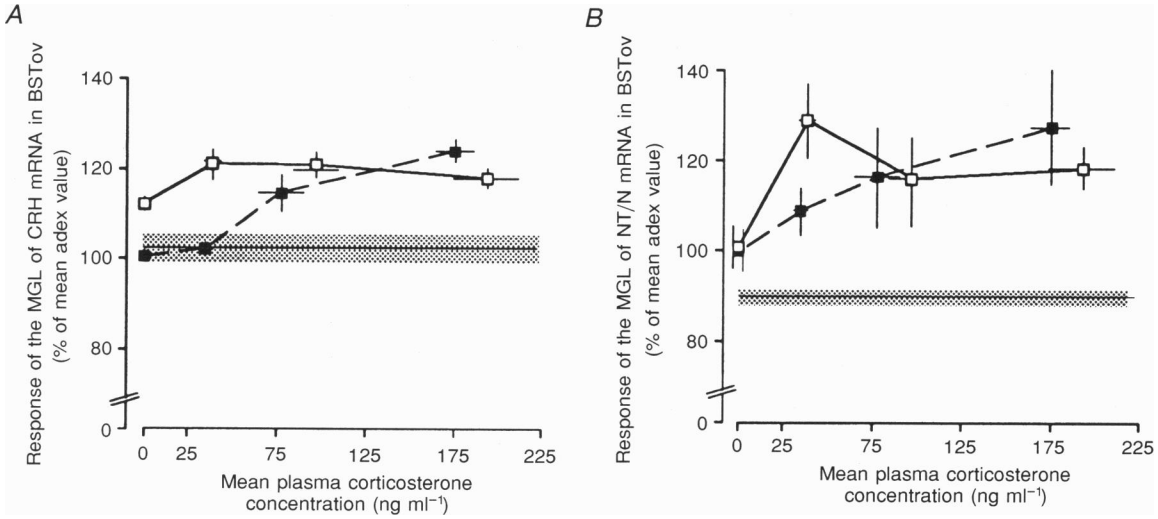

Figure 9. Dose–response relationship in the BST between CRH mRNA or NT/N mRNA and corticosterone

The mean (± S.E.M.) response of the mean grey level from CRH mRNA hybridization in the BSTov (*A*), and NT/N mRNA in the BSTov (*B*) to increasing plasma corticosterone in adrenalectomized animals (adex) either with (open squares, continuous line; $n = 20$) or without (filled squares, dashed line; $n = 20$) aldosterone. Animals are grouped according to the 4 ranges of plasma corticosterone (see Methods and Table 1 for details). Horizontal bars at each point represent the S.E.M. of the plasma corticosterone in that group. The mean and S.E.M. of intact animals is represented by the horizontal shaded bar.

these were not significant (Fig. 9*B*). NT/N mRNA levels in intact animals were significantly lower than those in group 4 animals without aldosterone ($P < 0{\cdot}01$), and groups 2 ($P < 0{\cdot}0001$), 3 ($P < 0{\cdot}005$), and 4 ($P < 0{\cdot}002$) with aldosterone.

NT/N mRNA – the fusiform subdivision. NT/N mRNA was not detected in the BSTfus of any animal.

Proenkephalin mRNA. Although cells in both the BSTov and BSTfus nuclei appeared to contain pENK mRNA (Fig. 8) there were no apparent differences with any steroid treatment, and levels were not quantitated.

Effects of adrenal steroid replacement on mRNA levels in the lateral hypothalamus

Corticotropin-releasing hormone mRNA. Although a few labelled cells were visible in group 1 animals, none were seen in intact animals or in animals treated with any combination of adrenal steroids (data not shown).

NT/N mRNA. Neither adrenalectomy nor corticosterone-replacement with or without aldosterone had any effect on the levels of NT/N mRNA levels in the lateral hypothalamus. NT/N mRNA in intact animals was not significantly different from any treatment group (data not shown).

DISCUSSION

Two broad conclusions emerge from our data. First, adrenal steroids can selectively and differentially regulate the levels of the mRNAs encoding CRH and NT/N, but not pENK in the PVHmpd, CEAl and BST. In the lateral hypothalamic area, however, the mechanisms governing CRH and NT/N mRNA are unaffected by circulating adrenal steroids in the experimental model used here. Second, the regulatory mechanisms responsible for these effects in the PVHmpd and CEAl are most sensitive to plasma corticosterone concentrations between 0 and 120 ng ml^{-1} (see also Swanson & Simmons, 1989), which correspond to those found during most of the normal diurnal secretory pattern away from the peak values (Dallman *et al.* 1987). We now show that these observations also extend to CRH and NT/N mRNAs in the CEAl. Together these results suggest that the plasma corticosterone concentrations found during most of the day in unstimulated animals play an important role in maintaining CRH and NT/N mRNA levels. Furthermore, they show that if plasma corticosterone concentrations are even slightly elevated above the daily mean and maintained for an extended period they can have a significant impact on the levels of peptide mRNAs. Before discussing our results in detail it is important to emphasize that we do not present evidence that corticosterone and aldosterone affect mRNA levels through a direct action on peptide gene transcription. However, we do show that corticosterone and aldosterone will selectively alter the accumulation rates of cytoplasmic mRNAs encoding CRH and NT/N in a cell-specific manner, and thus may selectively modify the amounts of mRNA available for translation into biologically active neuropeptides in different cell groups.

Despite increasing plasma corticosterone having opposite effects on CRH mRNA in the CEAl and PVHmpd, our results show that in adrenalectomized animals very similar concentrations of plasma corticosterone can maintain CRH mRNA in these two cell groups at levels seen in intact animals during the middle of the diurnal cycle. In addition, there is a close correlation between the levels of CRH hybridization in the CEAl and PVHmpd, as well as CRH and NT/N hybridization in the CEAl. Together these data strongly implicate plasma corticosterone as an important factor regulating the overall accumulation rates of these mRNAs in the CEAl and PVHmpd of unstimulated intact male rats. However, it is important to emphasize the existence of other mechanisms that also modulate levels of CRH mRNA on an hour-to-hour basis (Watts & Swanson, 1989; Kwak, Morano, Young, Watson & Akil, 1993). It is worth noting that the range of plasma corticosterone concentrations that can normalize neuropeptide mRNA levels appears to be narrow, and is comparable to the range required to normalize body and thymus weights and plasma ACTH concentrations after adrenalectomy (Dallman *et al.* 1987).

Two observations should be noted with regard to the BST. First, CRH mRNA in the BST appears to be much less sensitive to the effects of adrenal steroids than in the PVHmpd or CEAl, while NT/N mRNA in the BSTov is insensitive. Second, that NT/N mRNA in the BSTov of intact animals is always lower than in any of the adrenalectomized animals. Together these data illustrate differences in the nature of the BSTov and the CEAl, and implicate factors other than adrenal steroids in regulating levels of these mRNAs.

The effects we have described could be mediated directly by receptor mechanisms in the PVHmpd, CEAl and BST, indirectly by steroid receptor mechanisms modulating afferent neural inputs, or through a combination of both mechanisms. With regard to receptor mechanisms, two distinct receptor types have already been identified in the brain. The mineralocorticoid receptor (MR or type I) and glucocorticoid receptor (GR or type II) have similar specificities for aldosterone and corticosterone; however, while the type II has approximately equal binding affinity (K_d) for both steroids, type I has an approximately ten times higher affinity for corticosterone than does the type II (Reul & de Kloet, 1985). After binding corticosterone or aldosterone, both receptor types act as ligand-activated transcription factors (for references, see de Kloet *et al.* 1993), and can affect the transcription of CRH and NT/N genes when expressed in cultured cell lines, presumably

through the appropriate glucocorticoid response elements (Adler, Smas & Majzoub, 1988; Kislaukis & Dobner, 1990). The hippocampal type I population is at least 90% occupied at plasma corticosterone concentrations as low as 10 ng ml^{-1}, while the type II population shows increasing occupancy between 10 and 1000 ng ml^{-1} (Reul & de Kloet, 1985). In a series of elegant studies Dallman and co-workers have shown in adrenalectomized rats that corticosterone will reduce plasma ACTH concentrations with an ED_{50} of < 10 ng ml^{-1}, strongly suggesting a type I-related mechanism (Dallman *et al.* 1987; Levin, Akana, Jacobson, Kuhn, Siiteri & Dallman, 1987; Dallman, Levin, Cascio, Akana, Jacobson & Kuhn, 1989). That dexamethasone (a type II specific agonist) has little effect on CRH secretion into hypophysial portal blood of adrenalectomized rats again implicates a type I-related mechanism here (Fink, Robinson & Tannahill, 1988). On the other hand the response of thymus weight to increasing plasma corticosterone concentrations shows an ED_{50} of 60–80 ng ml^{-1} (Dallman *et al.* 1987, 1989; Levin *et al.* 1987) suggesting a type II specificity to this response. Our data also show an approximate ED_{50} of 60 ng ml^{-1} for the thymus response to corticosterone, while in the PVHmpd and CEAl the ED_{50} of corticosterone's effects on neuropeptide mRNAs was between 40 and 55 ng ml^{-1} in all cases (Figs 4 and 9). This observation, together with the data of Dallman *et al.* (1987, 1989), and the strong correlation between thymus weight and neuropeptide mRNA hybridization in the PVHmpd and the CEAl, would accord with the operation of type II- rather than type I-related mechanisms in regulating neuropeptide mRNA levels in both cell groups.

Data regarding a precise site of action for the effects of corticosterone point to a rather complex scenario. Studies employing biochemical and neuroanatomical approaches have shown that cells in the PVHmpd, CEAl and the BSTov have relatively high numbers of type II (Reul & de Kloet, 1985; Liposits, Uht, Harrison, Gibbs, Paull & Bohn, 1987; Arriza, Simerly, Swanson & Evans, 1988; Swanson & Simmons, 1989; Ahima & Harlan, 1990; Honkaniemi *et al.* 1992) thus providing anatomical data in support of type II-mediated effects directly in these cell groups. Similarly, dexamethasone applied either systemically or locally to the PVHmpd will inhibit adrenalectomy-induced increases in CRH mRNA and peptide (Young *et al.* 1986; Kovács & Mezey, 1987; Sawchenko, 1987). However, opposing a direct effect of corticosterone on the PVHmpd are the data of Kovács & Mezey (1987) who showed that local application of corticosterone to the PVHmpd was ineffective at reducing CRH mRNA. The data of Herman and co-workers (1989) show that the hippocampus is one possible site where corticosterone could influence CRH mRNA in the PVH. The question of whether corticosterone acts directly or indirectly to increase CRH and NT/N mRNA in the CEAl has not yet been addressed.

In the CEAl and BSTov, aldosterone appeared to amplify the effects of lower (< 120 ng ml^{-1}) but not higher concentrations of corticosterone, whereas in the PVHmpd aldosterone treatment increased CRH mRNA only in the absence of corticosterone. In the presence of corticosterone, the difference between the effects of aldosterone in the PVHmpd, and those in the CEAl and BSTov may be related to the opposing responses of these cell types to corticosterone, with aldosterone perhaps adding to the corticosterone-driven stimulatory effect already present in the CEAl and BSTov, but absent in the PVHmpd. Using an intraperitoneal saline injection as a stressor, Lightman & Young (1989) showed that CRH gene expression in the PVHmpd is actually not maximal in adrenalectomized animals and, with appropriate stimulation, can be increased even further. Here we observed a similar phenomenon; aldosterone treatment increased CRH mRNA levels in the PVHmpd above those already induced by adrenalectomy. Together with those of Lightman & Young (1989), our data point to the existence of mechanisms operating on PVHmpd neurones that can directly stimulate CRH mRNA accumulation, in contrast to mechanisms which might increase CRH mRNA by inhibiting the negative feedback effects of corticosterone. In the absence of corticosterone, the fact that aldosterone treatment increased CRH mRNA levels in all three cell groups suggests that a similar stimulatory mechanism might exist in other neurones expressing the CRH gene, whereas those mediating the inhibitory effects of corticosterone are clearly absent in the CEAl and BSTov.

The nature of the mechanism that increased CRH and NT/N mRNAs in the PVHmpd, BSTov, and CEAl after aldosterone treatment is unclear. As with the effects of corticosterone, those of aldosterone may involve direct receptor activation, since both type I and type II will bind aldosterone in the absence of corticosterone (Reul & de Kloet, 1985). However, since we did not obtain aldosterone dose–response data for neuropeptide mRNA levels, we cannot comment directly on the likelihood of type II- as opposed to type I-related mechanisms. Clearly, indirect mechanisms involving the effects of aldosterone on central and peripheral functions may also be significant, and further work is needed to clarify these issues.

Regarding the actions of aldosterone, two further points are worthy of note. First, PVH neurones contain the mRNA for 11β-hydroxysteroid dehydrogenase (Seckl, Dow, Low, Edwards & Fink, 1993), an enzyme able to impart aldosterone sensitivity to cells in the face of elevated corticosterone (for references see Seckl *et al.* 1993). Data obtained using a specific inhibitor suggests that 11β-hydroxysteroid dehydrogenase may modulate the feedback action of corticosterone on CRH secretion (Seckl *et al.* 1993). Since aldosterone did not affect the dose–response effects of corticosterone on CRH mRNA in the PVHmpd but did in the CEAl, our data imply that

the action of this enzyme might not be significant in regulating CRH mRNA levels in response to adrenal steroids in the PVHmpd, but may operate in the CEAl. Second, steroid-induced alterations of neuropeptide mRNA levels in the CEAl and BSTov may help modify goal-directed behaviours. In both the CEAl and BSTov, CRH- and neurotensin-immunoreactive neurones have bidirectional connections with the parabrachial nucleus (Moga & Gray, 1985; Moga *et al.* 1989, 1990), a brainstem pontine nucleus important for processing the visceral sensory information used to organize autonomic function and goal-directed behaviours, including sodium appetite (Schulkin, 1992). Thus, it is of interest that the levels of neuropeptide mRNAs are significantly increased in the CEAl and BSTov by an aldosterone treatment which significantly reduces sodium appetite.

Our results have interesting implications for the cell-specific regulation of neuropeptides in different physiological circumstances. In this context, we have already proposed that corticosterone may play a role in maintaining CRH and NT/N mRNAs in the PVHmpd, CEAl and BSTov of the unstimulated male rat. A variety of homeostatic disturbances can increase plasma corticosterone concentrations rapidly and in some cases for extended periods, and our data here emphasize that altered corticosterone secretion in these conditions may also function to regulate neuropeptide mRNA levels. Interestingly, with some stressors CRH mRNA in the PVHmpd still increases despite elevated corticosterone (Harbuz & Lightman, 1989; Lightman & Young, 1989; Imaki *et al.* 1991; Watts & Sanchez-Watts, 1995) emphasizing the complex nature of the mechanisms available to regulate this gene. On the other hand, it is now clear that corticosterone cannot be directly responsible for increasing NT/N and pENK mRNAs in the PVHmpd after various stresses (Lightman & Young, 1989; Watts, 1991; Watts & Sanchez-Watts, 1995), although glucocorticoids can modify the stress-induced increases in pENK mRNA (Lightman & Young, 1989).

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