An investigation of the effects of interleukin-1β on plasma arginine vasopressin in the rat: role of adrenal steroids

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Abstract

While the effects of cytokines on the hypothalamo-pituitary-adrenal axis have received a great deal of attention in recent years the effects of cytokines on posterior pituitary hormone release has been less well characterized. In the present study we have investigated the effects of a single i.p. injection of interleukin (IL)-1β on circulating levels of vasopressin (AVP) in the rat. We have found that the ability of IL-1β to increase plasma AVP is strongly influenced by circulating levels of glucocorticoid steroids. IL-1β did not affect plasma AVP in sham-operated control animals over the 4 h period of study. In contrast, following adrenalectomy we were able to stimulate AVP substantially with increases over the 4 h period. This effect was reduced by treatment of adrenalectomized rats with a low dose of dexamethasone and abolished with a high dose. These data suggest an inverse relationship between circulating levels of glucocorticoids and the ability of IL-1β to stimulate plasma AVP.

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Introduction

Arginine vasopressin (AVP) is synthesized in two distinct cell types in the paraventricular nucleus (PVN) of the hypothalamus. The parvocellular neurones are involved in the activation of the hypothalamo-pituitary-adrenal (HPA) axis in response to stress. The axons of these neurones project to the external zone of the median eminence (EZME). AVP and corticotrophin-releasing factor (CRF) are the major corticotrophin-releasing factors responsible for the activation of the pituitary-adrenal axis (Harbuz & Lightman 1992). AVP and CRF are co-localized in secretory vesicles in the EZME (Whitnall *et al.* 1985), released into the hypophysial portal blood (HPB) and carried to the anterior pituitary to evoke the release of adrenocorticotrophin (ACTH) from the corticotrophs. Projections from the classical magnocellular system terminate in the posterior pituitary where AVP is released directly into the peripheral circulation. Magnocellular AVP is the antidiuretic hormone which can also cause vasoconstriction and contribute to haemostasis. Plasma levels of AVP also increase in response to physical but not psychological stressors (Husain *et al.* 1979, Williams *et al.* 1985, Onaka *et al.* 1986, Ivanyi *et al.* 1991, Yagi & Onaka 1991).

The interaction of the immune and neuroendocrine systems has recently been the subject of much research interest. It is now firmly established that immunological challenges, e.g. administration of interleukin (IL)-1β, are able to activate the HPA axis as evidenced by increased circulating levels of ACTH and corticosterone, increased pro-opiomelanocortin (POMC) mRNA (the ACTH precursor) in the anterior pituitary and increased CRF mRNA in the parvocellular cells of the PVN (for review see Harbuz & Lightman 1992). These effects of IL-1β appear to be mediated by CRF, as CRF mRNA (Suda *et al.* 1990, Harbuz *et al.* 1992), turnover (Berkenbosch *et al.* 1989, Suda *et al.* 1990) and release into the HPB (Sapolsky *et al.* 1987) are increased following acute IL-1β administration and the effects of IL-1β can be blocked by CRF antisera (Berkenbosch *et al.* 1987, Sapolsky *et al.* 1987, Watanabe *et al.* 1990). In contrast, parvocellular AVP-containing neurones do not appear to be involved in this activation (Berkenbosch *et al.* 1989, Spinedi *et al.* 1992) even though they do respond to acute stressors. It has been suggested that the activation of the HPA axis by IL-1β occurs via an alternative mechanism to that evoked in response to stress (Whitnall *et al.* 1992), and it is likely that this activation occurs through an interaction with adrenal steroids (Chover-Gonzalez *et al.* 1993).

Whereas the effects of cytokines on the HPA axis have been well-documented, the effects on the magnocellular AVP system are less well-established. A number of *in vitro* studies have proposed a stimulatory role for IL-1β on AVP release from the posterior pituitary (Christensen *et al.* 1989, Nakatsura *et al.* 1991) although it appears that

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at higher concentrations this stimulatory effect is lost (Nakatsura *et al.* 1991). A stimulatory role for IL-1β has also been proposed *in vivo* (Naito *et al.* 1991). In the present study we investigated the time-course of the effects of acute administration of IL-1β *in vivo* on the release of AVP into the circulation in intact and adrenalectomized rats. We also determined the response following replacement of steroid in adrenalectomized rats using the synthetic glucocorticoid dexamethasone. Finally, the effect of restraint stress on plasma AVP in sham-operated and adrenalectomized rats was investigated.

Materials and Methods

Male Sprague–Dawley rats weighing 200–250 g were maintained on a 12-h light:12-h darkness cycle with free access to food and drink. All studies were begun between 0900 h and 0930 h. Recombinant human IL-1β, which was purified to homogeneity by high-pressure liquid chromatography (HPLC) and with a very low endotoxin level, was obtained from Otsuka Pharmaceutical Co. Ltd, Tokushima, Japan. It was diluted to a final concentration of 20 mg/ml with 0·9% (w/v) NaCl immediately prior to use. Adrenalectomy was performed under sodium pentobarbital anaesthesia via a dorsal approach. The animals were subsequently returned to their home cages and maintained on 0·9% saline. Sham-operated animals had their adrenals exposed but not removed.

Time-course study

Sixteen rats were randomly assigned to one of four groups, i.e. $n=4$ per group. Five days after surgery all rats were implanted with a cannula placed in the jugular vein. They were then individually housed with free access to food and water/0·9% saline. Forty-eight hours after cannulation (i.e. 7 days after surgery) the animals received either 2 μg IL-1β i.p. (in 100 μl saline) or 100 μl saline. Blood samples (0·4 ml) were collected immediately prior to injection (basal) and 30 min, 2 h and 4 h after IL-1β/saline administration. Plasma was stored at −20 °C until AVP determination by radioimmunoassay (Williams *et al.* 1985). A specific antiserum (A8; Ferring, Malmo, Sweden; cross-reactivity with oxytocin <0·1%, with DDAV $P<0{\cdot}01\%$) was used at a final dilution of 1/1 200 000. The percentage recovery was greater than 90%. Individual samples were assayed in duplicate. Inter- and intra-assay variations were below 10%. The detection limit was 1·25 fmol/ml.

Dexamethasone replacement study

Immediately following adrenalectomy, animals were given saline (0·9%), or either a low (0·1 mg/l) or high (5 mg/l) dose of dexamethasone (Sigma Chemical Co., Poole, Doreset, UK) in saline. Groups were comprised of six to eight animals per group. Sham-adrenalectomized animals were maintained on water. Seven days after surgery the animals received either 2 μg IL-1β i.p. (in 100 μl) or 100 μl saline. The animals were killed 4 h after injection and trunk blood was collected into chilled heparinized tubes and plasma stored at −20 °C until AVP determination.

Restraint stress study

Seven days after surgery one group of sham-operated and a group of adrenalectomized rats were placed in individual acrylic restrainers for 1 h and further groups of unstressed controls were left in their home cage. Groups were comprised of six to eight animals per group. The rats were killed by decapitation 4 h after the onset of stress and trunk blood was collected as described above.

Plasma osmolalities

Osmolality measurements were performed using fresh plasma by depression of freezing point on an Osmostat 030 (Clandon Scientific Ltd, Aldershot, Hants, UK).

Statistical analysis

All data are presented as the mean ± S.E.M. Statistical analysis was performed using the Mann–Whitney U rank sum test to compare groups following non-parametric analysis of variance (Kruskal–Wallis). $P<0{\cdot}05$ was considered significant.

Results

Plasma osmolalities were measured in all groups (data not shown). All the values obtained were in the range 290–303 mOsmol/kg and did not show any significant variation in any of the studies.

Time-course study

Under our experimental conditions IL-1β did not affect plasma AVP levels in the sham-operated animals at any of the time-points studied (Fig. 1). However there was a time-dependent increase in circulating AVP in adrenalectomized rats. This increase was significant from 30 min after administration ($P<0{\cdot}05$) compared with basal values and compared with values in the other three treatment groups at the appropriate time-points.

Dexamethasone replacement study

In the second study we were unable to determine any increase in plasma AVP in the sham-operated rats 4 h after the IL-1β injection (Fig. 2). In the adrenalectomized rats

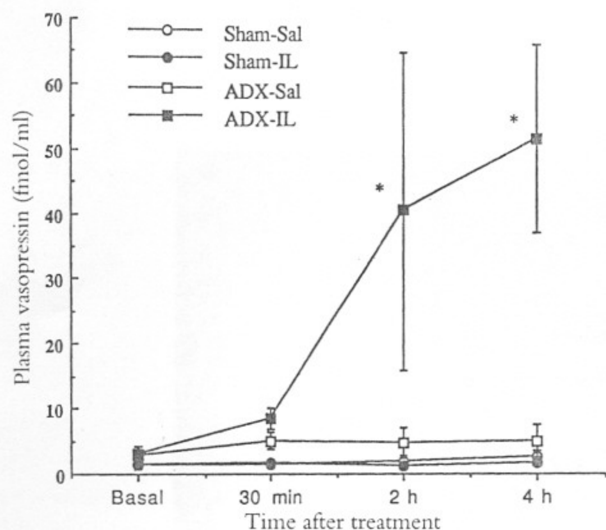

FIGURE 1. Time-related effect of interleukin (IL)-1β (solid symbols) or saline (Sal; open symbols) on plasma levels of vasopressin in normal (Sham; circles) and adrenalectomized (ADX; squares) rats. *$P<0.05$ compared with basal value and all other treatment groups at each individual time-point (Mann–Whitney U test following Kruskal–Wallis). Values represent the mean ± S.E.M. for $n=4$ per group.

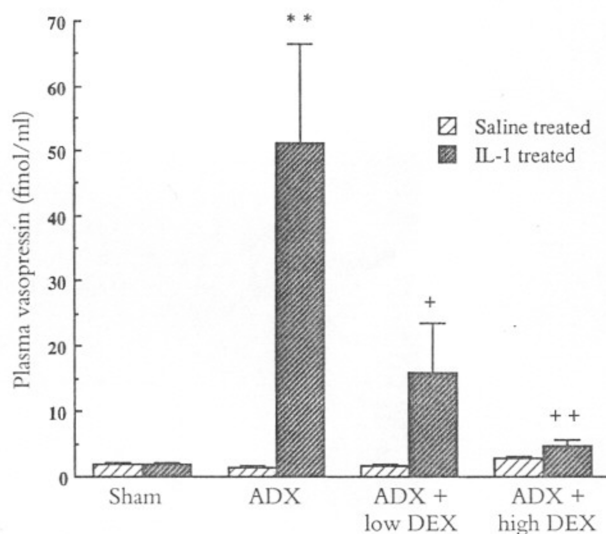

FIGURE 2. Effect of interleukin (IL)-1β on plasma vasopressin levels in adrenalectomized (ADX) rats and adrenalectomized rats given dexamethasone (DEX) replacement at low or high levels. **$P<0.005$ for ADX+IL compared with both the respective saline-injected group and the sham-operated rats given IL. $^{+}P<0.05$ comparing ADX+IL with ADX+low DEX+IL and $^{++}P<0.01$ with high DEX+IL. (Mann–Whitney U test following Kruskal–Wallis). Values represent the mean ± S.E.M. for $n=6$–8 per group.

there was a highly significant ($P<0.005$) increase in plasma AVP. This was significantly reduced by treatment with the low dose of dexamethasone ($P<0.05$ comparing adrenalectomized rats given IL-1β with adrenalectomized rats given the low dose of dexamethasone and IL-1β). The increase in circulating AVP was completely prevented by the high dose of dexamethasone ($P<0.01$; adrenalectomized rats given IL-1β with adrenalectomized rats given the high dose of dexamethasone and IL-1β).

Restraint stress study

Restraint stress did not result in an alteration in plasma AVP levels in the sham-adrenalectomized rats. Adrenalectomy caused a slight, but not significant, increase in plasma AVP and there was no significant difference between the levels of AVP in the adrenalectomized rats compared with the adrenalectomized rats given restraint stress (data not shown).

Discussion

In the present paper we report that the ability of a single i.p. injection of IL-1β to increase plasma AVP appears to be strongly influenced by circulating levels of glucocorticoid steroids. IL-1β was unable to elicit any increase in plasma AVP in sham-operated animals at any of the time-points studied despite this dose of IL-1β being effective in evoking the activation of CRF mRNA in the parvocellular PVN, POMC mRNA in the anterior pituitary and increasing circulating levels of ACTH and corticosterone (Harbuz *et al.* 1992). In contrast, following removal of endogenous glucocorticoids by adrenalectomy we were able to stimulate AVP substantially in a time-dependent manner. This effect could be reduced by treatment of adrenalectomized rats with a low dose of dexamethasone and abolished in the face of a high dose of dexamethasone. These data suggest an inverse relationship between circulating levels of glucocorticoids and the ability of IL-1β to stimulate plasma AVP.

Two AVP-containing systems are present in the hypothalamic PVN: the parvocellular and the magnocellular. The parvocellular PVN is involved in the response of the HPA axis to stress. Following acute stress not only is there an increase in CRF mRNA but there is also a corresponding increase in parvocellular AVP mRNA with no change in magnocellular AVP mRNA (Lightman & Young 1988). These parvocellular neurones are sensitive to steroid feedback, exhibiting an increase in AVP mRNA following adrenalectomy (Young *et al.* 1986). In addition, a number of studies have reported increased vasopressin immunostaining in the EZME following adrenalectomy and a decrease following steroid replacement (e.g. Stillman *et al.* 1977). In addition *in vitro* and *in vivo* studies

have reported that adrenalectomy increases AVP-positive terminals in the EZME (Sawchenko 1987, Whitnall *et al.* 1987) and also its release into the medium *in vitro* (Holmes *et al.* 1986); effects reversed by glucocorticoid replacement. The relatively low levels of AVP in the EZME make it unlikely to be the source of circulating AVP in the present study. Indeed, unlike the response to acute stress, the role of parvocellular AVP in the IL-1-induced activation of the HPA axis is controversial. An increase in the release of AVP from neurosecretory axons in the EZME in normal rats in response to IL-1β has been reported (Whitnall *et al.* 1992). Further supporting evidence for a stimulatory role comes from studies using push-pull perfusion of the median eminence, demonstrating increased AVP release following IL-1β (Watanabe & Takebe 1993). Whether this release derives from the magnocellular or parvocellular AVP neurones is unclear. In contrast, however, other authors have reported no effect of IL-1 on AVP release into the portal blood (Sapolsky *et al.* 1987), no alteration in AVP turnover in the EZME (Berkenbosch *et al.* 1989), no effect on the activity of electrophysiologically identified AVP neurones (Saphier & Ovadia 1990), and no effect on AVP release from the whole medial basal hypothalamus *in vitro* (Spinedi *et al.* 1992). It appears, therefore, that the evidence suggests that the effects of IL-1β on the HPA axis are mediated via CRF, the role of AVP being less clear.

A number of studies have suggested that glucocorticoids are able to inhibit magnocellular AVP synthesis and/or secretion. This may be a direct effect as type II glucocorticoid receptors have been identified in the ventral part of the supra-optic nucleus which contains the AVP magnocellular neurones (Kiss *et al.* 1988). It appears that the inhibition is effective in response to mild osmotic challenges (Raff *et al.* 1986) but can be over-ridden by more potent challenges such as hypoxia, haemorrhage or dehydration (Ahmed *et al.* 1967, Raff *et al.* 1984, Wood *et al.* 1984). Interestingly, the AVP response to osmotic challenge is not inhibited by short-term elevations of plasma steroid concentrations in the physiological range immediately prior to challenge, but requires longer overnight treatment with steroid for this inhibition to be effective (Raff *et al.* 1989).

Following adrenalectomy we were unable to determine any significant increase of plasma AVP. Similarly, we found no effect of restraint stress on plasma AVP in either sham-operated or adrenalectomized rats. Our findings are in accord with previous stress studies which have reported no effect on circulating AVP in response to predominantly psychological stressors such as restraint, noise, swimming or novel environment (Husain *et al.* 1979, Williams *et al.* 1985, Ivanyi *et al.* 1991, Yagi & Onaka 1991). The inability of these acute stressors to alter plasma AVP (i.e. magnocellular), despite the increase in HPA activity mediated by the parvocellular cells, suggests that the increase in the release of AVP in response to IL-1β in the present study is almost certainly from the posterior pituitary.

There have been few studies on the effects of IL-1β on the release of posterior pituitary hormones. Data from both *in vitro* and *in vivo* studies suggest a stimulatory effect of this cytokine on AVP release in normal animals. The present data are in conflict with this view. This may, however, be related to differences in experimental design, e.g. *in vitro* incubations require removal of tissue from circulating corticosteroids and the subsequent preincubation period will result in a further period of steroid withdrawal from the tissue. It is possible that the basal circulating levels of glucocorticoids are able to influence a strong negative control over the ability of IL-1 to release AVP. This may be similar to the inhibitory effects of glucocorticoids on AVP release to mild osmotic stimuli which can be over-ridden with more potent stimuli (Raff *et al.* 1989). A recent paper by Loxley *et al.* (1993), has reported increased AVP release from hypothalamic explants *in vitro* following exposure to IL-1β (presumably from magnocellular cells). This effect is increased by adrenalectomy and the hypersecretion overcome by preincubation with dexamethasone. These data support our findings in the present study of an inhibitory effect of glucocorticoids on the IL-1β-stimulated release of AVP.

The results in the present study appear to be at variance with the report of Naito *et al.* (1991) who reported a dose-dependent increase in plasma AVP in intact rats. The reasons for the discrepancy between the two studies are unclear but may reflect differences in the route of administration or the different strains of rat used. Comparing the effects of IL-1 in the two studies, it is interesting to note that when comparing the equivalent doses, Naito's control rats and our adrenalectomized rats had a similar response to IL-1 at 30 min. In the presence of steroid this increase is limited and returns to basal levels by 1 h (Naito *et al.* 1991), while in the absence of steroid there is a time-dependent increase. One might speculate that not only is the IL-1β stimulated release of plasma AVP dependent on the circulating level of corticosterone at the time of challenge, but may also be able to terminate the continuing release of AVP.

The physiological relevance of these findings are unclear. One might speculate that the check on the release of AVP in response to IL-1 may be a further manifestation of the protective effect of glucocorticoids as suggested by Munck *et al.* (1984). Clearly the mechanism underlying the release of AVP by cytokines and the role of glucocorticoid steroids in this activation require further investigation.

In summary, we were unable to demonstrate any increase in circulating AVP in intact rats. In contrast, we found a substantial increase in plasma AVP concentrations in adrenalectomized rats. This stimulation was reduced by treatment of adrenalectomized rats with a low dose of dexamethasone and abolished with high dose replacement.

This increase in plasma AVP was time-dependent resulting in a substantial increase over the 4 h studied.

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