The effects of metyrapone infusion on corticotropin-releasing factor and arginine vasopressin secretion into the hypophysial portal blood of conscious, unrestrained rams

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The effects of rapid changes of circulating cortisol levels on ACTH secretion and on corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) concentrations in hypophysial portal blood were studied in six adult rams. Pharmacological adrenalectomy was obtained by 3 h metyrapone infusion (100 mg·kg⁻¹·h⁻¹). Blockade of cortisol synthesis induced a tenfold increase of plasma ACTH levels accompanied by a moderate increase of CRF secretion (150% vs preinjection levels) and a large increment of AVP secretion (535% vs preinjection levels). ACTH levels remained high during the 3 h following the end of metyrapone infusion. During the same period, CRF secretion was still elevated (231% vs preinjection levels), while AVP secretion was further stimulated (2,151% vs preinjection levels). Subsequent hydrocortisone infusion (66 µg·kg⁻¹·h⁻¹) for 2 h induced a rapid decrease of both ACTH and AVP secretion, while CRF levels in hypophysial portal blood still remained elevated. These data suggest that changes in ACTH secretion induced by acute modifications of the negative glucocorticoid feedback are, in addition to the well documented direct effect of cortisol on the corticotropes, mainly mediated by variations of hypothalamic AVP secretion.

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The regulation of both basal and stress-induced anterior pituitary ACTH secretion and synthesis is a multifactorial process which involves mainly two hypothalamic neurohormones: corticotropin-releasing factor (CRF) and arginine vasopressin (AVP). Both CRF and AVP involved in ACTH regulation are synthesized in the parvocellular portion of the hypothalamic paraventricular nucleus (PVN). Axons from this region project to the zona externa of the median eminence and release their peptide content into the hypophysial portal blood (HPB, review in 1). Glucocorticoids represent the major inhibitory factor for ACTH synthesis and secretion. After removal of endogenous glucocorticoids by adrenalectomy (ADX), pituitary ACTH synthesis and secretion increase. These changes are reversed by subsequent treatment with natural or synthetic glucocorticoids. Although it is well established that glucocorticoids can modulate directly the corticotrope cells, it is known that part of the negative glucocorticoid feedback is exerted through a modulation of the hypothalamic neuropeptides that control ACTH secretion (2). Nevertheless, the respective role of CRF and AVP in mediating this feedback is still a matter of debate. Most of the studies exploring the negative glucocorticoid feedback, particularly changes in CRF and AVP secretion into HPB, have been performed in rats (3–7). However, HPB collection in rats requires anesthesia and acute surgical approach, with consequent activation of the hypothalamo-pituitary-adrenal axis. In addition, lesion of the pituitary stalk will sever AVP fibers originating from the magnocellular neurons and projecting to the posterior pituitary (8). Recently, methods for measuring neurohormones in HPB in conscious and unrestrained sheep have been available (9, 10). In this report we studied the effects of acute cortisol deprivation or replacement on CRF and AVP secretion in HPB and ACTH and cortisol release in peripheral blood in sheep. Acute glucocorticoid deprivation was obtained by metyrapone infusion. Metyrapone is a 11 β-hydroxylase inhibitor which acutely suppresses cortisol synthesis, with subsequent activation of ACTH and deoxycortic (S) secretion (11). Metyrapone testing is widely used in the clinical investigation of hypothalamo-pituitary-adrenal disorders (12, 13).
Materials and methods

Animals and surgery

The experiments were carried out on six 9–11-month-old Merinos Alps rams (45 kg bw). The rams were prepared for portal blood sampling under general anesthesia as previously described (10). A twin cannula was implanted through the transnasal route in front of the long portal vessels, above the anterior pituitary gland. After 7 days, catheters were inserted into the jugular veins of two animals, placed side by side in two small pens on the floor; 24 h later, one catheter was used for injection of heparin (25,000 IU at the beginning followed by 5000 IU every 30 min); the other was connected to a peristaltic pump and used for collection of peripheral blood. At 09.00 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. Paired samples of portal blood (1 to 2 ml, depending on the animals) and jugular blood (2 ml) were collected using a peristaltic pump every 20 min for 11 h.

Experimental procedures

Metyrapone (Ciba-Geigy, Basel, Switzerland), dissolved in normal saline, was iv infused using a peristaltic pump at a constant rate of 100 mg·kg⁻¹·h⁻¹ for 3 h. Jugular and portal blood were collected during the 2 h period.
Fig. 2. Variations in peripheral ACTH levels and CRF and AVP concentrations in hypophysial portal blood under basal conditions, during and after iv metyrapone infusion (100 mg·kg⁻¹·h⁻¹) and during hydrocortisone infusion (66 μg·kg⁻¹·h⁻¹) in a representative animal.
preceding metyrapone infusion (basal conditions), during metyrapone infusion and during the subsequent 3 h. Subsequently, hydrocortisone (Hydrocortisone Hemisuccinate, Roussel-Uclaf, Basel Switzerland), dissolved in normal saline, was iv infused using a peristaltic pump at a constant rate of 66 μg·kg\(^{-1}\)·h\(^{-1}\) for 2 h, while portal and jugular blood were still collected.

**Assays**

All hormone measurements were performed in the same assay using previously described extraction and radioimmunological techniques (14, 15). The intrassay coefficients of variation were: CRF, 6%; AVP, 5.5%; ACTH, 5%; cortisol, 10%; S: 8.5%. The limit of detection of the assays were: 1.05 pmol CRF/l plasma, 1.5 pmol AVP/l plasma, 1.1 pmol ACTH/l plasma, 2.21 nmol cortisol/l plasma, 0.75 nmol S/l plasma.

**Statistical analysis**

Mean plasma hormone concentrations were calculated for the 2 h of basal conditions, the 3 h during and after metyrapone infusion, and the 3 h during hydrocortisone infusion. Results are expressed as the mean ± se. Data were compared by analysis of variance followed by Fischer’s test using a computerized statistical program (Statview 512, BrainPower, Inc., Calabasas, CA).

**Results**

Fig. 1 shows a typical profile of cortisol and S concentrations in peripheral blood and Fig. 2 a typical profile of peripheral ACTH concentrations and CRF and AVP levels in HPB. Metyrapone infusion induced a dramatic and rapid decrease in plasma cortisol accompanied by a large increase in S, which lasted for the 3 h following the end of metyrapone infusion. Plasma ACTH concentrations increased rapidly after the onset of metyrapone infusion and remained elevated during the 3 h following the end of metyrapone infusion. CRF levels in HPB were moderately increased during metyrapone infusion and showed a larger increment during the 3 h following the end of metyrapone infusion. The pattern of AVP concentrations in HPB was different from that of CRF, showing an immediate, large, and long-lasting increase since the onset of metyrapone infusion. It should be noted that the plasma levels of ACTH increased just before significant increases in CRF and AVP were observed. This is probably due to the removal of the direct inhibitory effect of glucocorticoids on the corticotropes (2). Fig. 2 shows the effect of hydrocortisone infusion, starting 3 h after the end of metyrapone infusion, on peripheral ACTH levels, and CRF and AVP concentrations in HPB. Hydrocortisone induced a rapid and parallel decrease in ACTH and AVP levels, which returned to preinfusion levels, while CRF concentrations remained unchanged.

Fig. 3 shows the results obtained in the six animals.

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**Figure 3** Variations in ACTH, cortisol and S concentrations in peripheral blood and CRF and AVP levels in hypophysial portal blood under basal conditions, during and after iv metyrapone infusion (100 mg·kg\(^{-1}\)·h\(^{-1}\)), and during iv hydrocortisone infusion (66 μg·kg\(^{-1}\)·h\(^{-1}\)). Results are the mean ± se of six animals. * p < 0.05.
Cortisol concentrations decreased significantly (p<0.001) during (1.71 ± 0.25 nmol/l) and after metyrapone infusion (5.24 ± 3.42 nmol/l) compared with preinfusion levels (32.84 ± 3.61 nmol/l), while S concentrations increased (p<0.001) both during (35.05 ± 3.99 nmol/l) and after metyrapone infusion (53.33 ± 2.42 nmol/l) compared with preinfusion levels (9.19 ± 0.12 nmol/l). Plasma ACTH showed a large increase (p<0.001) both during (242 ± 39 pmol/l) and after metyrapone infusion (279 ± 38 pmol/l) compared with preinjection levels (28 ± 4 pg/ml). CRF levels in HPB increased slightly but significantly (p<0.05) during the 3 h of metyrapone infusion (15.05 ± 1.34 pmol/l), and showed a larger increment during the subsequent 3 h (23.35 ± 2.33 pmol/l, p>0.01 vs preinjection: 10.10 ± 1.03 pmol/l). AVP concentrations in HPB showed a significant increment during metyrapone injection (53.1 ± 9.3 pmol/l, p<0.001) and were further increased after the end of metyrapone administration (213.7 ± 31.6 pmol/l, p<0.001 vs preinjection: 9.9 ± 0.9 pmol/l). As expected, hydrocortisone infusion resulted in a dramatic increase of plasma cortisol concentrations (22.91 ± 9.11 pmol/l). During hydrocortisone administration, ACTH and AVP levels decreased significantly (78 ± 14 and 106 ± 25 pmol/l, respectively, p<0.01 vs the mean levels measured during the 3 h post-metyrapone infusion), while CRF concentrations were unchanged (19.9 ± 2.3 pmol/l).

Discussion

Our data indicate that, in the sheep, acute depletion of circulating glucocorticoids obtained by metyrapone infusion induces a rapid increase in plasma ACTH levels, as previously reported in other species (4, 11–13). Under these experimental conditions both CRF and AVP secretion in HPB were stimulated, supporting the hypothesis that, in addition to the well-documented direct effect of cortisol on the corticotropes (16), both neurohormones are necessary for the anterior pituitary response to removal of endogenous glucocorticoids (17). It is not clear from our experiment whether the increased CRF and AVP release were consecutive to changes in the pulsatility of secretion of these two neurohormones. Indeed, our timing of HPB collection does not allow study of the temporal variations of hypothalamic neurohormone secretion. CRF and AVP secretion in HPB did not vary in a parallel fashion after glucocorticoid removal or replacement. Indeed, a rapid decrease of plasma cortisol following metyrapone infusion modestly stimulated CRF concentrations in HPB, while AVP release was dramatically increased. This observation is consistent with the report of Holmes et al. (18), who showed that the ratio of AVP:CRF secretion from rat median eminence incubated in vitro increases after adrenalectomy (ADX). In addition, it is well documented that CRF and AVP become co-localized in the parvocellular neurons of the PVN after ADX (19). Contrary to this, hydrocortisone infusion, which under our experimental conditions shows a comparable time course to that of the intermediate phase of the physiological glucocorticoid feedback (2), significantly reduced AVP secretion, while CRF release was unaffected. This observation suggests that the respective roles of CRF and AVP in mediating the negative glucocorticoid feedback may differ in accordance with the time course and with the variations in plasma glucocorticoid levels. Indeed, it has been reported that in rats long-term surgical ADX stimulates both CRF and AVP secretion in HPB (5). It is well established that ADX induces an increase in CRF and AVP gene transcription in the paraventricular nucleus (20, 21) that is reversed by chronic glucocorticoid treatment (22). Dallman et al. (17) have demonstrated that, after hypothalamic deafferentation in the rat, there was no response of either proopiomelanocortin (POMC) mRNA or plasma ACTH to ADX. In the same animals, chronic CRF treatment was able to induce a post-ADX increase in anterior pituitary POMC mRNA levels comparable to that found in unlesioned ADX rats.

However, CRF caused only a 2.5-fold elevation of plasma ACTH in lesioned ADX rats, whereas ADX in intact animals brought about a 12-fold increase, suggesting that a hypothalamic factor other than CRF was responsible for the post-ADX increased secretion of ACTH. Taken together with the above-mentioned reports, our present findings suggest strongly that the increased AVP synthesis (21) and secretion (5) is responsible for the increased ACTH secretion following ADX. This also suggests that the increased CRF synthesis (20) and secretion (5) in response to ADX has little effect on ACTH secretion by itself but may potentiate the effect of AVP on the corticotropes, as indicated by the reduction in the post-ADX increase of plasma ACTH levels after passive immunization against CRF (23). It is also clear that increased CRF secretion in response to long-term glucocorticoid deprivation stimulates POMC gene transcription, and therefore ACTH synthesis (24). Contrary to this, in long-term ADX rats, 3 h dexamethasone treatment did not change CRF output, while AVP secretion was significantly decreased (5). Taken together with our data, this suggests that the intermediate glucocorticoid negative feedback induces a decrease in AVP but not CRF secretion into HPB. Contradicting this, it has been shown that treatment for 17 h with dexamethasone decreases CRF secretion in HPB (25). Therefore, one can hypothesize that during long-term glucocorticoid deprivation, increased CRF synthesis and secretion could serve to stimulate ACTH synthesis and therefore to allow the pituitary chronically to hypersecrete ACTH in response to increased AVP synthesis and secretion. On the other hand, during sudden and short-lasting increases of plasma glucocorticoid levels, i.e. during the intermediate phase of the physiological negative glucocorticoid feedback, decreased AVP secretion is sufficient to modulate ACTH secretion, changes in ACTH synthesis being of little benefit for the regulation of...
the corticotropic function. Such an acute regulation of ACTH secretion mainly driven by AVP has already been described in the sheep during exposure to stress (14, 26, 27).

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