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Evidence for arginine vasopressin as the primary activator of the HPA axis during adjuvant-induced arthritis

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1 Adjuvant-induced arthritis (AA) is an experimental inflammation of the joints that results in chronic activation of the hypothalamo-pituitary-adrenal (HPA) axis.

2 In this study the role of hypothalamic corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP) in the regulation of the HPA axis in this condition both in Sprague-Dawley (SD), and Piebald-Viral-Glaxo (PVG) rats has been further characterized.

3 The increase in AVP peptide content of portal blood (as early as day 11), just prior to the onset of arthritis is confirmed and further increases, peaking at day 16 are shown, coincident with the progression of inflammation in the PVG rats.

4 The increase in AVP is associated with a significant increase in the expression of AVP but not CRF mRNAs in the medial parvocellular division of the hypothalamic paraventricular nucleus (PVN) of arthritic SD rats.

5 In the presence of maximal inflammation of SD rats there was a significant decrease in the maximum binding of [^{125}I]-Tyr-oCRF to anterior pituitary membranes, whereas AVP receptor concentration in anterior pituitary membranes from both PVG and SD rats showed a significant increase with respect to controls.

6 The basal adrenocorticotrophin (ACTH) secretion *in vitro* was similar in both control and arthritic SD rats but that from arthritic PVG rat pituitaries was significantly greater than the respective controls (436 ± 91 v 167 ± 23 pg/tube). The ACTH response of pituitaries of arthritic PVG rats to CRF or the combination of CRF and AVP was significantly higher compared with the controls, although the ACTH response of arthritic SD rat pituitaries was unchanged.

7 The results are consistent with the view that activation of the parvocellular vasopressin system has an important role in the adaptation of the HPA axis to experimentally-induced chronic stress of arthritis.

Keywords: Arginine vasopressin (AVP); chronic stress; HPA-axis; adjuvant arthritis

Introduction

Arginine vasopressin was the first identified secretagogue of adrenocorticotrophin (ACTH) (Martini, 1966) but it is now generally considered that hypothalamic corticotrophin-releasing factor (CRF) is the primary and most potent activator of the pituitary-adrenal axis (Vale *et al.*, 1981; Rivier & Plotsky, 1986). CRF and AVP produced by the parvocellular neurones of the hypothalamic paraventricular nucleus (PVN) are secreted into the hypophysial portal blood (HPB) from axon terminals projecting to the external zone of the median eminence. On reaching the anterior pituitary corticotrophs, both peptides act synergistically to stimulate the release of ACTH which in turn stimulates the synthesis and release of glucocorticoids from the adrenal cortex. In control rats, AVP is co-localized in approximately half of the CRF positive parvocellular neurones and these neurones are believed to be the main source of AVP in the HPB (Whitnall, 1993). In addition to its intrinsic capacity to stimulate ACTH secretion, AVP has been shown to potentiate the stimulatory effects of CRF (Gillies *et al.*, 1982; Rivier & Vale, 1983) and to have an important physiological role in the control of ACTH secretion during stress. Release of CRF and AVP in response to acute stress is the first stage in the activation of the hypothalamo-pituitary-adrenal (HPA) axis. This activation of the HPA axis is also characterized by increased expression of CRF mRNA and AVP mRNA in the PVN, increased anterior pituitary ACTH precursor, pro-opiomelanocortin (POMC) mRNA, and increased plasma levels of ACTH and corticosterone (Antoni, 1986; Lightman & Young, 1988; Harbuz & Lightman, 1992).

Adjuvant-induced arthritis, (AA) which shares certain characteristics with rheumatoid arthritis, is a chronic inflammatory disease that can be induced in specific rat strains. We have recently reported that chronic arthritis induced in the rat by an intradermal injection of an oil emulsion of heat-killed *Mycobacterium butyricum* results in a persistent activation of the HPA axis with increased adrenal size, increased expression of anterior pituitary POMC mRNA, increased morning circulating ACTH and corticosterone and loss of the normal diurnal rhythm of these hormones (Harbuz *et al.*, 1992; Sarlis *et al.*, 1992; Stephanou *et al.*, 1992). This indicates that in this model of chronic inflammatory stress, the drive to ACTH secretion is increased, despite the chronic increase in corticosteroid negative feedback from the elevated secretion of corticosterone. Since CRF mRNA has been demonstrated to increase after repeated stress (Herman *et al.*, 1989; Lightman & Young, 1989; Imaki *et al.*, 1991; Mamalaki *et al.*, 1992), it is surprising that the chronic inflammatory stress of AA did not result in an increase in CRF mRNA in the parvocellular neurones of the PVN. In the PVG rat the situation is even more paradoxical, since in this strain CRF mRNA actually

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decreases during AA, irrespective of the adjuvant used (Harbuz *et al.*, 1992). In these animals we also found a consistent decrease in CRF-41 peptide release into the HPB, whilst the level of the other secretagogue AVP, was increased 28 days following the induction of AA. These results suggested that AVP might be the predominant factor responsible for the activation of the pituitary-adrenal axis in this condition. In the present study, therefore we have tested this hypothesis by performing repeated measures of AVP in hypophysial portal plasma throughout the time course of the syndrome and by studying the expression of both CRF and AVP mRNAs within the parvocellular division of the PVN at the time of maximal inflammation. We have used PVG and Sprague-Dawley rats, both of which are susceptible to AA, to investigate the role of hypothalamic AVP in the activation of the HPA axis during AA.

Since there is evidence that in several chronic stress paradigms the ACTH response to the primary stressor is reduced (Hashimoto *et al.*, 1988; Hauger *et al.*, 1990), we studied *in vitro*, the regulation of anterior pituitary CRF and AVP receptors and the response of anterior pituitary glands of arthritic and control animals to CRF and/or AVP. It is well established that adrenalectomy (ADX) results in increased CRF immunostaining and expression of CRF mRNA in the parvocellular division of the PVN, increased AVP mRNA in the CRF positive cells (Jingami *et al.*, 1985; Wolfson *et al.*, 1985; Young *et al.*, 1986; Sawchenko, 1987; Lightman & Young, 1989; Swanson & Simmons, 1989) and increased concentrations of CRF and AVP in the HPB (Plotsky & Sawchenko, 1987; Eckland *et al.*, 1988; Fink *et al.*, 1988). Therefore in the present study we also investigated the expression of the two peptide mRNAs in ADX arthritic rats. A preliminary account of these results was presented at the 158th meeting of the British Pharmacological Society in Brighton (Chowdrey *et al.*, 1995).

Methods

Animals

Age-matched adult male Sprague-Dawley rats (250 ± 20 g), or Piebald-Viral-Glazo (PVG) rats (100–150 g), (Bantin & Kingman, Bedfordshire, U.K.) were housed 4 to a cage with free access to food and water. All animals were kept in a 12 h light:12 h dark cycle (lights on at 07 h 00 min). All studies were begun between 09 h 00 min and 10 h 00 min.

Animal procedures

Adjuvant arthritis was induced in the animals by an intradermal injection of 0.1 ml of a 10 mg ml^{-1} suspension of ground heat-killed *Mycobacterium butyricum* (Difco, Detroit, Michigan, U.S.A.) in paraffin oil (Fluka Chemie AG, Buchs, Switzerland) into the tail base. Control animals were injected with vehicle alone.

ADX or sham-ADX was performed via the dorsal approach under fentanyl citrate (0.315 mg ml^{-1}): fluanisone (10 mg ml^{-1}): diazepam (5 mg ml^{-1}) anaesthesia (1:1:2 v/v, 0.8 ml kg^{-1} body wt.) Following surgery, sham-ADX animals received tap water for drinking whilst ADX animals were provided with 0.9% NaCl in tap water. Adjuvant or vehicle injection was given to the appropriate group at the time of the surgery. Following the injection of adjuvant, in the morning of day 14, animals were killed within 2 h of lights-on. Trunk blood was collected and plasma stored at −20°C for subsequent hormone measurements by radioimmunoassay (RIA). The brains were rapidly removed, frozen on dry ice and stored at −80°C for subsequent *in situ* hybridization.

Collection of hypophysial portal blood and RIA for AVP

On days 7, 11, 16 and 21 after the injection of adjuvant rats were anaesthetized with 10% urethane (1.3–1.8 g kg^{-1}, i.p.) and portal blood (30 min collection period) was obtained by a modification of the Worthington technique (Worthington, 1966), as described previously (Eckland *et al.*, 1988). Plasma was stored at −20°C for subsequent AVP measurements by RIA (Rooke & Baylis, 1982; Williams *et al.*, 1985).

In vitro *incubation of anterior pituitaries and RIA of ACTH*

Anterior pituitary incubations were performed essentially by the method described previously (Nicholson *et al.*, 1984). Briefly fresh anterior pituitaries from SD and PVG rats were quartered and two quarters incubated per well in 1 ml Krebs-Ringer medium containing bovine serum albumin (BSA, 0.1%) and ascorbic acid (0.01%) under 95% 0_2/5% CO_2 at 37°C. After a 60 min equilibration period in medium alone, pituitaries were incubated for 30 min in medium containing CRF (1×10^{-9} M), AVP (1×10^{-8} M) or a combination of both. Following incubation, medium samples were heated at 90°C for 10 min, diluted in assay buffer and ACTH content was measured by RIA (Jessop *et al.*, 1989). The antiserum used was Wellcome E4, kindly provided by Dr G. Court, Wellcome Research Laboratories, Beckenham, Kent. The intra- and inter-assay coefficients of variance for ACTH measurements were 9% and 13.5% respectively.

CRF receptor assay

Immediately after decapitation, anterior pituitaries were removed, frozen in dry ice and stored at −70°C for no longer than 6 days before the binding assay. Pituitaries were thawed and homogenized in 10 volumes of 50 mM Tris-HCl buffer, pH 7.4 containing 5 mM $MgCl_2$, 2 mM EGTA, 100 kiu ml^{-1} of aprotinin and 1 mM DTT, with 6 strokes of a mechanical homogeniser (Teckmar, Cinn., OH, U.S.A.), filtered through 40 μm nylon mesh. The filtrate was centrifuged at 30,000 *g* and the resulting membrane pellet resuspended in the same buffer; 100 μl aliquots of membrane suspension (50 to 100 μg protein) were incubated with approximately 100,000 c.p.m. (0.1 nM) of [^{125}I]-Tyr-oCRF (Dupont-NEN, Boston, MA, U.S.A.) in a total volume of 300 μl of the above 50 mM Tris-HCl buffer containing 0.1% BSA, in 1.5 ml polystyrene microfuge tubes, in the absence and presence of increasing concentrations of unlabelled CRF. Tubes were incubated for 60 min at 22°C and after addition of 1 ml of ice-cold Tris-Mg-EGTA buffer containing 7.5% polyethyleneglycol, bound radioactivity was separated by centrifugation at 12,000 *g* for 3 min. Pellets were washed twice with the same buffer, the tips of the tubes cut and the radioactivity measured in a γ counter; Nonspecific binding defined as residual [^{125}I]-Tyr-oCRF binding in the presence of 10^{-6} M unlabelled oCRF was less than 10% of the total activity.

AVP receptor assay

Pools of 5 pituitaries were homogenized in 10 ml of 20 mM ice cold sodium bicarbonate solution by 10 strokes in a Downce homogenizer, filtered through nylon gauze and sequentially centrifuge at 100 *g*, for 10 min at 40°C and at 30,000 *g* for 30 min. Pellets were resuspended in 10 ml 50 mM Tris-HCl, pH 7.4 containing 5 mM $MgCl_2$, 2 mM EGTA, 10 μg ml^{-1} of bacitracin, and incubated for 10 min at room temperature to dissociate any endogenously bound ligand. Preliminary experiments showed that bound radioactivity increased by about 10% by use of this preincubation step. Membrane suspensions were centrifuged for 20 min at 30,000 *g* and the pellet resuspended in 2.2 ml of Tris-Mg-EGTA-bacitracin buffer to give a protein concentration of 30 to 50 μg 50 μl^{-1}. Fifty μl aliquots of membrane suspension were incubated with 0.1 to

8 nM (1,500 to 150,000 c.p.m.) [^{3}H]-arginine vasopressin (NEN DuPont Research Products, Boston, MA, U.S.A. specific activity 50 μCi mmol^{-1}) in a total volume of 150 μl of 50 mM Tris-Mg-EGTA-bacitracin buffer containing 0.2% BSA, in the absence and presence of 1 μM unlabelled AVP (Peninsula Labs, Belmont, CA, U.S.A.). After 60 min incubation at 22°C, bound radioactivity was separated by filtration through GF/C glass fibre filters, washed twice with ice cold PBS and counted in a β-counter. Preliminary experiments showed that binding was linear with increasing protein concentrations up to 80 μg. Binding reached equilibrium between 50 and 60 min and remained stable for at least an additional 60 min.

CRF and AVP receptor binding data was analysed using LIGAND (Munson & Rodbard, 1980) and the specific binding was subjected to Scatchard transformation for estimation of B_{max} and K_d.

Concentrations and affinities of CRF and AVP receptors were calculated by computer analysis of the binding data using the programme, Ligand (Munson & Rodbard, 1980).

Analysis of hypothalamic CRF and AVP mRNA by in situ *hybridization*

Cryostat sections (12 μm thick) were cut in the cronal plane 1.8–1.9 mm caudal to the bregma (stereotaxic coordinates from Paxinos & Watson, 1982), and placed on gelatin-coated slides that were kept at −80°C until hybridization. The PVN subnuclei were characterized on cytoarchitectonic criteria from adjacent methylene blue stained sections (Swandon & Kuypers, 1980). *In situ* hybridization was performed as previously described (Young *et al.*, 1986; Harbuz & Lightman, 1989). The probes were synthetic 48-base oligonucleotides, complementary to part of the exonic mRNA sequences coding for CRF (bases 496–543) (Jingami *et al.*, 1985), and AVP bases coding for the last 16 amino-acids of the glycopeptide sequence (Ivell & Richter, 1984). The specificity of these probes has previously been demonstrated. The probes were labelled with ^{35}S-labelled-αthio-dATP (>3000 Ci mmol^{-1}; New England Nuclear) using terminal deoxynucleotidyl transferase to add a tail to the 3′ end of the probe. The specific activities of the

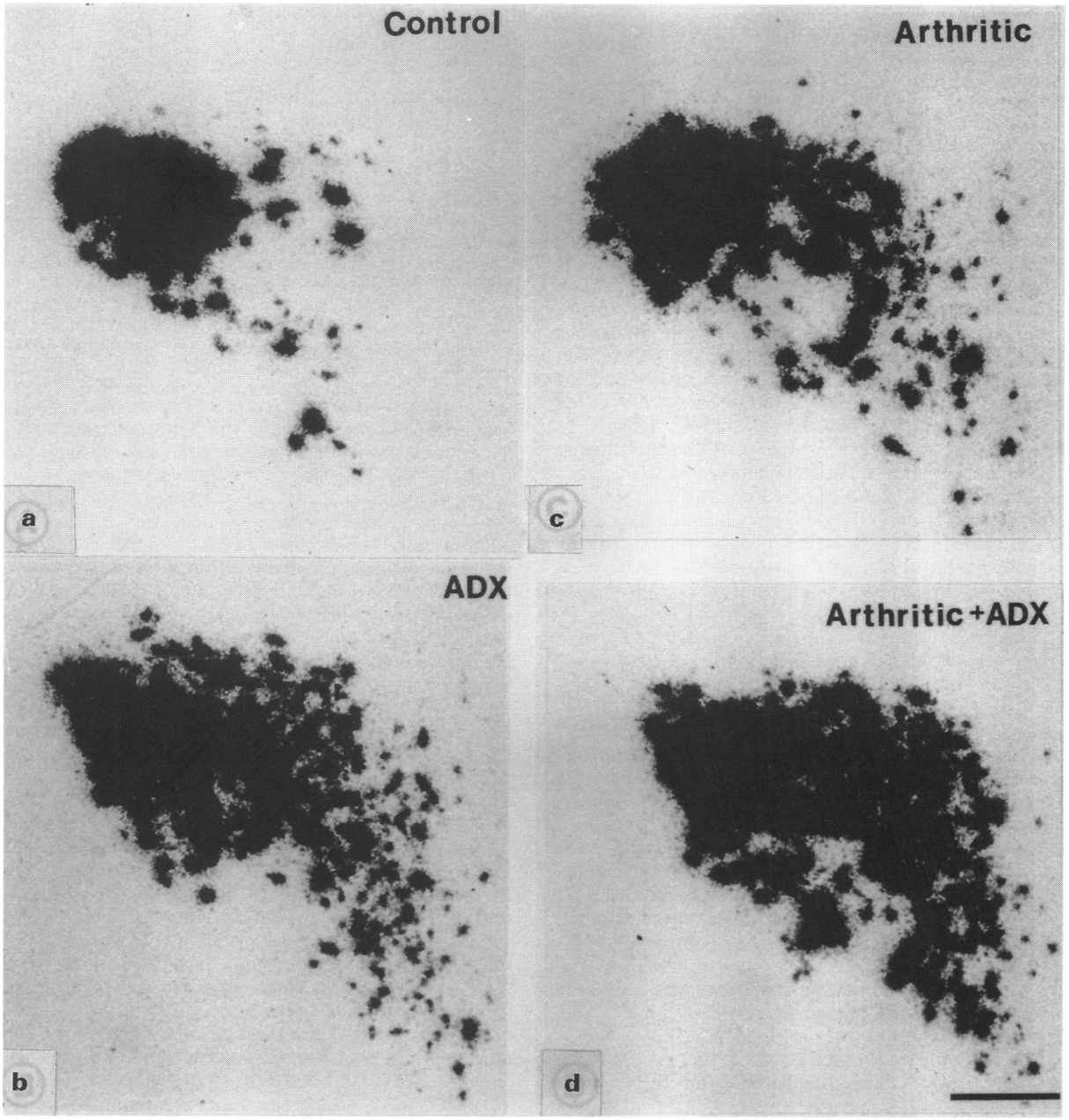

Figure 1 Representative photomicrographs demonstrating distribution of hybridized AVP mRNA in the paraventricular nucleus of Sprague-Dawley rats. Adjuvant arthritis (AA) was induced in control and adrenalectomized animals by an intradermal injection of 0.1 ml of a 10 mg ml^{-1} suspension of ground heat-killed *Mycobacterium butyricum* into the tail base. Control groups were injected with vehicle alone. Bar: 100 μm.

probes were approximately 2.0×10^{19} d.p.m. mol^{-1}. All control and experimental sections were hybridized in the same incubation reaction. The sections were hybridized overnight with 0.5 to 1.0×10^{7} d.p.m. ml^{-1} at 37°C in hybridization buffer (pH 7.2). After hybridization, slides were washed in 4×15 min changes of NaCl (0.5 mol l^{-1})-sodium citrate (0.015 mol l^{-1}) buffer (SSC; pH 7.2) at 55°C followed by two changes of SSC for 30 min each at room temperature to remove unbound and non-specifically bound probe. The sections were exposed to Hyperfilm MP autoradiography film (Amersham International plc, Amersham, Bucks) for an appropriate exposure time. A series of slides were dipped in Amersham LM-1R emulsion and exposed for 4 to 16 weeks before being developed. The autoradiographic images were quantified with a computer-assisted image analysis system (Image 1.41; Wayne Rasband, N.I.H., U.S.A.) and grain densities converted to d.p.m. mg^{-1}) wet weight using ^{35}S-brain paste standards as reference.

Results

Effects of chronic inflammatory stress on AVP and CRF mRNAs

The distribution of AVP mRNA within the PVN of control, ADX, AA and adrenalectomized AA Sprague-Dawley rats determined by *in situ* hybridization is shown in Figure 1. Light microscopy analysis of the slides after exposure to nuclear emulsion in control rats, revealed the characteristic localization with very high density of grains in the posterior magnocellular division of the PVN and scattered cells in the medial parvocellular division. Due to the extensive exposure time, the excitability of the photographic emulsion was saturated in areas overlying the magnocellular division rendering quantitative assessment of AVP mRNA in this region impossible. However, AVP transcripts in the medial parvocellular division were markedly increased in ADX and in AA rats (Figure 2). Compared with ADX alone or AA rats, there was a further increase in AVP transcripts in adrenalectomized AA rats. In PVG rats, AA also caused an increase in AVP transcripts in the parvocellular division of the PVN, and similar to Sprague Dawley rats the effect of AA was additive to that of ADX (data not shown). Consistent with previous reports, ADX markedly increased CRF mRNA in the parvocellular division of the PVN, but the levels in adrenalectomized AA animals were not significantly different from the increased levels after ADX alone (Figure 3).

HPB levels of AVP during the course of development of AA

These studies were very labour-intensive and were performed only in PVG rats. In adjuvant-injected animals, AVP release into the HPB gradually increased, reaching significance by day 11 compared with controls (Figure 4). Portal plasma AVP levels rose from a control value of 14 ± 2 to 42 ± 22 fmol ml^{-1} on day 7 and to 101 ± 30 on day 11 following injection of adjuvant. The portal plasma levels of AVP were further elevated by day 16 (217 ± 9) and were sustained on day 21 (224 ± 40), coincident with the period of maximal inflammation.

Effects of chronic inflammatory stress on anterior pituitary CRF and AVP receptors

The effect of AA on anterior pituitary CRF and AVP binding in SD rats is shown in Table 1. Compared with controls, in the presence of clinical signs of inflammation on day 14 there was a significant decrease in the concentration ($B_{max} = 579 \pm 21$ controls v 447 ± 25 AA animals) but no change in the binding affinity (K_d) of anterior pituitary CRF receptors. In contrast to the CRF receptor changes, AVP receptor concentration in anterior pituitary membranes from 14 day arthritic animals showed a significant increase with respect to controls. In PVG rats, the number of AVP binding sites was unchanged on day 7 and showed a small but statistically significant increase on day 14 after the injection of adjuvant {(87.5 ± 1.0, 88.5 ± 2.5 and 103 ± 5 fmol mg^{-1} in controls, day 7 and day 14 AA rats respectively) ($P < 0.05$)}.

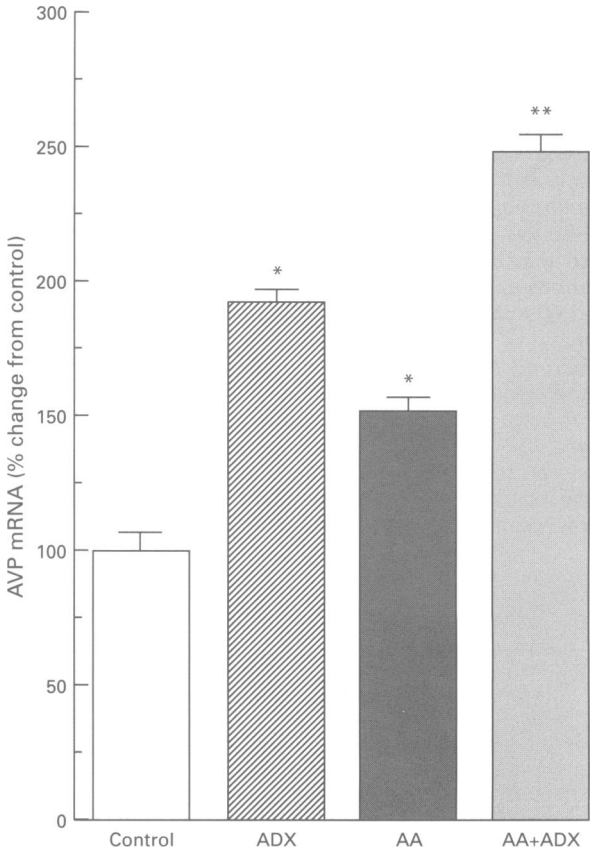

Figure 2 Quantitative *in situ* hybridization histochemical analysis of AVP mRNA in the medial parvocellular division of the paraventricular nucleus of control, adrenalectomized (ADX), arthritic (AA) and adrenalectomized arthritic (ADX/AA) rats 14 days following the injection of adjuvant or vehicle. mRNA values are expressed as percentage change from control which was ascribed a value of 100 and represent means ± s.e.mean (n = 6 to 8 animals). Comparison between groups were made with Kruskal-Wallis analysis of variance followed by Mann-Whitney U test. *$P < 0.001$ compared to control group. **$P < 0.001$ compared to AA or control group.

Effects of chronic inflammatory stress on the response of corticotrophs to CRF and AVP

The *in vitro* release of ACTH, a measure of the responsiveness of corticotrophs from pituitary quarters of control and arthritic animals in response to AVP and CRF is shown in Figure 5. The basal ACTH secretion was similar in both groups of SD rats but that from arthritic PVG rats was significantly greater than the respective controls (436 ± 91 v 167 ± 23 pg/tube). Although AVP was a weak secretagogue, the ACTH response to any of the treatments in the SD arthritic rat pituitaries was similar to that seen in the controls. In contrast, the ACTH response of pituitaries of arthritic PVG rats to CRF or the combination of CRF and AVP was significantly higher than in the respective non-arthritic controls.

Discussion

These studies provide evidence for a dominant role of AVP in maintaining the responsiveness of the pituitary corticotroph

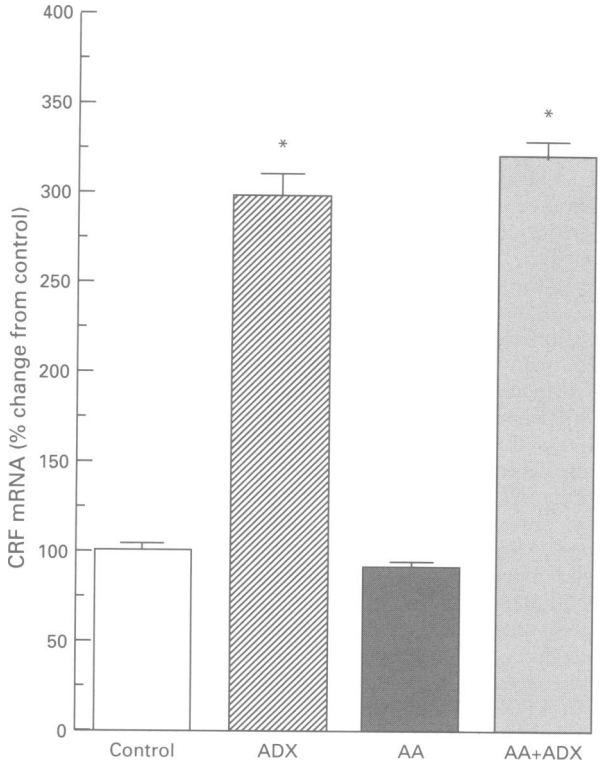

Figure 3 Quantitative *in situ* hybridization histochemical analysis of CRF mRNA in the medial parvocellular division of the paraventricular nucleus of control, adrenalectomized (ADX), arthritic (AA) and adrenalectomized arthritic (ADX/AA) rats 14 days following the injection of adjuvant or vehicle. mRNA values are expressed as percentage change from control which was ascribed a value of 100 and represent means ± s.e.mean (n = 6 to 8 animals). Comparison between groups was made with Kruskal-Wallis analysis of variance followed by Mann-Whitney U test. *$P<0.001$ compared to AA or control group.

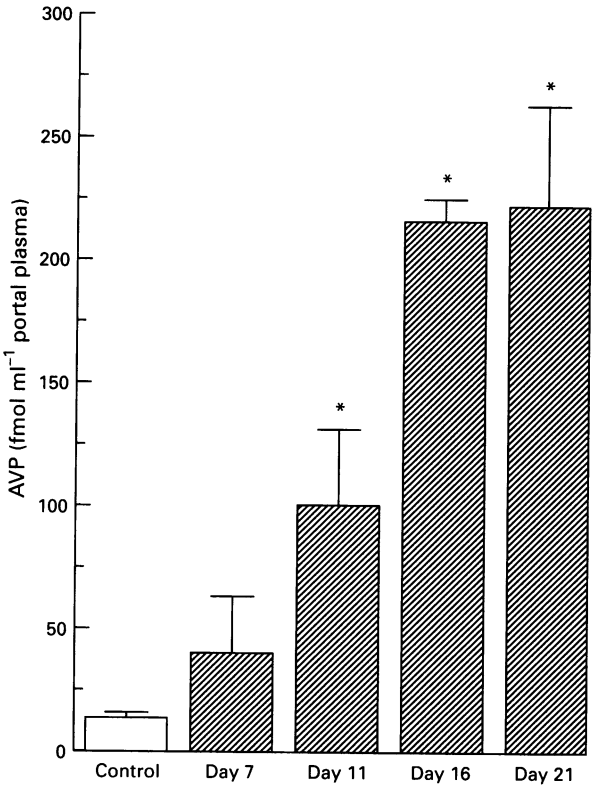

Figure 4 Plasma concentrations of arginine vasopressin (AVP) release into the hypophysial portal blood of control and arthritic animals on days 7, 11, 16 and 21 following the injection of adjuvant. Data are presented as hypothalamic release in a 30 min period. Values represent means ± s.e.mean (n = 6 to 8 animals). Comparisons between groups were made by one way analysis of variance followed by Fisher PLSD with a probability for significant difference set at the 5% level.

during chronic inflammatory stress. Thus, the dramatic increase in AVP mRNA in the parvocellular division of the PVN and the progressive elevation of irAVP in hypophysial portal circulation following the onset of the inflammatory process are indicative of marked activation of the parvocellular vasopressinergic system. In addition, the number of AVP receptors was significantly elevated in 14 day AA rats, which is consistent with previous studies showing a direct relationship between AVP receptor levels and corticotroph responsiveness in several chronic stress paradigms (Aguilera *et al.*, 1994).

A number of studies support an important role for AVP in the regulation of the pituitary corticotroph during chronic stress. Chronic intermittent exposure to immobilization, insulin-induced hypoglycaemia or psychological stress stimuli have been shown to increase the number of CRF cells containing AVP and to increase the ratio of AVP to CRF content within the zona externa of the median eminence (deGoeji *et al.*, 1991; 1992a,b,c). In chronically restrained rats, exogenous AVP but not CRF was found to increase plasma levels of both ACTH and corticosterone (Hashimoto *et al.*, 1988). In addition it has been suggested that endogenous AVP is essential for sustaining the pituitary adrenal stress response in circumstances when the pituitary is refractory to CRF stimulation (Scaccianoce *et al.*, 1991). However, detection of changes in AVP mRNA in parvocellular cells during chronic stress have been more elusive. For example, despite marked accumulation of irAVP in the external zone of the median eminence after repeated immobilization or i.p. hypertonic saline, no detectable changes in mRNA in parvocellular PVN are observed by regular light microscopy analysis of emulsion coated slides. Increases after immobilization have been described using sensitive computerized imaging at the cellular level. Therefore the clear increase in AVP mRNA in the parvocellular division of the PVN together with the clear increase in AVP mRNA in the parvocellular division of the PVN together with the increase in AVP in the portal blood shown in these experiments suggest that chronic inflammatory stress is associated with much larger stimulation of AVP than other stress models.

Table 1 Binding affinity (K_d) and capacity (B_{max}) for [^{3}H]-arginine vasopressin in [^{125}I]-Tyr-oCRF in SD rat anterior pituitary membrane preparations from either control or AA animals on day 7 and day 14 following the injection of adjuvant

	CRF receptors		*AVP receptors*	
Experimental group	K_d (nM)	B_{max} (fmol mg^{-1})	K_d (nM)	B_{max} (fmol mg^{-1})
Control	1.4 ± 0.1 (3)	579 ± 21 (3)	0.45 ± 0.1 (3)	255 ± 8.9 (3)
AA day 7	1.3 ± 0.2 (3)	549 ± 36 (3)	ND	ND
AA day 14	1.2 ± 0.1 (4)	447 ± 25* (4)	0.44 ± 0.1 (2)	309 ± 3.8** (3)

Values are mean ± s.d. of the data obtained from membranes of three pools of 3 to 5 pituitaries. n values in parentheses indicate groups of animals from separate experiments. ND, not detected; *$P<0.05$ lower than controls; **$P<0.01$ higher than controls.

In contrast, activation of the CRF system does not appear to play a role in the increased corticotroph responsiveness during chronic inflammatory stress. Previous studies in PVG rats showed a paradoxical decrease in CRF expression and secretion into the hypophysial portal blood associated with the increased pituitary ACTH responsiveness during the development of AA (Harbuz *et al.*, 1992). Pituitary portal levels of

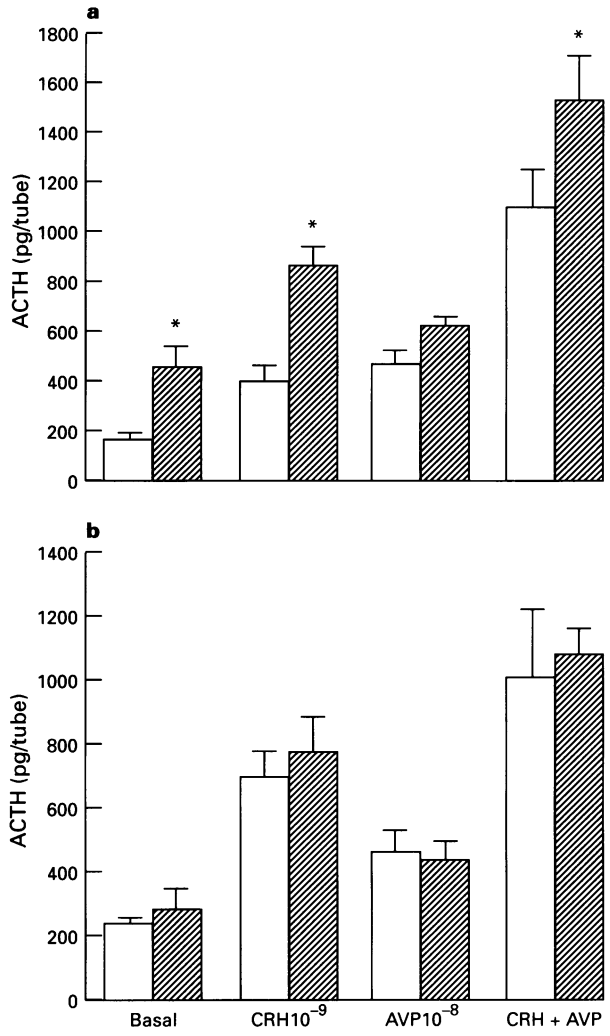

Figure 5 The *in vitro* ACTH release from pituitary quarters of control (open columns) and AA (hatched columns) PVG (a) and SD (b) rats in response to medium alone or medium containing CRF (1×10^{-9} mol l^{-1}), AVP (1×10^{-8} mol l^{-1}) or a combination of both. Values are means ± s.e.mean. ($n = 12$); *$P < 0.05$ compared to respective controls.

CRF were not measured in the present experiments in the SD rats, but the slight decrease in CRF mRNA levels in the PVN, suggest that CRF output is reduced or unchanged but not increased. The tendency of CRF expression to be inhibited during chronic inflammatory stress is at variance with observations in other stress paradigms with increased corticotroph responsiveness. In some models such as repeated hypertonic saline injection (Kiss & Aguilera, 1993; Lightman & Young III, 1987), electroconvulsive shock or foot shock (Imaki *et al.*, 1991) the increases are marked and sustained, while in others such as cold exposure and immobilization the increases are more transient and detectable only with sensitive quantitative methods after repeated stress (Bartanusz *et al.*, 1993; Lightman & Harbuz, 1992).

The corticotroph activation in AA, despite a decreased CRF output is intriguing. Although the ability of AVP to potentiate CRF stimulation of ACTH release is well established, it is clear that only CRF can stimulate POMC transcription and that this effect is not potentiated by AVP (Levin *et al.*, 1989). Thus in the light of decreased CRF, there is no simple explanation for the increases in POMC mRNA in AA rats. In the presence of permissive levels of CRF, the increased expression of POMC mRNA and increased ACTH secretion is presumably due to the combined effect of high levels of AVP and the presence of locally produced (Stephanou *et al.*, 1993) or circulating cytokines, which are likely to be elevated during chronic inflammation. In this regard it has been shown that prolonged exposure of AT-20 cells or rat cultured pituitary cells to interleukin-1 (IL-1) potentiates β-endorphin responses to subsequent incubation with CRF (Fagarasan *et al.*, 1989).

Adrenalectomy is also known to increase both the number of AVP expressing cells in the parvocellular PVN (Sawchenko *et al.*, 1984) and the levels of AVP mRNA in these cells (Wolfson *et al.*, 1985; Swanson & Simmons, 1989) and to increase the concentration of AVP in the HPB (Plotsky & Sawchenko, 1987; Eckland *et al.*, 1988; Fink *et al.*, 1988). There is also down-regulation of both AVP receptors (Antoni *et al.*, 1985) and AVP-induced phosphatidyl-inositol turnover in the anterior pituitary (Todd & Lightman, 1987). In the present study we confirmed that adrenalectomy resulted in marked increases in both AVP and CRF mRNAs in the parvocellular neurones of the PVN. We have previously shown in PVG rats that the additional stimulus of AA in ADX rats actually decreases the elevated expression of CRF mRNA seen following ADX alone (Harbuz & Lightman, 1992). In the present study, despite the increased inflammation seen following AA in adrenalectomized SD rats, there was no further increase in the elevated expression of CRF mRNA due to ADX alone. This confirms that it is not simply change in sensitivity to corticosteroid feedback that causes the differential effects on AVP and CRF mRNAs. Indeed in adrenalectomized AA rats the AVP mRNA was significantly greater than in ADX alone animals.

Although we did not test corticotroph response *in vivo*, our *in vitro* studies show that compared with controls, AVP was less potent than CRF in producing ACTH release from AA rat pituitaries as has been demonstrated in *in vitro* control rat pituitaries (Gillies & Lowry 1979). Compared with controls, arthritic PVG rat pituitaries showed a significantly higher ACTH response to CRF and to the combination of CRF and AVP. These increased responses of the arthritic PVG rat pituitaries are of interest but the importance of this to the pathology of AA in this rat strain is unclear. In contrast to the PVG strain, the ACTH response to these secretagogues in the SD rat was similar in the arthritic and control groups. There are numerous reports in the literature demonstrating the effects of AVP on CRF-stimulated ACTH release to be either synergistic or additive (Antoni, 1986). In the studies we have performed, our data are suggestive more of an additive than a synergistic effect. These results indicate that increased sensitivity of the corticotrophs, at least in the PVG rats, may partly be responsible for the increased circulating levels of ACTH seen following AA, even though this does not correlate with our binding studies.

The downregulation of CRF receptors observed in 14-day arthritic rats is consistent with results in other chronic stress paradigms with increased corticotroph responsiveness such as continuous or repeated immobilization and repeated i.p. hypertonic saline (Hauger *et al.*, 1988; 1990; Kiss & Aguilera, 1993). The mechanism of CRF receptor loss is likely to involve exposure of the pituitary to increased AVP levels. It is well known that activation of AVP receptors in the corticotrophs has a marked potentiating effect on CRF receptor down-regulation following adrenalectomy or exogenous CRF administration (Hauger & Aguilera, 1993; Holmes *et al.*, 1986). Therefore it is possible that even in conditions with low CRF output, the high AVP levels in the hypophysial portal blood may influence CRF receptor levels. It must be noted however that the decrease in CRF receptor numbers is actually correlated with an increased responsiveness to CRF, presumably reflecting changes in signal transduction mechanisms.

Glucocorticoids have also been shown to decrease pituitary CRF receptors *in vivo* (Wynn *et al.*, 1985; Hauger *et al.*, 1987) and *in vitro* (Childs *et al.*, 1986; Schwartz *et al.*, 1986). The high circulating glucocorticoid levels could contribute to the decrease in the pituitary CRF receptors during chronic inflammatory stress. However, this is unlikely since CRF receptors are unchanged in other conditions with high glucocorticoid levels such as water deprivation or food deprivation

(Hauger & Aguilera, 1993; Kiss & Aguilera, 1993). While AVP binding sites are reportedly unaffected by acute changes in AVP secretion (Landgraf *et al.*, 1991), data from previous studies in which AVP levels were chronically altered indicate changes in pituitary AVP binding sites (Antoni *et al.*, 1985; Shewey & Dorsa, 1986). Furthermore a number of studies have shown that alterations of the glucocorticoid milieu results in changes in the binding characteristics of pituitary AVP receptors (Antoni *et al.*, 1985; Koch & Lutz-Bucher, 1985) and their associated intracellular second messenger systems (Todd & Lightman, 1987). A positive regulatory effect of glucocorticoids on pituitary AVP receptors is suggested by studies showing that glucocorticoid replacement reverses the adrenalectomy-induced reductions in pituitary AVP receptors (Antoni *et al.*, 1985; Koch & Lutz-Bucher, 1985). Our results demonstrating an upregulation of pituitary AVP receptors in the presence of chronically elevated portal plasma levels of AVP as well as raised circulating corticosterone, lend further support to the above studies. It is also possible that during chronic stress, changes in the expression of several neuropeptides co-localized with AVP in the parvocellular neurones could contribute to the upregulation of AVP receptors.

In conclusion, our results demonstrating increased expression of AVP mRNA in the parvocellular PVN, chronically elevated HPB levels of the peptide during the time course of development of AA, and upregulation of pituitary AVP receptors, and an enhanced ACTH release to the combination of AVP and CRF from PVG arthritic rat pituitaries *in vitro*, provide further support for the view that activation of the parvocellular vasopressinergic system has an important role in the adaptation of the HPA axis to chronic stress.

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