Endocrine and behavioural factors affecting water balance in sheep subjected to isolation stress

R. F. Parrott, S. N. Thornton, M. L. Forsling* and C. E. Delaney
AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT
*Department of Physiology, Middlesex Hospital Medical School, London W1P 6DB
RECEIVED 10 June 1986

ABSTRACT

The effect of stress on drinking, water balance and endocrine profile was studied using ten castrated rams. Individual sheep were exposed to 30-h periods of total isolation (psychological stress) or physical separation from their social group (control). Plasma was analysed for haematocrit, osmolality, electrolyte levels and concentrations of cortisol and arginine vasopressin. Isolation stress significantly reduced water intake, increased haematocrit and plasma concentration of cortisol, but did not alter osmolality or vasopressin concentration. The physiological effects of this self-imposed water restriction contrast with those obtained by depriving the sheep of water for 24 h under conditions that were not stressful, i.e. by keeping them grouped together. These results suggest that cortisol may act to defend plasma volume in sheep exposed to acute stress. The results also indicate that vasopressin probably should not be considered to be a 'stress hormone' in the sheep.


INTRODUCTION

Numerous studies have sought to determine the endocrinological response of the sheep to stress. Several of these have been concerned with effects of husbandry procedures (Kilgour & de Langen, 1970; Purchas, 1973; Thurley & McNatty, 1973; Fulkerson & Jamieson, 1982) and environmental factors (Panaretto & Vickery, 1970; Guerrini & Bertchinger, 1982) on the pattern of cortisol release. However, little attention has been paid to the physiological and behavioural changes that may accompany alterations in plasma concentrations of 'stress hormones'.

In sheep stressed by exposure to various combinations of temperature and humidity, highly significant correlations have been found between conditions that increase plasma concentrations of cortisol and those that reduce water intake and urine output (Guerrini & Bertchinger, 1982). These findings suggest that cortisol may affect water balance in the sheep. In contrast, treatment of sheep for 5 days with individual gluco- or mineralocorticoids had little effect on water intake or related physiological indices (Fan, Coghlan, Denton et al. 1975) although combined steroid administration did produce a transient inhibition of drinking. Thus it would appear that the effects of stress on fluid balance cannot readily be stimulated by the administration of exogenous corticosteroids. Nevertheless, the possibility that endogenous glucocorticoids can influence extracellular fluid homeostasis in the sheep is of interest and requires further investigation.

The antidiuretic hormone, arginine vasopressin (AVP), has often been said to be influenced by stress, although this is not supported by recent findings in the rat (Lang, Heil, Ganten et al. 1983). In the sheep, as in other species, there is a positive relationship between plasma AVP concentration and osmolality (Weitzmann & Fisher, 1977) and a restriction of daily water intake, insufficient to inhibit feeding and therefore not especially stressful, raises AVP levels (Blair-West, Gibson, Woods & Brook, 1985). However, it has not been established whether AVP is directly involved in the response of the sheep to stress.

In the present study, the effect of stress-induced alterations in plasma concentrations of cortisol and AVP on the maintenance of water balance in the sheep has been examined. Since physical stressors, such as extreme temperatures, might have a direct effect on water intake, the animals were subjected to a psychological stress. The sheep, being a social species, is sensitive to changes in group structure and separation from the flock is known to be particularly stressful (Kilgour & de Langen, 1970). Accordingly, determinations of water intake, plasma cortisol and AVP, haematocrit, plasma osmolality and electrolyte concentrations have been made in sheep kept in total...
isolation. These results have been compared with those obtained by holding individual sheep within sight and sound of, but separate from, their companions.

MATERIALS AND METHODS

The animals used were ten adult fully fleeced Clun Forest wethers (rams castrated in early life) from two long-established social groups (five sheep per group) housed in separate enclosures in a large barn. The sheep were maintained under natural light, except during cleaning and blood sampling, and the experiment was carried out during December and January when the recorded ambient temperature did not exceed 9 °C.

Individual sheep were subjected to a 30-h period of separation or isolation. Separation involved moving an animal to a single pen (1·8 x 1·8 m) at one end of the communal enclosure; this situation permitted limited social interaction with other members of the group. Isolation was carried out by transferring a sheep to a pen (2·3 x 1·7 m) in a nearby wooden hut; in this situation even vocal communication was not possible. During periods of separation or isolation, the sheep received their usual twice-daily ration of hay and concentrates and water was available ad libitum in 4 litre containers. Two sheep from the same social group were tested at the same time, one separated and one isolated, and all animals were exposed to both conditions with a minimum interval of 6 days between treatments.

Blood was collected at 10.30 h in the group enclosure, the sheep were then taken to the separation or isolation pens and further samples were taken 2, 4, 6, 24, 26, 28 and 30 h later. On each occasion the amount of water consumed was measured and the container refilled.

Blood was collected in 10 ml heparinized syringes (Sarstedt, 'Monovette', Beaumont Leys, Leics) and, after removal of aliquots for haematocrit readings, the remainder was centrifuged. Plasma samples for radioimmunoassay were stored at -20 °C and those for use in other determinations were kept at 4 °C. Haematocrit tubes were centrifuged and packed cell volume percentages determined using a haematocrit reader (Hawksley & Son Ltd, Lancing, Sussex). Osmolality was measured using an osmometer (Roebling, Camlab, Cambridge), concentrations of sodium and potassium were determined using a flame photometer (Model 405 Corning, Halstead, Essex) and chloride levels were quantified using a chloride analyser (Model 925 Corning). Cortisol concentrations were measured using a previously described method (Heap, Silver & Walters, 1981) and the radioimmunoassay for AVP was as described by Forsling & Williams (1984). Statistical analysis was carried out using the paired t-test (two-tailed).

RESULTS

Cumulative water intake over the 30-h experimental period is shown in Fig. 1. The sheep drank little during the first 6 h of separation or isolation (mean intake ± S.E.M., 320·5 ± 141·1 ml, separated; 54·0 ± 49·7 ml, isolated). However, during the 18-h period between the final record on day 1 (6 h) and the first record on day 2 (24 h), the sheep drank significantly (P<0·02) more when separated (1579·0 ± 324·1 ml) than when isolated (453·0 ± 245·5 ml). This trend was maintained throughout the second day (total intake, 1183·0 ± 372·3 ml, separated; 464·0 ± 178·7 ml, isolated). Five of the ten animals did not drink during the first 24 h of isolation and four of these did not consume any water at all during their period of isolation. The sheep appeared to eat very little when isolated, although this was not quantified.

Plasma cortisol concentrations are shown in Fig. 2. Although these values are low in comparison with those reported for other breeds of sheep, they are in close agreement with those described for normal and cold-stressed Clun Forest sheep, using the same assay (Thompson & Goode, 1981). On five out of seven sampling occasions during the experimental period, isolation resulted in significantly higher cortisol levels than separation, the effect being most marked in the first sample after isolation (2 h). The failure to detect a significant difference in the final sample of both days (6 h and 30 h) may reflect the finding that cortisol concentrations in the sheep are lowest in the afternoon (Fulkerson & Tang, 1979).

Plasma AVP concentrations are given in Fig. 3. There was considerable variation between animals and no significant treatment effects were detected. Comparison with Fig. 2 indicates that the patterns of release of cortisol and AVP seem to be inversely related. However, the correlation coefficient just failed to reach significance at the 5% level in a two-tailed t-test (r = -0·678, t = 2·262).

The haematocrit (percentage packed cell volume; mean ± S.E.M.) appeared to decrease during separation (34·8 ± 0·8 at 0 h, 33·2 ± 0·5 at 30 h) and to increase slightly during isolation (34·8 ± 0·8 at 0 h, 35·2 ± 0·6 at 30 h). On three sampling occasions (2, 6 and 30 h), the values were significantly higher (P<0·02, 0·05, 0·05 respectively) in isolated sheep. Plasma osmolality, however, did not differ between treatments at any time during the 30-h period; values (mosmol/kg; mean ± S.E.M.) ranged from 303·9 ± 1·4 to 307·2 ± 1·4 during separation and from 302·6 ± 1·4 to 305·3 ± 1·7 during isolation. Concentrations of plasma electrolytes (mmol/l; mean ± S.E.M.) were also similar during both conditions. Plasma Na+ ranged from 154·0 ± 2·9 to 156·3 ± 2·9 (separation) and from 154·1 ± 2·4 to 157·5 ± 2·5 (isolation); plasma K+ values were in the
FIGURE 1. Cumulative amount of water drunk by sheep \((n = 10)\) individually subjected to physical separation from their social group (▲) or total isolation (●) for a 30-h period beginning at 10.30 h. Values shown are means ± S.E.M. *\(P < 0.01\) compared with total isolation (two-tailed paired \(t\)-test).

FIGURE 2. Plasma concentrations of cortisol in sheep \((n = 10)\) subjected to physical separation (▲) or total isolation (●) for a 30-h period. Values shown are means ± S.E.M. *\(P < 0.05\), **\(P < 0.02\) compared with separated sheep (two-tailed paired \(t\)-test).
range of 4.1 ± 0.2 to 4.4 ± 0.2 for both conditions; plasma Cl\(^{-}\) ranged from 102.3 ± 0.8 to 104.9 ± 0.8 (separation) and from 102.7 ± 0.9 to 104.0 ± 1.1 (isolation). These findings indicate that isolation stress had relatively little effect on body fluid tonicity and composition, even though water intake was markedly reduced (Fig. 1). This is particularly obvious when the four sheep that refused to drink during isolation are considered separately (Table 1). Because this failure to show a physiological response to 30-h partial or total water restriction is most unusual (Blair-West et al. 1985; Park, Congiu, Denton & McKinley, 1985) the sheep were retested to demonstrate that they were capable of responding normally to water deprivation when they were not unduly stressed. The animals were, therefore, kept in their social groups and blood samples were taken before and after a 24-h period in which water was withheld. These results, which are also presented in Table 1, show that group deprivation produced the expected increases in osmolality and plasma electrolyte concentrations.

**DISCUSSION**

The 30-h period of isolation stress produced the anticipated increase in plasma cortisol concentrations and a trend towards a corresponding decrease in AVP.
levels. Isolation stress also resulted in reduced and, in some animals, an absence of drinking, but the usual physiological changes associated with dehydration did not occur. Acute stress may, therefore, activate a mechanism that enables the volume, tonicity and ionic composition of the extracellular fluid in the sheep to be maintained in the face of a severe reduction in water intake.

When non-stressed sheep are deprived of water, increases in plasma osmolality and plasma Na⁺ concentrations are apparent after about 24 h (Blair-West et al. 1985; Park et al. 1985) and the haematocrit shows a paradoxical decrease (Blair-West, Gibson, Woods & Brook, 1985; S. N. Thornton & R. F. Parrott, unpublished observations). However, when sheep are stressed to the extent that plasma cortisol levels are raised, an increase in haematocrit occurs (Guerrini & Bertchinger, 1982), which is probably caused by splenic contraction due to emotional excitement (Turner & Hodgetts, 1959), rather than a change in plasma volume. The present results, whilst confirming these various findings, also show that when sheep are subjected to acute stress they remain in water balance, even though drinking is reduced (Guerrini & Bertchinger, 1982). Since sheep normally drink after feeding, at a time when plasma osmolality is increased (Ternouth, 1967, 1968), stress-induced reductions in food intake may indirectly affect fluid balance. The isolated sheep in this study appeared to eat less than normal and it is therefore possible that the results can be explained by an inhibitory effect of cortisol on food intake. However, it also seems likely that cortisol may have a direct physiological action on water balance, as has been suggested previously (Fan et al. 1975).

There is evidence from other species for the existence of a phenomenon of this kind. For example, in the rat (Moses, 1965) and the dog (Swingle, Da Vanzo, Glenister et al. 1959) it has been shown that glucocorticoids can bring about shifts in body water which lead to plasma volume expansion and a recent report has indicated that adrenocorticotropic hormone and cortisol can produce similar effects in man (Connell, Whitworth, Davies et al. 1986). Although the underlying mechanism is unknown, in the sheep there is a distinct possibility that the rumen, which acts as a reservoir for up to 15% of body water (Shkolnik, Maltz & Choshniak, 1980), may be involved in the response. Under normal conditions, water movement between the rumen and plasma is minimal (von Engelhardt, 1970); however, factors within the animal can alter permeability and water movement across the rumen can occur against an osmotic gradient (Dobson, Sellers & Shaw, 1970). Since cortisol is known to aid transfer of water across the sheep placenta (Leake, Stegnner, Palmer et al. 1984), it might also affect those cells of the ruminal epithelium that normally provide a barrier to water movement (von Engelhardt, 1970). Although further studies are required to substantiate this hypothesis, it is interesting to note that both rumen motility and water intake are reduced in sheep following intracerebroventricular injection of corticotrophin-releasing factor (Ruckebusch & Malbert, 1986) at a dose level sufficiently high to raise plasma cortisol levels (Donald, Redekopp, Cameron et al. 1983).

With regard to the possible role of AVP as a 'stress hormone' in the sheep, under the conditions of short-term stress imposed in the present experiment there was evidence of an interaction between cortisol and AVP. High cortisol concentrations in isolated sheep were associated with low AVP levels and AVP concentrations tended to be higher in the less stressed separated animals. This response differs from that observed when sheep are exposed to a prolonged stress. For example, in sheep subjected to 4 weeks of environmental stress, not only is the plasma concentration of cortisol raised and drinking inhibited, but there is also evidence of antidiuresis (Guerrini & Bertchinger, 1982), suggesting augmented AVP release. This could occur in response to a sustained increase in osmolality due to reduced water intake, but it could also result from enhanced mineralocorticoid secretion, since AVP concentrations rise after about 3 weeks in sheep bearing deoxycorticosterone implants (Brooks, Share, Crofton et al. 1985). However, the reason that a similar pattern of response is not seen during acute stress seems to be that cortisol raises the osmotic threshold for AVP release (Aubry, Nankin, Moses & Streeten, 1965). This has the effect of delaying the increased pituitary output of AVP that would normally occur in response to rising osmolality resulting from decreased water intake. Thus, in the sheep, it would seem that AVP may not respond directly to stress but, rather, to stress-induced changes in circulating cortisol and its associated physiological effects.

In conclusion, in the acutely stressed sheep cortisol appears to reduce thirst and maintain water balance. An effect of this nature would be highly adaptive because it would eliminate the need to find water when the flight response is activated. Any effect of stress on AVP release, however, is complex and involves interactions with cortisol itself and the effects of cortisol on water intake. These observations are not consistent with the view that AVP has a primary role as a 'stress hormone' in the sheep.

ACKNOWLEDGEMENTS
We are grateful to Mr K. J. Baker for practical assistance. M.L.F. gratefully acknowledges financial assistance from the Central Research Fund of the University of London.
REFERENCES


