

## Effect of endotoxin on the hypothalamic–pituitary–adrenal axis in sheep

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### Abstract

Endotoxin has been shown to stimulate ACTH and cortisol secretion through an action at the hypothalamic level. However, the nature of hypothalamic neurohormones, corticotropin-releasing hormone (CRH) and especially arginine vasopressin (AVP), involved in that regulation is still controversial. The purpose of this study was to determine the effects of an acute i.v. endotoxin administration on CRH and AVP secretion into hypophysial portal blood (HPB). The experiment has been performed in sheep since it is possible to collect HPB and quantify CRH and AVP secretion in this animal under physiological conditions. The release of both peptides into HPB was stimulated by endotoxin injection, the increase in portal AVP being more pronounced than that of CRH. An initial, transient, increase in jugular AVP concentrations was observed, probably due to the activation of magnocellular AVP neurons. In conclusion, our data indicate that the activation of the pituitary–adrenal axis after endotoxin injection is associated with an increased release of both CRH and AVP into HPB. Magnocellular AVP neurons are initially stimulated while parvocellular CRH and AVP neurons are stimulated throughout the experiment.

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### Introduction

The hypothalamic–pituitary–adrenal (HPA) axis plays an important role in maintaining homeostasis under stressful conditions such as microbial infections and endotoxin shock. Interleukin (IL)-1, IL-6 and tumor necrosis factor- $\alpha$ , the major inflammatory cytokines produced under these situations, stimulate the HPA in rats as shown by increased plasma adrenocorticotropin (ACTH) and glucocorticoids and pro-opiomelanocorticotropin mRNA in the anterior pituitary gland (1–3). The regulation of ACTH secretion is a multifactorial process which involves mainly two hypothalamic neurohormones, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) (4, 5). There is now convincing evidence that in the rat cytokines stimulate hypothalamic CRH secretion as shown by *in vivo* (6, 7) and *in vitro* (8, 9) studies. However, the influence of cytokines on hypothalamic AVP release remains controversial. Indeed, IL-1 has been shown *in vitro* to exert no effect on AVP release from hypothalamic fragments (8, 9). Other authors have reported no effect of IL-1 on AVP secretion into hypophysial portal blood (HPB) (6), no change in AVP turnover in the median eminence (7) and no effect on the activity of electrophysiologically identified AVP

neurons (9). In contrast, other *in vivo* experiments support a stimulating action of cytokines on AVP release. Indeed, Whitnall *et al.* (10) have reported that IL-1 deplete secretory vesicles at the level of the paraventricular nucleus (PVN) parvocellular neurons, not only from AVP-deficient CRH neurons but also from AVP-expressing CRH neurons (10). In addition, peripheral administration of IL-1 evokes AVP rise in systemic plasma (11, 12) and in perfusates of hypothalamus (13). In the latter studies, the stimulation of AVP release probably occurs at the level of magnocellular neurons of the PVN. Thus, although the effect of endotoxin administration on the stimulation of CRH neurons has been well established, its effect on AVP neurons remains controversial. AVP may play an important role in stress adaptation by its action on the pituitary gland and also through its peripheral action on vasoconstriction. Therefore, it was of interest to evaluate AVP secretion in portal as well as in systemic blood.

During the past few years, a method for measuring CRH and AVP in HPB under physiological conditions has been validated in conscious, unrestrained sheep (14, 15). In this species, the ACTH-releasing potency of AVP has been reported higher (16) or similar (17) to that of CRH. Both peptides act synergistically for

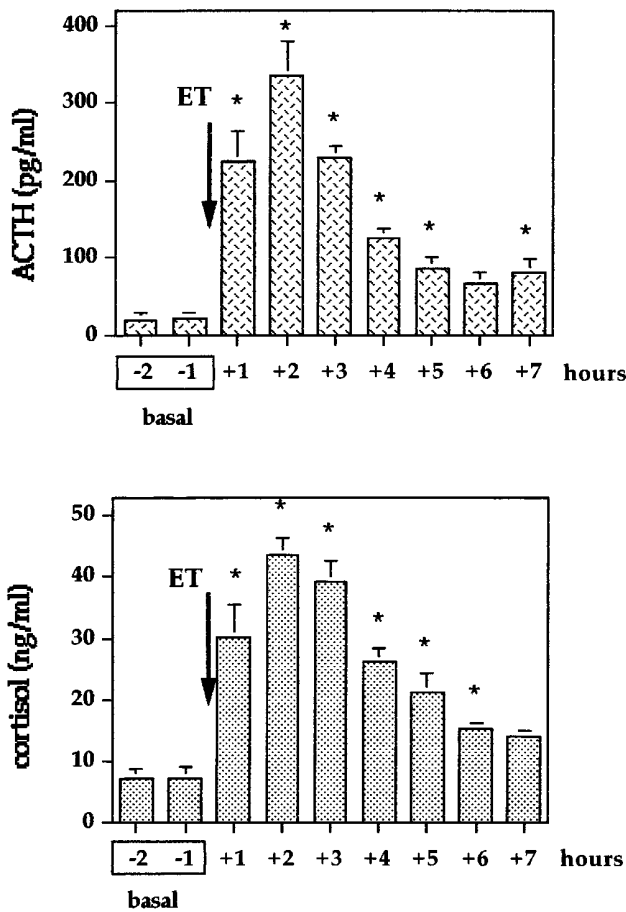
releasing ACTH (18) and have been involved in the physiological response of ACTH and cortisol to stress (19, 20). Using this method, we have directly measured CRH and AVP secretion into HPB after intravenous injection of endotoxin. Endotoxin, a potent stimulator of the immune system, is a lipopolysaccharide (LPS) found as a principal component of Gram-negative bacteria, the main mediator of septic shock. We have also measured AVP levels in jugular plasma (as an index of the secretion of magnocellular AVP neurons) in order to determine if magnocellular AVP neurons are activated by the immune challenge.

**Materials and methods**

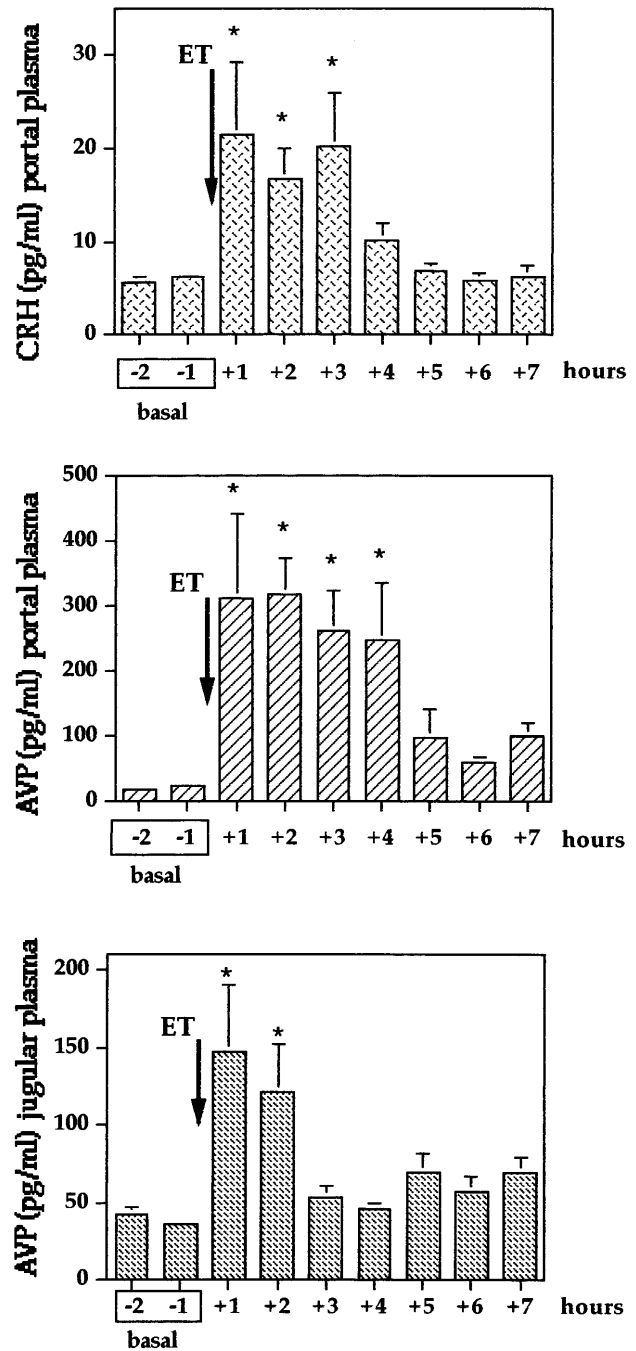
**Animals and surgery**

The experiments were carried out on five 9- to 11-month-old Merinos Alps rams (45 kg body weight). The experimental protocol was approved by the Ethical Committee on Animal Studies of the University of Aix-Marseille II. The rams were prepared for portal blood

sampling under general anesthesia, as previously described (15, 21). A twin cannula was implanted through the transnasal route in front of the long portal vessels, above the anterior pituitary gland. After 10–12 days, catheters were inserted into the jugular veins of two animals, placed side-by-side in two



**Figure 1** Effect of endotoxin (ET) injection (400 ng/kg i.v.) on mean ( $\pm$ S.E.M.) hourly plasma ACTH and cortisol levels in sheep ( $n = 5$ ). \*  $P < 0.01$  vs basal ACTH and cortisol levels.



**Figure 2** Effect of endotoxin (ET) injection (400 ng/kg i.v.) on mean ( $\pm$ S.E.M.) hourly CRH and AVP levels in HPB, and AVP levels in jugular plasma ( $n = 5$ ). \*  $P < 0.01$  vs basal portal CRH and AVP and jugular AVP levels.

small pens on the floor; 24 h later, at 0600 h, a needle was inserted into the upper cannula to create a lesion of hypophysial portal vessels, and the resulting portal blood was collected through the lower cannula. One jugular catheter was used for injection of heparin (25 000 IU at the beginning, followed by 5000 IU every 30 min) and endotoxin; the other was connected to a peristaltic pump and used for collection of peripheral blood. Paired samples of portal (0.8–1.2 ml, depending on the animals) and jugular blood (2.0 ml) were collected using a peristaltic pump every 10 min for 8 h.

### Experimental procedures

Endotoxin (*Escherichia coli* O55: B5; Sigma, St Louis, MO, USA) was reconstituted in phosphate-buffered saline with 0.1% BSA to a stock concentration of 0.1 µg/µl. Endotoxin was administered by a bolus injection (400 ng/kg) in 2 ml saline through the jugular cannula, followed by 5 ml saline as previously described (22). Jugular and portal blood were collected during the 2 h period preceding endotoxin or saline injection and during the subsequent 6 h. Blood samples were immediately centrifuged at 4 °C for 10 min and the resulting plasma was stored at –20 °C until assayed.

### Assays

All hormone measurements were performed in the same assay using previously described extraction and radio-immunological techniques (23). The intra-assay coefficients of variation within the measurement range of each assay were: CRH, 6%; AVP, 5.5%; ACTH, 5%; and cortisol 4.7%. The limits of detection of the assays were 5 pg/ml for CRH and AVP, 10 pg/ml for ACTH, and 0.5 ng/ml for cortisol.

### Statistical analysis

The mean hourly plasma hormone concentrations were calculated for the period of basal secretion and the period following endotoxin administration. Results are expressed as the means ± S.E.M. Data were compared by analysis of variance followed by Fisher's test using a computerized statistical program (Statview 512, Brain Power Inc., Calabasas, CA, USA).

### Results

The i.v. administration of endotoxin at the dose of 400 ng/kg led to increased respiration, intermittent cough and diarrhea. High fever (41–42 °C) was recorded in all animals and lasted for 6 h. Mean plasma ACTH and cortisol levels increased rapidly and significantly. The maximum increase was reached for both hormones during the second hour, with a 10- and

4.5-fold increase for ACTH and cortisol respectively (Fig. 1). CRH and AVP release into HPB were both stimulated by endotoxin administration. The mean portal CRH levels increased rapidly with a peak during the first hour. CRH levels remained high during the first 3 h and then returned to initial values. The stimulation of AVP release was much more pronounced. High levels were already reached within the first hour. Then, AVP levels were still high during the next 3 h, before decreasing rapidly to levels which were still moderately higher than basal values. Jugular AVP levels increased after endotoxin administration; the increase was rapid and transient, lasting for only 2 h (Fig. 2). Figure 3 shows a typical profile of ACTH and cortisol in peripheral blood and Fig. 4 a typical profile of CRH and AVP portal levels and jugular AVP in the same representative animal.

### Discussion

Our data indicate that, in the sheep, acute parenteral administration of endotoxin stimulates ACTH and cortisol secretion and the release of CRH and AVP into HPB. The pattern of stimulation of AVP release into the peripheral circulation is different from that into HPB, being transient and limited to the first 2 h. The source of AVP measured in jugular blood or HPB is probably different. Indeed, peripheral AVP is secreted by the posterior pituitary gland and reflects the activity of magnocellular neurons in

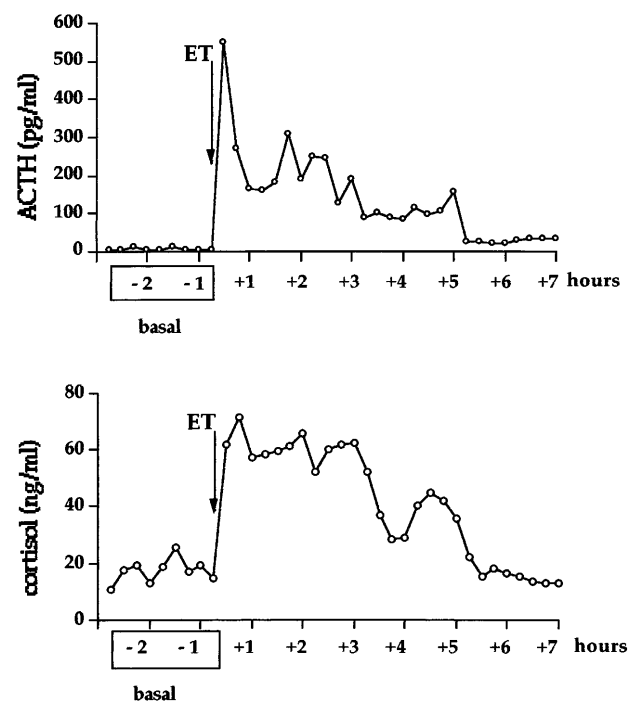


Figure 3 Effect of endotoxin (ET) injection (400 ng/kg i.v.) on jugular plasma ACTH and cortisol levels in one representative animal.

the PVN and supraoptic nucleus (5). AVP in hypophysial portal vessels originates mainly from the parvocellular part of the PVN, although the contribution of magnocellular neurons to portal AVP cannot be excluded (24, 25). The analysis of the pattern of jugular and portal AVP levels following endotoxin administration suggests that magnocellular AVP neurons are rapidly and transiently activated and that the parvocellular portion of the PVN accounts mainly for the increased AVP levels in portal blood after the third hour.

The temporal variations in the activation of the different cellular groups should be better defined by *in situ* hybridization for hypothalamic CRH and AVP mRNA. However, studies on CRH and AVP cellular localization in ovine hypothalamus are rather limited and no information is yet available on AVP and CRH co-localization in this species. Using this technique in the rat, the activity of

neurons of the parvocellular portion of the PVN expressing CRH and AVP has been shown to increase after endotoxin administration. However, in the same animals, there was no evidence for the activation of the magnocellular part of the PVN. An expression of *c-fos* was detected in magnocellular neurons of the PVN, but the transcript was localized mainly in oxytocin neurons and only in few AVP cells (26). In conscious sheep, the intravenous administration of LPS is accompanied by a significant upregulation of *c-fos* and IL-1 $\beta$  mRNA in the choroid plexus, *c-fos* CRH and oxytocin mRNA in the PVN and *c-fos* mRNA in the supraoptic nucleus. There was no clear upregulation of AVP mRNA in the PVN or supraoptic nucleus (27). However, it is generally accepted that endotoxin injection is followed by an increased production of humoral and hypothalamic cytokines which have been shown to stimulate the HPA axis. A stimulating action of cytokines at the level of AVP magnocellular neurons has been observed in intact (11–13) and adrenalectomized rats (28) as well as in humans (29). Indeed, the stimulation of AVP release into the peripheral circulation in both species has been attributed to the activation of magnocellular neurons since this population of hypothalamic neurons is considered to be the source of AVP in the general circulation. Thus, our results are in good agreement with those previous *in vivo* studies performed in rat and humans. The physiological importance of AVP from magnocellular hypothalamic neurons is still unproven. In rats, it has been possible to induce chronic hyponatremia and inhibit magnocellular hormone secretion while activation of parvocellular neurons appears to be unaffected (30). Under such condition, the stimulation of ACTH secretion induced by either stress or hypertonic saline injection is reduced, indicating that it is partially mediated by magnocellular AVP (31). Similar data have been observed in neuroleptomized rats (32).

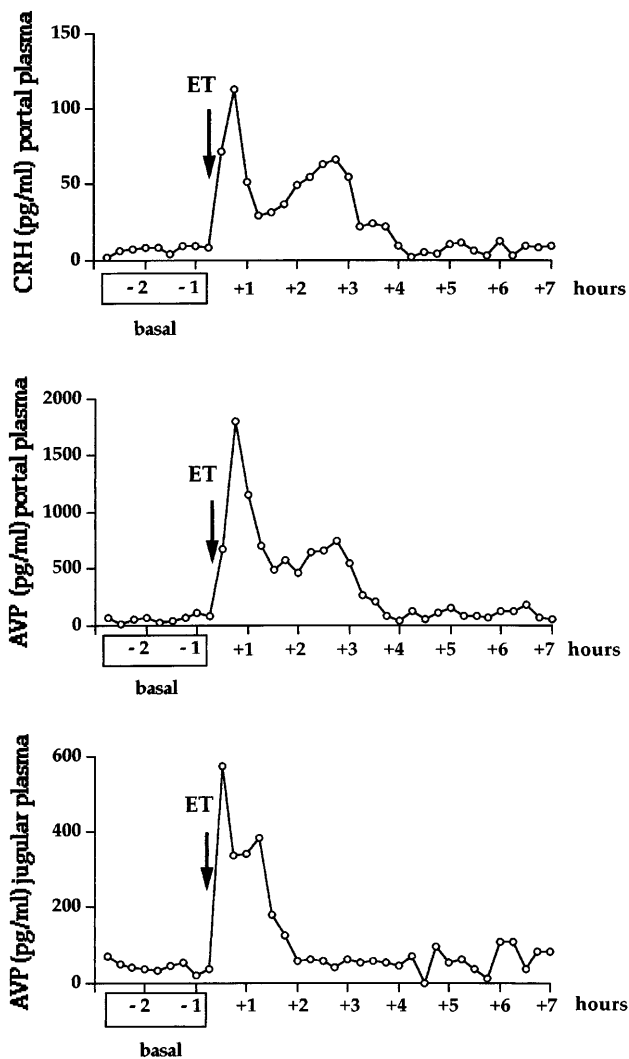
In summary, we have observed that the parenteral administration of endotoxin stimulates the hypothalamic release of CRH and AVP into the HPB. The simultaneous determination of peripheral and portal AVP suggests that AVP secreted by magnocellular neurons increases transiently, shortly after endotoxin administration.

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**Figure 4** Effect of endotoxin injection (400 ng/kg i.v.) on CRH and AVP levels in HPB and AVP concentrations in jugular plasma in one representative animal.

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