ACTH STIMULATION TESTS IN RED DEER

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ABSTRACT

The use of the ACTH stimulation test was evaluated in a variety of classes of red deer. Using adult farmed hinds responses to doses of 100, 250 and 500 μg ACTH were established. While the dose of ACTH did not affect the peak response, the duration of response increased with dose. In wild hinds with high basal plasma cortisol concentrations exogenous ACTH was ineffective in causing a further elevation in plasma cortisol concentration. Dexamethasone was effective in reducing endogenous plasma cortisol concentrations in calves. However, the cortisol response to subsequent ACTH stimulation was unaffected by pre-treatment with dexamethasone. In the first of two studies using calves there was a significant decline in basal cortisol concentrations over a 12-week period, suggesting that animals adapted to their environment or management. ACTH caused an elevation in plasma cortisol concentrations at weeks 0 and 12 and there was a difference in response at week 12 in relation to management regime. However, a second study using calves failed to demonstrate a difference in response due to management. When the test was used as part of a study to evaluate the domestication of wild hinds, although the high basal cortisol levels declined over a 4-month period, there was no effect of ACTH administration. It is concluded that both basal cortisol concentrations and responses to ACTH stimulation are variable and that the use of both as indices of stress may be appropriate measures of adrenal responsiveness in red deer only in certain circumstances.

INTRODUCTION

Knowledge of the stress that semi-intensive environments and routine management practices may have on farmed deer is required to satisfy consumer concerns about farming systems. Although stress in deer has been shown to be caused by both conventional management procedures and investigative procedures, such as blood sampling required to obtain physiological data (Van Mourik & Stelmasiak, 1984), interpretation of behavioural changes attributable to stress requires an understanding of underlying physiological mechanisms which may influence not only behaviour but also metabolism, reproduction and immunity and hence have long-term consequences to the animal (Blecha & Baker, 1986; Kelley, 1988).

Adrenal cortex activity has been widely used as a quantitative index of stress (Stevens, 1980; Barnett & Hemsworth, 1990). Although there have been a number of reports on plasma cortisol profiles in species such as cattle and sheep (Johnston & Buckland, 1976; Fulkerson & Jamieson, 1982), there are few reports of plasma cortisol changes in deer (Seal *et al.*, 1983; Van Mourik & Stelmasiak, 1984; Matthews & Cook, 1991). The release of cortisol following injection of ACTH has been used to provide an index of adrenal sensitivity but Rushen (1991) questioned the reliability of data based on the activity of the pituitary-adrenocortical axis when making assessments of long-term stressors and animal welfare. Smith and Bubenik (1990), however, suggested that animals with a good response to ACTH stimulation would be best suited to domestication.

The successful application of an ACTH stimulation test requires preliminary research to 1) identify an appropriate dose of ACTH and a sample collection regime, 2) assess its validity in a range of animal types (eg. highly nervous (wild) animals or those accustomed to farm conditions and of quiet temperament) and 3) determine the effect of a variety of management regimes on adrenal sensitivity. The aim of this work was to evaluate the use of the ACTH stimulation test in red deer (*Cervus elaphus*) and in particular to address these questions.

MATERIALS AND METHODS

Animals and treatments

Experiment 1: dose response

Twenty adult farmed red deer hinds (mean live weight (± se) 77.6 ± 2.0 kg), accustomed to routine handling procedures, were used to determine the response in plasma cortisol concentration to a range of exogenous ACTH doses so that an appropriate dose and sampling regime could be established for this species. They were brought into the handling area from an adjacent paddock and confined as groups within a small collecting area at least two hours before the start of the experiment. To enable blood sample collection the hinds were subjected to minimal manual restraint within this area. Groups of five individuals (allocated at random) were injected (iv) with 100, 250 or 500 μg synthetic ACTH (Synacthen, 250 μg tetracosactrin/ml, Ciba, Basle, Switzerland) and five animals were untreated. Blood samples were collected by jugular venepuncture immediately before ACTH injection and at 30, 60, 90, 120 and 180 minutes after injection. Plasma samples were assayed for cortisol concentration.

Experiment 2: wild hinds

Forty-two adult red deer hinds (mean live weight (± se) 64.1 ± 1.0 kg) were captured by enclosure on an estate in north-east Scotland four months prior to calving and the response to ACTH tested. They were herded into successively smaller areas until they entered a standard deer handling system which they passed through twice. On the first occasion a blood sample was collected from all animals by jugular venepuncture and every second animal was injected (iv) with 500 μg ACTH, whilst manually restrained in a confined area. A second blood sample was taken when the hinds passed through the system approximately 80 minutes later. Plasma samples were assayed for cortisol.

In a second study ninety red deer hinds (mean live weight (± se) 68.7 ± 0.8 kg) caught from the wild were used in an assessment of the effect of domestication on adrenal responsiveness 7 months post-capture. They had been maintained in five groups and subjected to a range of conventional management systems. They were subjected to an ACTH stimulation test using 100 μg ACTH on the day their calves were weaned. The test was repeated one week later. The hinds were then rutted in three large groups before being turned out to graze an extensive hill area at a remote location. Four months after the first ACTH stimulation test the hinds were gathered and the test repeated.

Experiment 3: effect of dexamethasone
Thirty female calves aged approximately 7 months (mean live weight (± se) 54.1 ± 0.8 kg) were used to evaluate the effect of suppression of endogenous ACTH release prior to an exogenous challenge in order that the importance of basal cortisol levels to the subsequent response could be established. All animals were manually restrained and bled by jugular venepuncture after which 15 randomly selected animals received 2 mg dexamethasone sodium phosphate (Dexadreson, Intervet UK Ltd.) administered iv. Three hours later a second blood sample was collected and then 100 μg ACTH was administered iv. A third blood sample was collected approximately 60 minutes later.

Experiment 4: studies using calves
Two studies to assess the value of ACTH stimulation tests in studies on calf welfare were conducted. In the first study, 40 male red deer calves, aged approximately 4 months (mean live weight (± se) 44.8 ± 1.2 kg), were used. After weaning all calves were housed for a one-week period. Pairs of calves of comparable liveweight were randomly allocated to each of two groups (Housed; H and Pasture; P). Prior to the treatments being imposed blood samples were collected by jugular venepuncture from all animals and 100 μg ACTH was administered (iv) to each animal. A second sample was collected from each animal approximately 70 minutes later. After six weeks the animals of Group P were housed and after a further six weeks all animals were again subjected to the ACTH stimulation test as before.

In the second study 40 mixed sex red deer calves, aged approximately 4 months and derived from either wild-caught or farmed hinds, were studied in the post-weaning period. In a replicated 2 x 2 factorial study calves were weighed and allocated according to sex and liveweight to 8 groups of 5 calves: groups of calves from each of the two sources (wild; W and farmed; F) were housed at two stocking densities (1.8 m^2/head; H and 4.5 m^2/head; L) for the study period. ACTH stimulation tests were conducted in weeks 1 and 20 as described above, with an interval of approximately 60 minutes between ACTH administration and the second blood sample.

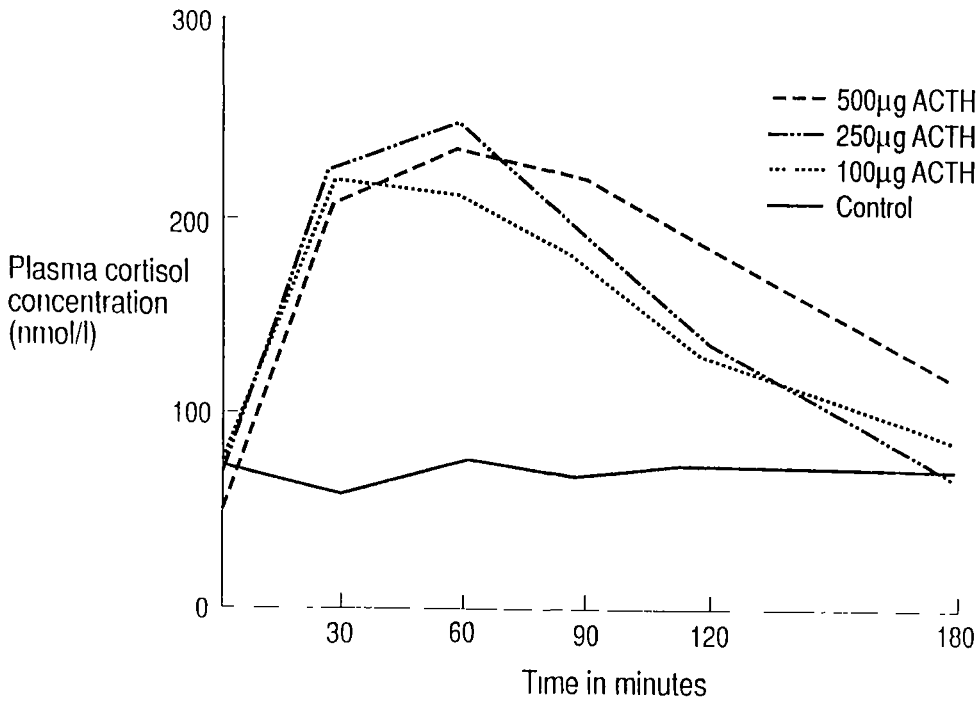
Assay procedure
Samples from Experiments 1 and the first study in Experiment 2 were analyzed for total circulating cortisol (hydrocortisone) using a serozyme EIA method (Serono diagnostics SA, Switzerland). Standards were prepared in charcoal stripped deer plasma. The sensitivity of the assay was 2.76 nmol/l and intra- and inter-assay coefficients of variation were 5% and 6% respectively. Samples from Experiment 3 and the second study in Experiment 4 were analyzed for total cortisol using a radioimmunoassay (Coat-a-count Cortisol, Euro/DPC Ltd.). The sensitivity of the assay was 2.68 nmol/l and intra- and inter-assay coefficients of variation were 11% and 18% respectively. Samples from the first study in Experiment 4 and the second study in Experiment 2 were analyzed for total cortisol by radioimmunoassay according to the method of Thomas and Rodway (1983). The detection limit was 1 nmol/l and the intra- and inter-assay coefficients of variation were 11% and 15% respectively.

RESULTS AND DISCUSSION

Experiment 1: dose response
There was no change in basal cortisol concentrations in undisturbed farmed deer suggesting

that the sample collection procedure did not cause adrenal stimulation. After 30 minutes the mean cortisol concentrations in all animals injected with ACTH were significantly greater than control animals (P <0.001) (Figure 1). The magnitude of the response was unaffected by dose but the duration of the peak response increased with dose. The pattern of response was similar to that reported by Jopson *et al.* (1990).

FIGURE 1 Mean plasma cortisol concentrations (n = 5 per group) in red deer hinds before and after injection (i.v.) with three different doses of synthetic ACTH (Synacthen) at time 0.



These results indicate that, in practice, if the time of blood sample collection in relation to ACTH administration can be accurately controlled, a dose of 100 µg would be appropriate, but if the re-sampling time cannot be accurately controlled, a larger dose of ACTH would provide a longer window during which the maximal response would be measured. It would be interesting to explore the effect of doses below 100 µg to determine whether the magnitude of the response was related to adrenal preconditioning since Fulkerson and Jamieson (1982) showed that, in sheep, at lower doses of ACTH peak response was dose-related.

Experiment 2: wild hinds

In wild hinds at capture the mean plasma cortisol concentration at the time of first sampling was high (238 nmol/l) and comparable to that of the farmed hinds following ACTH stimulation in Experiment 1. Thus, as a result of the handling procedure, wild hinds appeared to be secreting cortisol at the maximum possible rate. Eighty minutes later (ie. within the period of maximal response as predicted from Experiment 1) plasma cortisol concentrations had declined significantly (P < 0.01) in both the ACTH-treated (194 ± se. 18.0 nmol/l) and untreated (138 ± se. 18.2 nmol/l) animals. These values were significantly different ($P < 0.05$). It is concluded that in animals with maximally elevated plasma cortisol concentrations, exogenous ACTH was unable to induce a further elevation and so the test is unlikely to be of use in the assessment of adrenal preconditioning in these animals, as proposed by Rushen (1991). We conclude that the test was inappropriate for newly captured wild hinds.

When ACTH stimulation tests were conducted in wild-derived hinds which had been conventionally managed for 7 months basal cortisol concentrations were still high. There was no difference in either the basal cortisol concentrations or the response to ACTH administration when the deer were sampled at weaning and one week later (Table 1). However, four months later, following management at a remote hill location but with daily contact with a stocksman, the basal plasma cortisol concentrations had fallen significantly. This may have been due to a seasonal effect (as with the decline in basal cortisol levels in the first study in Experiment 4) although we are not aware of studies on seasonal cortisol output in red deer and no distinct seasonal rhythms have been found in either white-tailed deer (Bubenik *et al.*, 1975) or Eld's deer (Monfort *et al.*, 1993). Although the response to ACTH was greatest following the third stimulation test, there was no significant difference between the three occasions. In this instance, pre-treatment with dexamethasone to suppress endogenous ACTH release may have provided additional information.

TABLE 1 Plasma cortisol concentrations (nmol/l) before and after ACTH injection (iv) of 100µg ACTH (T; treated) or control (C) in wild-derived hinds at weaning of their calves and one week and four months later.

		Weaning	+ 1 Week	+ 4 Months	SED	Sig.
Pre-ACTH (n = 90)	T	324	317	232	17.4	***
	C	313	302	207	20.4	***
Post-ACTH (n = 90)	T	343	354	297	25.7	NS
	C	319	293	221	19.7	***

*** $P < 0.001$
NS non-significant

Experiment 3: effect of dexamethasone

One possible solution to the problem of elevated basal concentrations would be suppression of endogenous cortisol secretion prior to ACTH challenge. The effect of inhibiting endogenous ACTH output on the subsequent response to an ACTH stimulation test is shown in Table 2. Three hours after intravenous dexamethasone the treated deer calves had a significantly lower plasma cortisol concentration than untreated animals (P < 0.001). However, the magnitude of the cortisol response following exogenous ACTH was similar in both groups, although less than that recorded for adult hinds. This suggests that knowledge of the pre-ACTH plasma cortisol concentration may not be a prerequisite to assessing adrenal responsiveness and that differences in plasma cortisol concentration may not be the most appropriate index of adrenal responsiveness. Since pre-treatment with dexamethasone imposes additional experimental constraints, we have not routinely used it in our subsequent studies. However, it would be valuable to explore its use in animals with a substantial cortisol output at the time of testing to determine whether the effect depends on the circumstances of the particular test.

TABLE 2 Plasma cortisol concentrations (nmol/l ± se) before and after dexamethasone injection (iv) (2 mg) and the subsequent response to 100µg ACTH in 7-month old calves.

	Before dexamethasone	After dexamethasone	After ACTH
Dexamethasone (n = 15)	40.8 ± 6.8	4.3 ± 0.7 ***	131.9 ± 7.1
No dexamethasone (n = 15)	43.7 ± 7.2	23.1 ± 4.6 ***	130.3 ± 10.3

*** P < 0.001

Experiment 4: studies using calves
The ACTH stimulation test was used as part of two studies of deer calf management. In the first, animals of both Groups H and P showed a significant decline (P < 0.001) in mean basal plasma cortisol concentrations and in the response to ACTH over the 12-week period (Table 3). This suggests that the animals may have adapted to their environment or management, although it is impossible to eliminate possible age effects. There was a significant response by both groups of calves to ACTH injection on both occasions (P < 0.01) and group H exhibited a greater response to ACTH at week 12 (P = 0.05) (Table 2) suggesting that the adrenal glands of group H calves were more sensitive. Using an ACTH stimulation test in bovine calves, Dantzer *et al* (1983) and showed that a 'low-initiative' environment was associated with a more active adrenal gland. Arguably, therefore the P calves in the present study had a richer environment than the H calves. It is likely that the observed effects were due to both age and management factors.

TABLE 3 **Mean plasma cortisol concentrations (nmol/l ± se) on two occasions in farmed red deer calves maintained indoors for 12 weeks (H) or at pasture for 6 weeks and indoors for 6 weeks (P) before and after injection (iv) of 100µg ACTH.**

	Week 0		Week 12	
	H	P	H	P
Number	20	20	20	20
Pre-injection	63.1 ± 7.5	43.4 ± 14.3	27.5 ± 4.4	10.2 ± 2.9
Post-injection	142.4 ± 14.4	140.9 ± 33.0	71.0 ± 8.2	37.0 ± 7.1
Difference	79.2 ± 12.4	97.5 ± 26.8	43.5 ± 5.8*	26.9 ± 6.0*

* P = 0.05

The results of the second study using weaned calves investigating effects of source and stocking density are summarised in Table 4. In week 1, wild-derived calves had a higher mean plasma cortisol concentration after ACTH injection than farm-derived calves ($P < 0.05$). The difference between concentrations before and after ACTH injection was significantly greater in H than L calves ($P < 0.05$) at this time. No differences were detected at week 20 which suggests that the treatments applied in this study were not of sufficient magnitude to alter adrenal responsiveness.

TABLE 4 **Plasma cortisol concentrations (nmol/l) before and after ACTH injection (iv) of 100µg ACTH (at weeks 1 and 20) in wild (W) and farmed (F) calves housed at high (H) and low (L) stocking densities.**

Week	ACTH	W	F	H	L	SED	WvF	HvL
1	BEFORE	97.0	54.0	65.2	85.8	19.8	NS	NS
	AFTER	137.1	76.7	109.5	104.3	18.1	*	NS
	INCREASE	40.1	22.8	44.3	18.5	7.0	NS	*
20	BEFORE	45.1	97.0	76.3	65.7	37.5	NS	NS
	AFTER	138.0	168.0	166.0	140.0	49.0	NS	NS
	INCREASE	93.1	70.7	89.7	74.1	15.9	NS	NS

* P < 0.05
NS non-significant

CONCLUSIONS

Taken together, these results attest to the variable nature of the basal cortisol concentration. The steady basal cortisol response from untreated deer in Experiment 1 contrasts with the observations of Jopson *et al* (1990) whose control deer showed a decline in plasma cortisol over the 4-hour sampling period following mechanical restraint to facilitate sampling, indicating that the sampling procedure itself can elicit a variable effect. Basal cortisol concentration therefore has a limited value as an index of stress (Rushen and Passille, 1992).

Neither endogenous cortisol secretion nor that induced by exogenous ACTH will always provide a clear assessment of a stressor. Hargreaves and Hutson (1990) showed that in sheep, while habituation to a potentially adverse stimulus did not affect the peak endogenous cortisol output, the response to exogenous ACTH increased, possibly through adrenal hypersensitivity. The effects of different stressors on the modulation of the ACTH response remain to be elucidated.

This study shows the potential for applying the ACTH stimulation test to red deer and indicates the most appropriate doses of ACTH for different sampling regimes. The results suggest that in circumstances where endogenous cortisol concentrations are very high, the ACTH stimulation test may not be appropriate. It may also be inappropriate to make comparisons over periods of time. However, in some circumstances the post-ACTH plasma cortisol concentration may be used to obtain a description of the stress response of deer. This may be particularly so if it is combined with other physiological and behavioural observations. We consider it important to investigate more fully the relationship between basal cortisol concentration and adrenal responsiveness before the test can be recommended.

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