Plasma concentrations of adrenocorticotropin-related peptides after corticotropin-releasing hormone and vasopressin injections in sheep

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Abstract. Ovine corticotropin-releasing hormone (1 µg/kg body weight) and arginine vasopressin (1 µg/kg) were injected iv in sheep, both separately and in combination. Plasma were sampled just before and 5, 15 and 30 min after the injection. Adrenocorticotropin-related peptides were isolated by Sephadex G-50 column chromatography and measured by RIA. Cortisol and aldosterone were determined on the same plasma samples. Three molecular forms of immunoreactive ACTH (IR-ACTH) were isolated: ‘big’ (> 20 000 mol wt), ‘intermediate’ (= 8000 mol wt) and ‘little’ (= 4500 mol wt). Following CRH injections, the three molecular forms of ACTH were enhanced, particularly the ‘little’ form, whereas ‘intermediate’ IR-ACTH was highly and specifically responsive to AVP. After a simultaneous injection of CRH and AVP, additive increases occurred for ‘intermediate’ and ‘little’ IR-ACTH. The release of different molecular forms of IR-ACTH after stimulation by CRH or AVP of corticotrope cells suggests that ACTH-related peptides could be stored in different intracellular pools or secreted by different pituitary cells.

Among the hypothalamic factors controlling ACTH release, CRH is playing the dominant role. This peptide isolated by Vale et al. (1981) from the ovine hypothalamus has been used to stimulate the pituitary-adrenal system in several species, specially in sheep (Kalin et al. 1983; Pradier et al. 1986). Numerous previous reports have shown that AVP could act as a CRH-like peptide (for review see Lutz-Bucher et al. 1981). Recent works confirmed the CRH activity of AVP, with (De Bold et al. 1984; Rivier & Vale 1983; Redekopp et al. 1986) or without (Bähr et al. 1988) additive effects of both peptides on pituitary activity. Such discrepancies may be due to the doses used in vivo as well as to differences in the in vitro preparations. After CRH injection into sheep, Donald et al. (1983) and Pradier et al. (1986) observed a rise in plasma aldosterone. Thus, the effects of CRH and AVP on the pituitary-adrenal axis were compared in sheep (Redekopp et al. 1985; Pradier et al. 1986) and both studies suggested that AVP acted similarly to CRH on plasma immunoreactive ACTH (IR-ACTH), cortisol and aldosterone concentrations. It was, however, interesting that increases in plasma ACTH and aldosterone concentrations were greater after AVP than after CRH, whereas the opposite was observed for cortisol. This difference of response to CRH and AVP could be due to the release by the pituitary of different immunoreactive ACTH-like peptides.

Furthermore, two different anatomical sites of the anterior pituitary have been described to secrete ACTH (Lutz-Bucher et al. 1981) responding specifically to CRH or AVP (Lutz-Bucher & Koch 1983). Thus, in the sheep pituitary, Silman et al. (1979) found a heterogeneity of the immu-
noreactive ACTH content. In the present study we have compared the effects of CRH and AVP injections on the plasma concentrations of the different molecular forms of IR-ACTH in the adult sheep.

Materials and Methods

Treatment of animals and sampling of plasma

This experiment was performed on 20 Ile de France ewes, age 2 years, weight about 50 kg, which were neither pregnant nor lactating. Each animal was housed in an individual box, was fed hay and grain concentrate, and had free access to tap water. Animals were divided into 4 groups which received, respectively, CRH (1 µg/kg), AVP (1 µg/kg), CRH + AVP (1 µg of each/kg) or isotonic saline (0.1 ml/kg) (injection vehicle).

CRH and AVP (Sigma Chemical Co, St. Louis, MO) were dissolved in 0.001 N HCl and diluted ten times with sterile saline 0.9% containing 0.25% bovine serum albumin (final dilution 10 mg/l). Catheters were implanted into the left and right jugular veins the day before treatment, one being used for injection, the other for blood sampling. Blood was collected on EDTA just before the injection and then 5, 15 and 30 min after. After centrifugation at 4°C, a 1-ml sample of plasma, acidified to pH 1.5 by addition of 200 µ1 1.6% glycin in 1 N HCl (for the ACTH chromatography) and the remaining plasma (for steroid measurement) were stored at −20°C until assayed.

Separation of different forms of ACTH

The different forms of ACTH were obtained by subjection of plasma to gel filtration at 4°C on Sephadex G50 fine (Pharmacia, Uppsala, Sweden) using 1% formic acid as buffer according to Ratter et al. (1980). As described previously (Yalow & Berson 1973; Orth & Nicholson 1977; Chatelain & Cheong 1987) we used Sephadex G50 rather than G75 or G100 in order to obtain a better separation of the low molecular weight forms of ACTH. The separation of the different high molecular weight forms (> 20 000) containing a very low corticosteroidogenic activity (Roebuck et al. 1980; Chatelain & Cheong 1987), was of a minor interest.

Columns (Pharmacia K9/60, 60 × 0.9 cm) were first equilibrated and then eluted with 1% formic acid containing polypeptide (1 g/l, polyep 5115, Sigma Chemical Co) at a flow rate of 10 ml/h. The pre-acidified plasma sample was thawed, centrifuged and added on the top of the column. One milliliter fractions were collected and evaporated to dryness in a Speed Vac Concentrator (Savent, Formingdale, NY).

According to previous data (Coslovsky & Yalow 1974; Orth & Nicholson 1977; Chatelain & Cheong 1987), the following nomenclature of the different molecular forms of ACTH has been used:

The molecular form of ACTH eluted at the same position as ACTH1-39 was called 'little' IR-ACTH.

High molecular forms of ACTH eluted near the void volume of the column (indicating an apparent molecular weight of at least 20 000) were called 'big' IR-ACTH.

The peak of immunoreactive ACTH, eluted midway between the two previous peaks, was designated 'intermediate' IR-ACTH.

The apparent molecular weight of the different forms of ACTH was determined by gel filtration on column calibrated with: dextran blue 2000 (Pharmacia), α-chymotrypsinogen A (25000, Sigma Chemical Co) α- and β-lactalbumin (16500 and 36000, gift of Dr Leveux, INRA Theix, France), cytochrome C (12400, Sigma Chemical Co), synthetic human ACTH1-39 (4570, Giba-Geigy, Basel) and vitamin B12 (1355, Sigma Chemical Co) (Fig. 1). The apparent molecular weight was calculated from the equation $K_{av} = \frac{(V_c - V_0)}{(V_i - V_0)}$ where $K_{av}$ = partition coefficient, $V_c$ = elution volume, $V_o$ = void volume and $V_i$ = total volume of the gel.

The recovery of the different peptides added to acid-treated plasma containing no immunoreactive ACTH and applied to the column was over 90%.

Radioimmunoassays

Plasma cortisol and aldosterone concentrations were estimated according to Dalle & Delost (1976) and Pradier et al. (1986), respectively. The sensitivity of the cortisol assay was 0.10 pmol/tube and that of the aldosterone assay 50 fmol/tube. The inter- and intra-assay CV were, respectively, 10 and 8%, for cortisol and 15 and 10% for aldosterone.

The fraction tubes were reconstituted with assay buffer and radioimmunoassay of ACTH was performed using ACTH1-24 standard as previously described (Pradier et al. 1986). Sensitivity was 0.5 fmol/

Results

Molecular forms of IR-ACTH in sheep plasma (Fig. 1)

Radioimmunoassay of ACTH in eluted fractions showed three peaks corresponding to 'big', 'intermediate' and 'little' IR-ACTH. Between these three peaks possessing ACTH immunoreactivity only blank values were obtained.

Determination of apparent molecular weight gave values of 4500 for 'little' ACTH, 8000 for 'intermediate' ACTH and higher than 20 000 for 'big' ACTH.

The mean plasma concentrations of these three different molecular forms of IR-ACTH were expressed as pmol/l of IR-ACTH1-39. Values measured in twenty adult normal sheep were 6.90
Elution profile of immunoreactive ACTH, in 1 ml plasma of sheep, 15 min after injection of saline, CRH, AVP or CRH+AVP. Arrows indicate the elution positions of calibration markers (DB: dextran blue; βL: β-lactalbumin; αC: α-chymotrypsinogen A; αL: α-lactalbumin; C: cytochrome C; ACTH: ACTH₁₃₉; B₁₂: Vitamin B₁₂).

± 1.10 pmol/l for ‘big’ IR-ACTH, 3.10 ± 0.70 pmol/l for ‘intermediate’ IR-ACTH and 31.8 ± 4.7 pmol/l for ‘little’ IR-ACTH.

Integrated responses of IR-ACTH peptides after CRH and AVP injections (Fig. 2)

During the 30 min of the sampling period sheep injected with CRH, in contrast to the control animals, presented a significant rise in the three molecular forms, of equivalent magnitude. Highest values were obtained at 15 min for the dominant form: from 32 ± 5 pmol/l before injection, ‘little’ IR-ACTH concentrations reached 65 ± 6 pmol/l. The animals injected with AVP showed a spectacular sharp rise in ‘intermediate’ IR-ACTH, whereas ‘little’ IR-ACTH remained unchanged. Thus, after AVP treatment, ‘intermediate’ IR-ACTH became the dominant molecular form of IR-ACTH in sheep plasma. It demonstrated a sharp significant rise as soon as 5 min after injection (+750%), reaching the top value at 15 min (105 ± 12 pmol/l, +2400%). In animals receiving the combination of CRH and AVP, rises in the ‘intermediate’ and the ‘little’ forms were equivalent to the addition of both single specific actions.

Changes in cortisol and aldosterone concentrations in the sheep plasma after CRH or AVP injection (Fig. 3)

During the sampling period, plasma cortisol concentrations increased significantly from 53 ± 8 at 0 to 97 ± 12 nmol/l at 30 min (P < 0.05) whereas plasma aldosterone levels remained unchanged (400 ± 25 pmol/l) in the control group.

After either CRH or AVP alone and after injection of both peptides together, plasma cor-
tisol and aldosterone concentrations increased significantly as early as 5 min after injection, reaching top levels after 15 min.

Other observations
Whatever the treatment and the sampling time, we did not observe any changes in plasma renin activity, or in osmolality (298 ± 2 mosmol/l), in Na and K concentrations (respectively, 138 ± 1 and 3.7 ± 0.2 mmol/l) or in hematocrit (32 ± 1%).

Discussion
This experiment in sheep have allowed us to compare the effects of CRH and AVP on plasma concentrations of the different molecular forms of IR-ACTH, cortisol and aldosterone. The results confirmed the efficiency of AVP on ACTH-releasing activity, as already demonstrated in vivo in sheep (Redekopp et al. 1985; Pradier et al. 1986), in the rat (Rivier & Vale 1983) and in vitro especially in the rat (for review, see Antoni 1986).
The biosynthesis of ACTH from its large precursor proopiomelanocortin (POMC) by enzymatic cleavage, is accompanied by the release of lipotropin and peptides with ACTH-immunoreactivity. They were identified in ovine pituitary gland by Silman et al. (1979). Differences were demonstrated between the ACTH-releasing actions of AVP and CRH, especially at the pituitary level (Antoni 1986). It has been shown that pituitary corticotrope cells AVP receptors in the rat were clearly distinct from those binding CRH and represented a novel type of receptors, different from V1 (pressor) and V2 (renal) (Koch & Lutz-Bucher 1985; Baertschi & Friedli 1985; Mormede et al. 1985; Jard et al. 1986). Furthermore, several pools of POMC-related peptides in cultured pituitary cells have been demonstrated (Ham & Smyth 1985). These authors observed that the secretion occurring after CRH contained a much higher proportion of ‘little’ IR-ACTH than the basal secretion. Our results agree well with these data. Our original contribution to this field of research concerns the preferential secretion of ‘intermediate’ IR-ACTH after AVP (80% of the secreted IR-ACTH compared with 10% in controls). Thus, the activation of different receptors may produce the release of different intracellular pools of ACTH or may stimulate different corticotrope cells.

Previously, Pradier et al. (1986) demonstrated that cortisol release was lower after AVP than after CRH. In the present work, the responses were similar with both peptides. The discrepancy may be due to the time course of sampling (0–30 min against 3 h for the older data).

At the adrenal level, it appears that ‘little’ and ‘intermediate’ IR-ACTH have the same biological activity on cortisol as on aldosterone secretion. Similar results have been obtained studying corticosterone production in the fetal rat (Chatelain & Cheong 1987).

In conclusion, these results show that CRH and AVP stimulation of sheep pituitary induced the release of different immunoreactive forms of ACTH.

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References


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