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Pattern of Cortisol Release in Sheep following Administration of Synthetic ACTH or Imposition of Various Stressor Agents

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Abstract

This paper describes experiments with sheep, in which changes in plasma cortisol, after imposition of various stressor agents, were compared to the changes following administration of synthetic ACTH. The influence of stress associated with shearing, yarding, oestrogen administration (30 μg oestradiol benzoate, i.v.), feeding and fasting on the plasma concentration of cortisol was monitored in four mature Merino ewes. They were placed in the experimental environment for 21 days before monitoring began. The ewes were treated in pairs, each pair being visually and acoustically isolated from the other. One pair of ewes was exposed to the particular stress and the remaining pair acted as their own controls. The treatments were reversed 2 days later. In the second part of the experiment, 0, 0·01, 0·1, 1·0 or 10·0 i.u. synthetic ACTH were injected as an intravenous bolus, after endogenous secretion had been suppressed by administration of synthetic glucocorticoid. All blood samples were taken via an indwelling jugular catheter. A comparison of cortisol release—estimated from a plot of cortisol in plasma versus time—following imposition of various stressor agents and administration of synthetic ACTH, allowed stress to be defined in terms of synthetic ACTH equivalents. The most severe stress was associated with shearing (0·84 i.u. synthetic ACTH equivalents), less stress was imposed by yarding and handling (0·45 i.u.), and there appeared to be no effect attributable to feeding, fasting or oestrogen administration. The similarity in the pattern of cortisol release following ACTH administration offers the possibility of defining acute, but not chronic, stress in terms of ACTH equivalents.

Introduction

Many attempts have been made to define stress quantitatively. The extent of cortisol release (Kilgour and De Langen 1970) and the general activity of the adrenal gland (Ganong 1963; MacKenzie *et al.* 1975) have been used as indicators of stress. These measurements assume that the degree of stress imposed is directly proportional to the amount of ACTH released and hence adrenal cortex activity (Selye and Heusen 1955; Dallman and Yates 1969). However, data on the relationship between stress, ACTH and cortisol have often been based on single or infrequent blood samples taken after imposition of various stresses. The episodic nature of cortisol release, found in most species studied (Hellman *et al.* 1970; Holaday *et al.* 1977; Fulkerson and Tang 1979), would suggest that infrequent blood sampling could give misleading results.

Apart from finding a suitable indicator of stress, the measurement of stress is further complicated by the fact that the physiological response of an animal to stress depends on past experience associated with that stressor agent and the degree of adaptation to it (Reid and Mills 1962; Bassett and Hinks 1969). In other words, the degree of stress 'experienced' by an individual to a given stimulus is a function of its past

and present environment. It would therefore appear that frequent blood sampling under strictly controlled conditions is a prerequisite before changes in the level of cortisol in blood can be validly related to stress.

The aim of the present experiment was to compare the pattern of cortisol release, estimated from changes in plasma cortisol concentration, following the administration of ACTH or the imposition of various stressor agents. If these patterns are similar it may be possible to quantify stress in terms of ACTH equivalents.

Materials and Methods

Animals

Four mature Merino ewes, weighing 35·6 ± 4·4 kg (mean ± s.e.), were placed in metabolism crates for 21 days to allow them to become familiar with their environment and the routine of blood sampling. Each pair of ewes was isolated, visually and acoustically, in separate rooms and fed oaten chaff and lupin grain *ad libitum* at 1000 h each day except where otherwise stated.

Table 1. Nature, duration and order of various stressor agents imposed and time of day of application
Blood samples were collected at the start of treatment, then every 4 min for 40 min, followed by every 10 min for 80 min

Order of treatment	Time of day (h)	Period between treatments (days)	Duration of stress (min)	Description of stress
1	1100	—	4–5	Shearing—normal mechanical.
2	1100	7	Rapid physiological response	Oestrogen—intravenous injection of 30 μg oestradiol benzoate in 0·5 ml ethanol (Schering A.G. Berlin, W. Germany).
3	1000	4	5	Feeding—normal feeding, with control ewes not being fed.
4	1600	3	Extended	Fasting—commencement of blood sampling was 32 h after last feed. Control ewes were fed 6 h previously.
5	1100	10	5	Yarding—each ewe was run into unfamiliar yards, drafted and handled with the usual noise associated with working sheep. No dogs were used.

Experimental Procedure

Changes in plasma cortisol levels following imposition of various stressor agents

One pair of ewes was exposed to the various stressor agents, while the other pair was kept in isolation and acted as their own controls. The roles were reversed 2 days later. Details of the stresses imposed are given in Table 1.

Changes in plasma cortisol levels in response to Synacthen

To block endogenous ACTH secretion, 3 mg of the synthetic glucocorticoid Dexafort (dexamethasone phenyl propionate and betamethasone sodium phosphate, 3·2 mg ml^{-1}, Intervet Labs Ltd, Cambridge, U.K.) were injected intravenously (see Beaven *et al.* 1964). Blood samples were taken at regular intervals after injection, to monitor the decline in plasma cortisol level.

Four hours later, by which time the endogenous level of cortisol had fallen to negligible levels (see Fig. 1), 0, 0·01, 0·1, 1·0 and 10·0 i.u. of the synthetic ACTH Synacthen (tetracosactrin, 0·25 mg ml^{-1}, Ciba Geigy, Lane Cove, N.S.W.) were injected intravenously to ewes on consecutive days. The relative potencies of Synacthen in terms of ACTH units are based on the response of male rat adrenal tissue (see British Pharmacopoeia 1973, Appendix XIVc, p. A108). Synacthen was diluted with acidified saline [0·9% (w/v) NaCl, pH = 2·5; see Beaven *et al.* 1964] so that each dose was administered as 1 ml of solution. Blood samples were taken at 4-min intervals following injection of Synacthen.

Blood sampling and cortisol assay

All blood samples were collected and hormones injected via in-dwelling polyethylene catheters (with internal diameter of 0·8 mm Disposable Infusion sets, Terumo Corporation, Tokyo, Japan) inserted at least 4 h before commencement of blood sampling, thus allowing cortisol levels, elevated by the stress of cannulation, to return to basal values (W. J. Fulkerson, unpublished data). The plasma was stored at − 16°C pending analysis for cortisol by radioimmunoassay, without prior extraction, using the method described by Endocrine Sciences, Tarzana, California, U.S.A. The coefficient of variation for within- and between-assays was 2·6 and 9·0% respectively, at 30 ng ml^{-1}, with a sensitivity of 3 ng ml^{-1}.

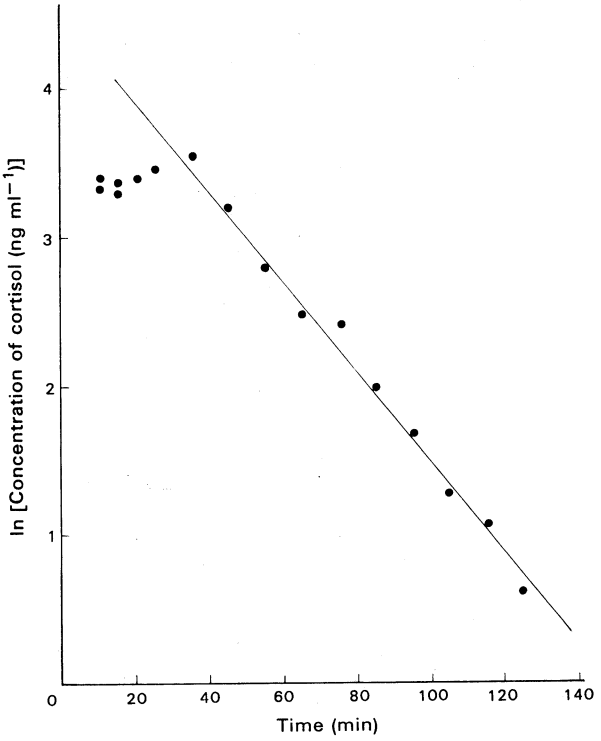

Fig. 1. Natural logarithm of concentration of cortisol in plasma of ewes, following intravenous injection of 3 mg Dexafort at time zero. Plotted points represent means of four ewes. Equation to regression line is $y = 4{\cdot}5 \pm 0{\cdot}14 - (0{\cdot}032 \pm 0{\cdot}005)x$.

The areas under the curve of the plot of cortisol (ng ml^{-1}) versus time (min), estimated by weighing the relevant areas of paper, following injection of each dose of Synacthen minus the effect of placebo injection (acidified saline), was determined for each of the four ewes. The mean values for all four ewes were then plotted against the natural logarithm of the dose of Synacthen administered, and these data were subjected to regression analysis. Similarly, the area under the curve of the plot of cortisol (ng ml^{-1}) versus time (min) following the imposition of the various stresses minus their own corresponding control values was calculated for each ewe. The mean of these values over all four ewes was then used to estimate the Synacthen equivalents released in response to each stress, using the above response curve.

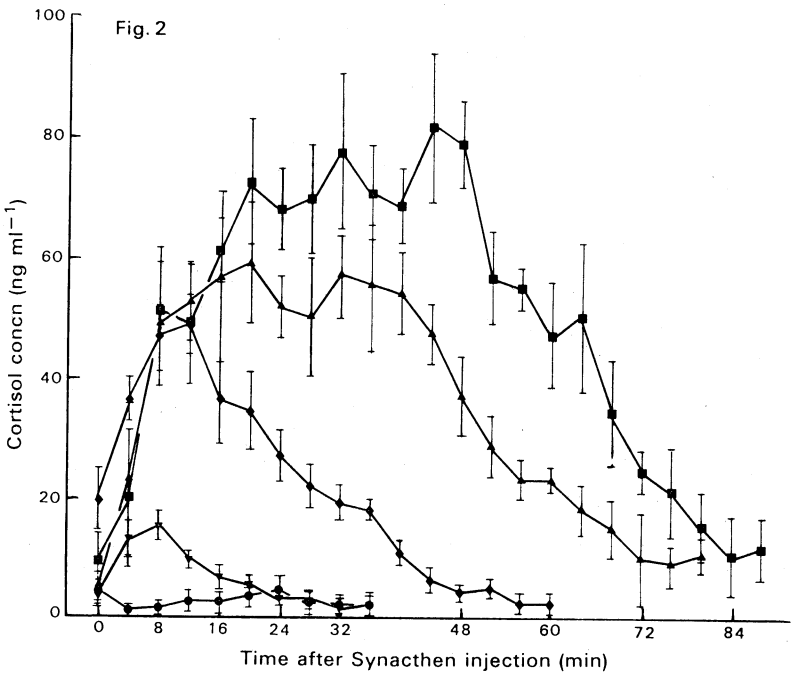

Fig. 2. Concentration of cortisol in plasma of ewes, injected 4 h previously with Dexafort, after intravenous injection of zero (●), 0·01 i.u. (▲), 0·1 i.u. (♦), 1·0 i.u. (▲) and 10 i.u. (■) Synacthen. Plotted points represent means of four ewes and standard errors are indicated by vertical bars.

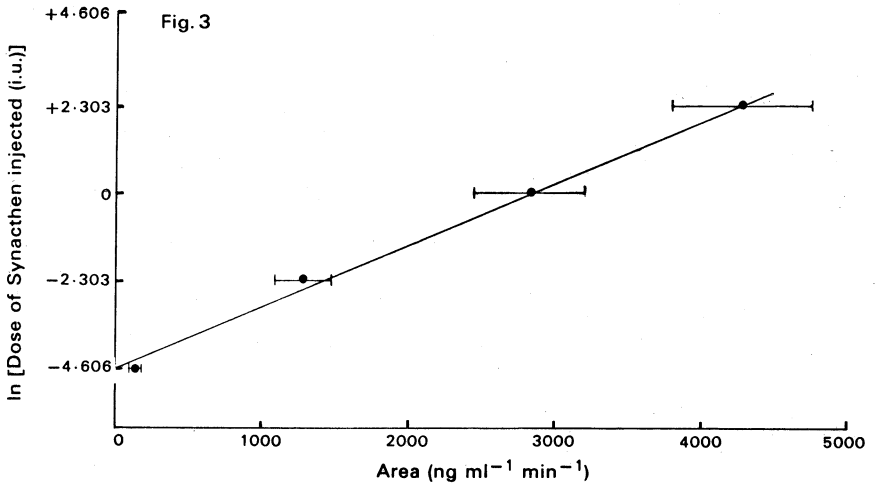

Fig. 3. Areas under curves of plot of cortisol in plasma versus time versus natural logarithm of dose of Synacthen injected, corrected for area under curve following placebo injection (acidified saline, pH = 2·5). Equation to regression line is $y = -4{\cdot}606 + 0{\cdot}0016x$.

Plasma concentrations of cortisol were subjected to analysis of variance. The mean values at any particular time were tested by 'least significant difference' technique (Snedecor and Cochran 1972).

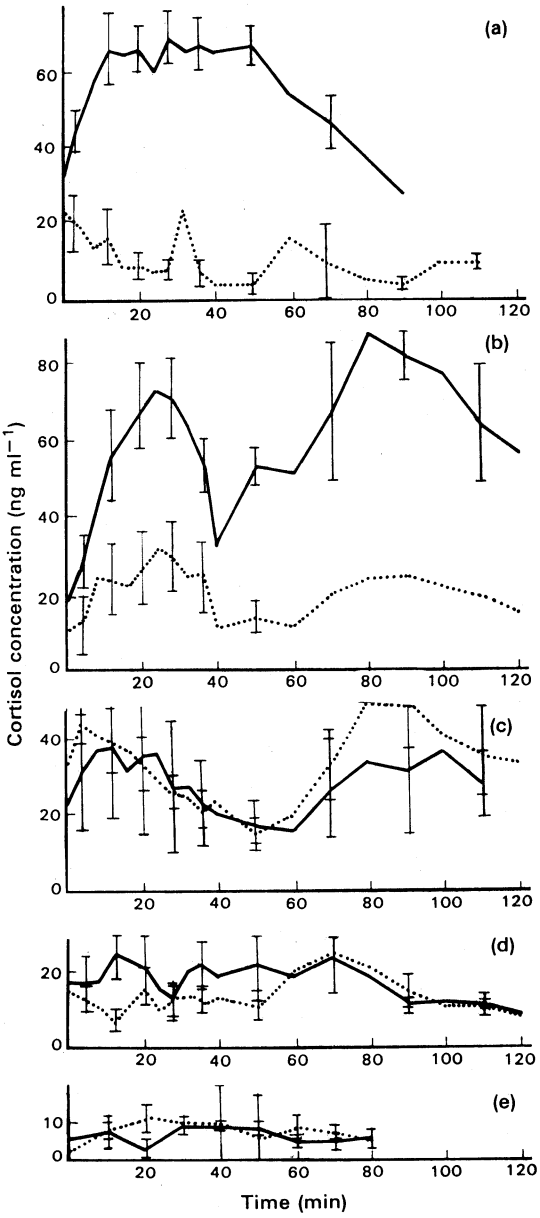

Fig. 4. Concentration of cortisol in plasma of ewes exposed to stress (———) associated with shearing (*a*), yarding (b), oestrogen administration (*c*), feeding (*d*), or fasting (*e*), together with values for untreated control ewes (- - - -). Each point represents mean for four ewes, and standard errors are indicated by vertical bars.

Results

Decline in Plasma Cortisol Levels following Injection of Dexafort

Commencing 35 min after administration of Dexafort, there was a significant linear decline in the natural logarithm of plasma cortisol concentration ($r = 0{\cdot}996$, $P < 0{\cdot}001$, residual standard deviation $= 0{\cdot}084$) over the next 100 min (see Fig. 1).

As a result of these findings, no attempt was made to stimulate endogenous cortisol secretion by administration of Synacthen for 4 h after Dexafort injection.

Plasma Cortisol Levels following Administration of Synacthen

Following injection of Synacthen, the maximum level of cortisol rose with increasing dose and the interval from injection to the time taken to return to basal levels also increased (Fig. 2).

The area under the curves in Fig. 2 was linearly related to the natural logarithm of the dose of Synacthen administered [see Fig. 3: $r = 0{\cdot}997$, $P < 0{\cdot}001$, residual standard deviation $= 0{\cdot}26$].

Effect of Stress on Plasma Cortisol Levels

Plasma cortisol levels rose sharply following shearing, from about 30 ng ml^{-1} to more than 60 ng ml^{-1} within 30 min, and then returned to pre-treatment levels over the next 90 min. The pattern of change in plasma cortisol was similar for yarding except that the response appeared to be more sustained. Oestrogen administration, fasting and feeding had no significant effect on cortisol release (see Figs 4*a*–4*e*).

When cortisol release was taken as the area under the curve of cortisol in plasma versus time for ewes subjected to various stresses *minus* the area for untreated 'control' ewes (see Table 2), shearing had a more potent effect than yarding, and oestrogen administration, feeding or fasting had no significant influence on cortisol release.

Reference to Fig. 3 allows an estimate to be made of synthetic ACTH equivalent under the various stress situations and these estimates are also tabulated in Table 2. It should be noted, however, that as cortisol concentrations had not returned to pre-stress values when blood sampling ceased following shearing or yarding, the effect of these stresses would have been underestimated.

Discussion

The results of the present experiment reveal the inherent dangers of simply using changes in plasma cortisol as an indicator of stress. To be meaningful, cortisol concentrations can only be used if day to day variation and episodic fluctuations are taken into consideration. This may be achieved by frequent sampling and by using each animal as its own control. Also, estimating cortisol release from changes in plasma cortisol is only valid if metabolic clearance rate for cortisol does not alter. Although this may be true in the short term (Beavan *et al.* 1964), it may not be the case over longer periods, e.g. 1–2 days; this technique is probably restricted to the determination of short-term effects of stress. Other studies have found that both imposition of stress (Panaretto 1974) and infusion of ACTH (Panaretto *et al.* 1973) lead to an increase in metabolic clearance rate.

ACTH release appears to provide a valid index of stress because ACTH is the mediator between stress imposed and cortisol released. Cortisol release was found to be linearly related to the natural logarithm of the dose of synthetic ACTH administered as an intravenous bolus. It would seem valid to equate only acute stress to this response curve; another dose-response curve, using prolonged infusion of ACTH, would be required to relate to chronic stress.

The most severe stress, in terms of cortisol release of ACTH equivalents, was found to be shearing. The initial rise in cortisol following shearing has been reported

previously by Kilgour and De Langen (1970) and Purchas (1973), although in these studies only peak cortisol levels were considered. Yarding and handling provoked a milder stress, whereas fasting, feeding and oestrogen administration appear to have had no real effect on cortisol release. The response to shearing and yarding in terms of ACTH equivalents would be slightly underestimated in the present study as the level of cortisol at termination of sampling had not returned to pre-treatment values. The lack of response to fasting is similar to results reported by Reid and Mills (1962) and Bassett (1974) in sheep but contrasts with results of other work in sheep (Purchas 1973) and in other species (Slater 1962; Alleyne and Young 1967). It appears that the response will depend on the severity of the fast—24 h probably being only mild in sheep—and previous feeding regime, i.e. *ad libitum* or periodic feeding. Data presented by Purchas (1973) indicate that starvation increases the concentration of cortisol as a consequence of psychological stress rather than as a consequence of metabolic disruption. This probably also applies to the effect of feeding where anticipation of feeding may promote stress.

Table 2. Effect of various stresses imposed on ewes on estimated cortisol release and estimated synthetic ACTH equivalents

Stressor agent	Estimated cortisol release[A] (ng ml^{-1} min^{-1})	ACTH[B] (i.u.)
Shearing	2500±440	0·850
Handling and yarding	1700±540	0·405
Feeding	160±366	—[C]
Fasting	80±100	—[C]
Oestrogen	214±796	—[C]

[A] Mean difference (±s.e.) in areas under the curve of the plot of cortisol (ng ml^{-1}) versus time (min) between animals exposed to stress and under control conditions.
[B] Synacthen equivalents.
[C] Not significantly different from zero.

The ewe, in which endogenous cortisol secretion has been suppressed by administration of glucocorticoids, provides a convenient bioassay medium for measurement of ACTH. A linear relationship is produced if the area under the curve of cortisol in plasma versus time is plotted against the natural logarithm of the dose of synthetic ACTH administered. Synacthen, at doses as low as 0·01 i.u., significantly raised the concentration of plasma cortisol. Likewise, Bassett and Hinks (1969) found an increase in plasma corticosteroids in normal sheep following injection (i.v.) of 0·02 i.u. Synacthen but not with less than 0·01 i.u., and Kilgour and De Langen (1970) found no additional rise in plasma cortisol after injections greater than 10 i.u. synthetic ACTH. Evidently the responsive range lies between 0·01 and 10 i.u. Synacthen. A greater sensitivity can be obtained by measuring cortisol level in venous blood of an adrenal gland transplanted to the neck of the sheep (Beaven *et al.* 1964), although the surgical preparation makes this technique less attractive.

Just as it is imprecise to use assay results of single or infrequent blood samples to estimate cortisol release following imposition of stress, infrequent blood sampling following ACTH administration will also give misleading results. The timing and amplitude of the peak level of cortisol in plasma, as well as the rate of decline, changes with dose of ACTH administered. For example, injection of 0·01 i.u. ACTH leads

to peak cortisol values of 15·6 ng ml^{-1} 8 min later, with the level returning to basal values after a further 12 min, and an injection of 10 i.u. ACTH leads to peak cortisol values of 75 ng ml^{-1} at 44 min and a return to basal values after more than 40 min.

Acknowledgments

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