Naloxone affects the release of cortisol, but not of vasopressin or oxytocin, in dehydrated sheep

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Abstract. Ovariectomized ewes (N = 7) were dehydrated for 24 h and then given iv injections of saline vehicle or 8 or 64 mg naloxone hydrochloride in saline. Blood samples were taken by jugular venepuncture before and after dehydration and at intervals during the 90 min period directly following naloxone treatment. Plasma concentrations of AVP, OT and cortisol were measured by radioimmunoassay. Plasma AVP levels and osmolality increased with dehydration, OT concentrations showed no consistent change, and cortisol levels were unaffected. After administration of naloxone, AVP and OT concentrations did not alter. The sampling procedure increased plasma cortisol levels and the duration of this response was prolonged by the 64 mg dose of naloxone.

Evidence that neurohypophysial secretion in the rat is under tonic opioid inhibition was provided by studies showing that oxytocin (OT) release during suckling (Clarke et al. 1979) could be enhanced by an opiate antagonist, naloxone, and that vasopressin (AVP) release after electrical stimulation of the pituitary stalk could be inhibited by morphine (Iversen et al. 1980). Since then, in vivo experiments using conscious rats have demonstrated that naloxone, which acts primarily as a μ-opiate receptor antagonist, can increase OT secretion under basal conditions, and in response to a rise in cerebrospinal fluid sodium concentration (Summy-Long et al. 1986), and AVP release under basal and osmotically-stimulated conditions (Forssling et al. 1988).

In contrast to these findings in the rat, different results have been obtained in other species. For example, in the dog, naloxone has recently been reported to have no effect on AVP release following hypertonic saline infusion (Hellesbrekers et al. 1988), although other workers (Wade 1985; Wade & Hunt 1986) did find a stimulatory effect on AVP secretion after both dehydration and hypertonic saline injection. In addition, studies in man indicate that naloxone has no effect on posterior pituitary hormone release under either basal (Honer et al. 1986; Dunne et al. 1987) or osmotically stimulated conditions (Seckl et al. 1988). Similarly, naloxone has also been shown to have no influence on basal levels of OT or AVP in the goat (Seckl & Lightman 1987). However, its action in ruminant species subjected to osmotic challenge remains to be investigated.

In addition to their effects on posterior pituitary function in rodents, endogenous opioids also affect the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. For example, the μ-opiate agonist, morphine, stimulates ACTH release in the rat and this effect is antagonized by naloxone (Buckingham & Cooper 1986). However, again, the opposite situation exists in man, since morphine decreases (Banki & Arato 1987) and naloxone raises (Morley et al. 1980) plasma cortisol concentrations. Similarly, although few studies have been carried out in ungulates, it has been found that naloxone will increase plasma cortisol in the pig (Barb et al. 1986) and preliminary evidence suggests that morphine blunts cortisol release in stressed sheep (Parrott & Thornton 1988).

The objectives of the present study were to investigate further the influence of endogenous
opioids on posterior and anterior pituitary function in ruminants. To this end, naloxone was administered to sheep osmotically stimulated by 24 h dehydration and, since this procedure does not activate the pituitary/adrenocortical axis (Thornton et al. 1987), it was also possible to obtain information on the effect of naloxone on plasma cortisol secretion in this species.

Materials and Methods

The experimental animals were 7 adult Clun Forest breed ewes ovarioctomized at least 6 months before the start of the experiment; gonadectomized females were used to eliminate variations in oxytocin release associated with the estrous cycle (Garcia-Villar et al. 1983). They were housed in individual pens and fed on hay and concentrates with water available ad libitum, except during dehydration and subsequent sampling.

Blood samples were collected by jugular venepuncture into 10-ml heparinized tubes. The first sample was taken at 14.00 h, just before water removal, and a second sample was taken 24 h later (time zero). This was immediately followed by an iv injection of 1 ml physiological saline vehicle, or saline containing naloxone hydrochloride (Dupont Ltd, Stevenage, UK). Additional blood samples were collected 5, 10, 20, 30, 60 and 90 min later. Naloxone was given at a low dose (8 mg; 0.1 mg/kg), based on that reported to reduce food intake in sheep (Baile et al. 1981) and at a high dose (64 mg; 1.0 mg/kg) equivalent to that found to induce cortisol release in the pig (Barb et al. 1986). All animals received each of the treatments; the low dose of naloxone was administered 5 weeks after saline and the high dose was given 10 weeks after the low dose.

Plasma obtained by centrifugation of the samples was divided and stored at −30°C pending analysis for osmolality and for radioimmunoassay to determine concentrations of AVP, OT and cortisol, as previously described (Parrott et al. 1988). The intra- and inter-assay coefficients of variation (%), respectively, were as follows: AVP 11.5, 12.6; OT 9.7, 9.3; cortisol 5.3, 5.4. Changes in osmolality after dehydration and of AVP, OT and cortisol after dehydration and naloxone administration were analysed using the two-tailed paired t-test. The effects of naloxone at each individual sampling point were only analysed if a preliminary analysis indicated an overall treatment effect.

Results

After 24 h dehydration, plasma osmolality increased from 300.0 ± 2.1 (X ± SEM) to 308.9 ± 1.2 mosmol/kg (P<0.01), from 298.9 ± 1.0 to 304.6 ± 1.8 mosmol/kg (P<0.02), and from 299.1 ± 1.1 to 308.7 ± 1.3 mosmol/kg (P<0.001) before treatment with saline, 8 or 64 mg naloxone, respectively. These changes were accompanied by significant increases in AVP (Fig. 1), but there were no subsequent alterations in AVP secretion after saline or naloxone administration (P > 0.05). It is also apparent from these data that dehydration produced a greater increase in osmolality, and especially AVP, in the test with 64 mg naloxone. Since this part of the experiment was done several months (March) after the tests with saline and 8 mg naloxone (November—December), there is a distinct possibility that external factors, such as changes in diet and ambient temperature, may have influenced the results.

Plasma OT concentrations did not change with dehydration before treatment with saline or 64 mg naloxone but did show a small increase before administration of 8 mg naloxone (Fig. 1). There were, however, no further changes in OT secretion after injection of saline or either dose of naloxone (P > 0.05).

Plasma cortisol levels were unchanged after 24 h dehydration but did rise during subsequent sampling (Fig. 1). The overall increase during the post-treatment period was significant for saline (P < 0.02), 8 mg naloxone (P < 0.05) and, especially for 64 mg naloxone (P < 0.001). Examination of individual time points revealed that this elevation in cortisol lasted for only 30 min after saline or 8 mg naloxone. However, following the 64 mg naloxone injection, cortisol concentrations were increased for a further 30 min (Fig. 1). A statistical comparison across treatments of the difference from pre-treatment values, although not strictly valid because of the long intervals between the treatments (see above), revealed that the cortisol levels observed 60 min after administration of 64 mg naloxone were greater than those seen following injection of saline (P < 0.05) or 8 mg naloxone (P < 0.01). There were, however, no significant differences between the cortisol responses to saline and 8 mg naloxone at any sampling time.

Discussion

The observation, in the present experiment, that 24 h dehydration produced consistent increases in plasma osmolality and concentrations of AVP, but
not of OT, is in agreement with previous findings on the effects of water deprivation (Thornton et al. 1987) and salt-loading (Carman et al. 1988) in sheep. In addition, the results also show that AVP and OT secretion in dehydrated sheep were not affected by the administration of 8 or 64 mg naloxone. Thus, although there are circumstances in which naloxone will enhance stimulated AVP and OT release in ruminants (Seckl & Lightman 1987), dehydration is not one of them. The sheep is, therefore, similar to man (Seckl et al. 1988) and different from the rat (Summy-Long et al. 1986; Forsling et al. 1988) with respect to the effects of naloxone on AVP and OT secretion after osmotic stimulation.

The blood sampling procedure used in this study, i.e. repeated capture, restraint and venepuncture, has previously been shown to produce a small elevation in plasma cortisol, lasting for not more than 30 min, in euhydrated sheep (Parrott et al. 1988). The present results indicate that this response also occurs in dehydrated sheep and, in ad-

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**Fig. 1.**

Plasma concentrations (± SEM) of vasopressin, oxytocin and cortisol in sheep (N = 7) when euhydrated (shaded columns) and after 24 h dehydration (open columns). The dashed lines indicate the time when saline vehicle or naloxone injections were given. *P < 0.05, **P < 0.02, 0.01, ***P < 0.001 (paired t-test, 2-tailed). Open asterisks indicate comparisons with euhydrated state (-24 h), shaded asterisks indicate comparisons with dehydrated state (0 min).
dition, confirm previous observations (Thornton et al. 1987) that 24 h without water does not elevate plasma cortisol levels in sheep.

Although blood collection by venepuncture stimulates cortisol release in this species, there was no evidence that the extent or duration of the response was increased following administration of the low dose of naloxone. However, when the sheep received the high dose, although the resulting increase in cortisol concentrations was of a similar magnitude, the enhanced secretion was maintained for a further 30 min. These stimulatory effects of a high dose of naloxone on cortisol release in water-deprived sheep are similar to those reported in euhydric pigs (Barb et al. 1986) and men (Morley et al. 1980) and also in both dehydrated and water replete dogs (Wade 1985). Such findings, however, contrast with those described in the non-dehydrated rat (Buckingham & Cooper 1986).

In conclusion, the present results do not support the view that opioidergic mechanisms modulate the posterior pituitary response to osmotic stimulation in the sheep. However, as suggested by experiments using exogenous opiates (Redekopp et al. 1985; Parrott & Thornton 1988), this study does provide evidence to indicate that endogenous opiates may be involved in the regulation of the pituitary/adrenal axis in this species. The results further indicate that the role of opioidergic neurones influencing posterior and anterior pituitary function may be fundamentally different in rodents and larger mammals.

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References


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