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Interrelationships between the Thyroid Gland and Adrenal Cortex during Fear, Cold and Restraint in the Sheep

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Abstract

To examine the relationship between the functioning of the adrenal and thyroid glands in sheep, plasma cortisol concentration, concentration of protein-bound ^{125}I from thyroid vein plasma, heart rate and blood pressure were measured in ewes bearing exteriorized thyroid glands. During these measurements stresses were imposed on the animals: fear induced by pistol shots or by a barking dog, cold by cooling and wetting, and physical restraint by a loose harness.

Increases in plasma cortisol concentration of 2–6 μg/100 ml were observed with each type of stressor, the response rapidly decreasing with habituation of the animal. Increases in the concentration of protein-bound ^{125}I from thyroid vein plasma were also observed repeatedly during cooling and wetting, occasionally after the introduction of a barking dog, and during continued restraint.

Cooling and wetting was the only stress causing consistent parallel activation of the adrenal cortex and thyroid gland; the other stressors resulted in independent fluctuations of secretions, as indicated by plasma cortisol concentration and concentration of protein-bound ^{125}I from thyroid vein plasma. No reciprocal relationship between thyroid gland and adrenal cortex activity was detected.

It was concluded that these ewes, which had been accustomed to normal experimental procedures for a period of 2 years, demonstrated functional independence of thyroid and adrenal cortical secretions when subjected to stress.

Introduction

The effect of externally applied stress on adrenal cortical function has been investigated by many workers with the consistent overall conclusion that a centrally mediated ACTH release occurs which activates adrenal steroid secretion (for review see Fortier 1965). By contrast the results of investigations of the effect of stressful stimuli on thyroid function have shown a very diverse pattern. In laboratory rodents and lagomorphs thyroid secretion and TSH release have been shown to be inhibited by stress from a variety of agents (Brown-Grant *et al.* 1954; Brown-Grant and Pethes 1960; Salaman 1966) apart from cold which has a stimulatory effect (Kajihara *et al.* 1972). For man no clear conclusion has been reached about the effect of stress on thyroid secretion and even cold stress has resulted in conflicting data (Ermans and Camus 1966; Wilson *et al.* 1970). In the sheep transient emotional stimuli have been shown to result in rapid activation of thyroid secretion (Falconer and Hetzel 1964), but no studies of the response to continuing or physical stimuli have been reported.

The present paper describes studies of thyroid and adrenal function in sheep subjected to the stresses of noise, a barking dog, cold or to physical restraint. The results of these studies are interpreted to provide evidence of the functional independence of these two components of the endrocine system. Preliminary papers concerning part of this work have already appeared (Falconer 1972; Falconer and Jacks 1972).

Materials and Methods

This study was carried out using six ewes in which the thyroid gland had been exteriorized with a jugular vein and carotid artery loop on the side of the neck (Falconer 1963). The opposing carotid artery was also exteriorized in a skin-covered loop to give easy access for blood pressure measurement. The ewes were acclimatized to the experimental procedures, including intermittent occupation of a metabolism crate, over a period of 2 years. Each animal was given an intramuscular injection of 50 μCi of ^{125}I in 1 ml 0·9% (w/v) NaCl and 4–8 days were allowed to elapse before any measurements were made. Cannulation of the loop containing the thyroid gland allowed collection of thyroid vein blood, which was analysed for total plasma ^{125}I concentration and protein-bound ^{125}I concentration as previously described (Falconer and Hetzel 1964). Plasma cortisol concentration was measured by the method of Murphy (1967) on the same blood samples. Heart rate was recorded using a 'Cardioline' electrocardiograph (Associated Electrical Industries, London), and arterial blood pressure was measured by a sphygmomanometer cuff applied to the exteriorized carotid artery on the opposite side to the thyroid loop. A period of 2–3 weeks elapsed between experiments on an individual animal.

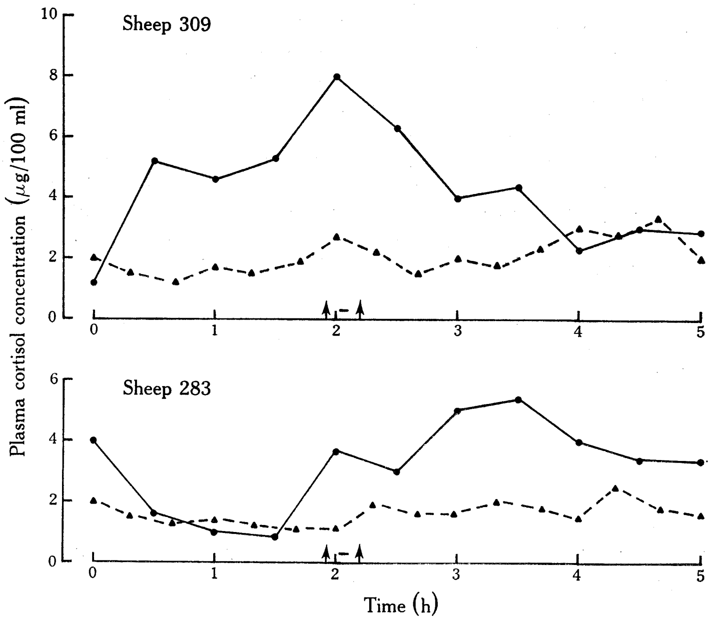

Fig. 1. Plasma cortisol concentration in two ewes exposed to a barking dog (↑ to ↑) on the first (●) and third (▲) occasions of exposure.

Fear

Transient emotional stress was imposed in four experiments by sudden noise (pistol shots), and in eight experiments by a barking dog, on four sheep held loosely in metabolism crates in a controlled-temperature room at 15–20°C. Blood samples from the thyroid vein and the jugular vein were collected at 20- and 30-min intervals over a period of 5 h. The stress was introduced *c*. 2 h after blood sampling commenced. Statistical assessment of the data was done by *t*-test, comparing mean hormone concentrations before and after application of stress.

Cold

The ewes were acclimatized in metabolism crates in a controlled-temperature room at 24 ± 1°C for 1 week prior to experiment. Cannulation of the thyroid vein was carried out as before. Thermistors were placed on the mid-back skin and in the rectum for temperature recording, and used with a calibrated potentiometer (Grant Instruments, Cambridge). The accuracy of recording of the skin temperature was ±0·5°C, and of the rectal temperature ±0·05°C. The experimental procedure was to allow 1 h to elapse after cannulation before setting the room temperature control to 0°C. Over a period of 1½ h the room cooled to 7°C, and after a further 4½ h it reached 0°C. Six experiments were carried out (two on fleeced sheep and four on shorn sheep) in which skin and rectal temperatures were measured, and thyroid vein and mixed venous (jugular) blood samples collected.

A further six experiments were performed in which a heavy spray of water at 0–5°C was applied to the sheep during cooling. Four of these experiments were carried out with fleeced sheep and two with shorn sheep.

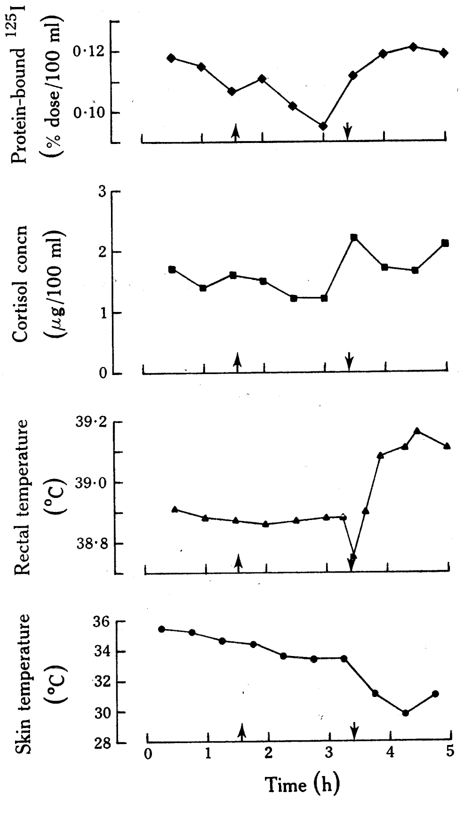

Fig. 2. Effect of cooling and wetting a fleeced sheep on the concentration of protein-bound ^{125}I from thyroid vein plasma (◆), plasma cortisol concentration (■), rectal temperature (▲) and mid-back skin temperature (●). Cooling was began at ↑ and ambient temperature decreased from 24 to 8·5°C over 4 h. A heavy spray of cold water was applied to the sheep at ↓. Average air velocity was 25 cm/s.

Restraint

A total of 16 experiments utilizing restraint were carried out, the sheep being restrained for the whole period of blood sampling (25 h) by a loose harness which prevented them turning or lying down. In addition to hourly sampling of thyroid vein blood, every 3 h a sample of jugular vein blood was taken and analysed for total and protein-bound ^{125}I concentration. During the experiments the animals were in continuous light.

Results

Fear

When a pistol was discharged near the sheep non-significant increases in mean plasma cortisol concentration occurred in two experiments, and significant ($P < 0\cdot05$) increases occurred in two experiments. In only one experiment was there a significant increase in plasma protein-bound ^{125}I concentration and this was in an animal in which there was also a significant increase in plasma cortisol concentration.

Use of a barking dog as a stimulus was employed in eight experiments. A feature of this series of experiments was the habituation of the sheep to both the experimental procedures and to the dog. Fig. 1 shows the plasma cortisol determinations on the first and third occasions for two sheep used in these experiments. Only on the first occasion of use of a dog were significant increases in concentration of protein-bound ^{125}I from thyroid vein plasma recorded; in all the later experiments no significant changes occurred.

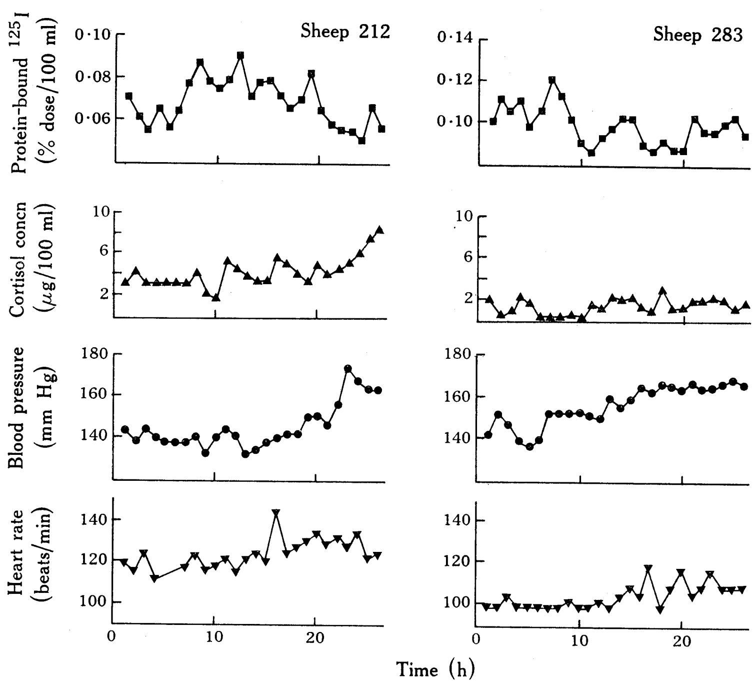

Fig. 3. Effect of 25 h of physical restraint in a standing position on the concentration in thyroid vein plasma of protein-bound ^{125}I (■) and cortisol (▲) and on the systolic blood pressure (●) and heart rate (▼) in two ewes with exteriorized thyroid glands.

Cold

Consistent increases in concentration of protein-bound ^{125}I from thyroid vein plasma and in plasma cortisol concentration were observed in all six experiments with wetting during cooling (Fig. 2). A sharp rise in rectal temperature occurred simultaneously. The mean change in mid-back skin temperature after wetting was $-7\cdot5$°C with a range of $-4\cdot0$ to $-12\cdot0$°C.

No comparable changes in hormone concentrations were detected in the absence of wetting though rectal temperatures rose in all six experiments. No differences between shorn and fleeced sheep were observed.

Restraint

The results of two experiments on different ewes are illustrated in Fig. 3 which shows the changes in concentration of protein-bound ^{125}I from thyroid vein plasma,

plasma cortisol concentration, blood pressure and heart rate during 25 h of continuous restraint. The most significant features of these results are the differences between the sheep in thyroid and adrenal steroid secretions despite the general trend towards increased blood pressure and heart rate apparent towards the end of all the experiments. Examination of the changes with time in plasma cortisol concentration and concentration of protein-bound ^{125}I from thyroid vein plasma in all eight experiments over 25 h of measurement showed no evidence for consistent simultaneous or reciprocal activation of the adrenal and thyroid glands.

The physical restraint employed in these experiments provided a considerable stress to the sheep, as shown by the blood pressure measurements (normally 120 mm Hg) which rose to almost 170 mm Hg during the later part of the period of restraint. The heart rate of sheep in an unstressed environment was *c.* 60–70 beats/min, but during these experiments a rate of 130 beats/min was observed in six of the eight experiments. Plasma cortisol concentration increased to 3–6 times above normal for unstressed sheep (Bassett and Hinks 1969).

Discussion

The effect of physical and emotional stress caused by an unfamiliar environment on plasma cortisol and blood glucose concentrations in the sheep has previously been described by Reid and Mills (1962). In their experiments the effect of transfer of sheep from pasture to indoor pens was an increase in plasma cortisol concentration within 2 h of transfer, which in fed animals returned to normal between 24 and 48 h. Transporting sheep from pasture in a road vehicle caused an even more marked rise in plasma cortisol concentration to 2–6 μg/100 ml within 2 h. After transporting, a rapid fall in plasma cortisol concentration occurred in all animals, to half or less of the raised concentration within 4 h of the end of the ride.

Other stressful stimuli, such as venipuncture, have also been shown to cause increased plasma cortisol concentration in the sheep from approximately 1 to between 1·5 and 4·0 μg/100 ml within 15 min (Bassett and Hinks 1969). Similar increases in sheep subjected to pistol shots and to a barking dog are described in this paper, though marked habituation to the stimulus was observed (Fig. 1). Earlier studies reported thyroid stimulation due to fear induced by a barking dog (Falconer and Hetzel 1964) but no measurement of blood cortisol concentration was carried out. In these experiments simultaneous activation of thyroid and adrenal glands was observed in sheep only before they became accustomed to the experimental procedure. Habituation to stress has been noted in other studies on the sheep (Bassett and Hinks 1969; McNatty and Thurley 1973), which makes it impossible to utilize stressful stimuli of a minor type to obtain repeated endocrine responses from the same animal.

Unlike emotional stress, the stress of cold has been shown in many species to cause a reproduceable stimulation of thyroid and adrenal gland activity (Straw and Fregly 1967; Fortier *et al.* 1970). In the present studies cold associated with wetting, which produces a consistent abrupt rise in rectal temperature, was found to stimulate an increase in both plasma cortisol concentration and concentration of protein-bound ^{125}I from thyroid vein plasma, beginning within 30 min of wetting (Fig. 2). The studies of Fortier *et al.* (1970), which also showed thyroid and adrenal gland activation by cold exposure, included in further experiments dexamethasone to block ACTH release. Fortier *et al.* concluded from their data that 'the acute secretion of ACTH and TSH are not inversely related'. The studies on fear and cold reported in this paper support this conclusion in that both independent and coincident changes in thyroid and adrenal gland activity have been observed after imposition of stress on the sheep.

To clarify the responses of the thyroid and adrenal glands to stressful stimuli, the experiments on continued physical restraint were carried out. This restraint was apparently not sufficient to cause pain, but prevented the animal from moving forward or back, turning or lying down. Under these conditions sheep go through periods of restlessness and periods of calm cud-chewing. Blood sampling and blood pressure and heart-rate measurements did not appear to disturb the sheep, as judged by preliminary experiments, but the initial cannulation was a cause of fear and discomfort, as described previously (Falconer and Hetzel 1964).

The effects of continued restraint shown in Fig. 3 demonstrate the absence of any clear relationship between thyroid secretion, adrenal secretion and blood pressure in these experiments. Marked changes in all the measured factors were observed, and the magnitude of the changes fell into the ranges previously reported for protein-bound ^{125}I concentration (Falconer and Hetzel 1964) and plasma cortisol concentration (Reid and Mills 1962; Bassett and Hinks 1969; McNatty and Thurley 1973) during stress. In particular, no consistent reciprocal relationship between the activity of the thyroid and the adrenal cortex was seen, both glands appearing to act independently during this type of continued stimulus.

The studies described in this paper support the hypothesis that there is no fixed relationship between thyroid and adrenal cortical secretion in response to stress in sheep that have been acclimatized to experimentation. Earlier work on the sheep has indicated that direct interaction between the circulating concentrations of adrenaline, noradrenaline (Falconer 1967), vasopressin (Falconer 1968) or corticosteroids (Falconer and Jacks 1975) and the function of the thyroid is unlikely. It must, therefore, be concluded that any activation of thyroid secretion by stress in the sheep is centrally mediated through the pituitary gland and that this response is not related directly, or reciprocally, to the function of the adrenal cortex.

Acknowledgments

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