Aust. J. Biol. Sci., 1988, **41**, 297–301

Effect of Myiasis and Acute Restraint Stress on Plasma Levels of Immunoreactive β-Endorphin, Adrenocorticotrophin (ACTH) and Cortisol in the Sheep

D. A. Shutt[A], *A. I. Smith*[B], *C. A. Wallace*[B], *R. Connell*[A] *and L. R. Fell*[A]

[A]Department of Agriculture, Hawkesbury Agricultural Research Unit, P.O. Box 217, Richmond, N.S.W. 2753.

[B]Medical Research Centre, Prince Henry's Hospital, Melbourne, Vic. 3004.

Abstract

Cutaneous myiasis in sheep arising from the activity of *Lucilia cuprina* larvae can result in significant physiological changes in susceptible animals. The stress imposed on the pituitary–adrenal axis of the sheep in response to myiasis and acute restraint is the subject of this investigation. Merino wethers were exposed to handling restraint, and blood sampling, during examination for blowfly strike; where necessary, they were treated for cutaneous myiasis. Significant changes in the plasma concentrations of immunoreactive β-endorphin (β-EP), ACTH and cortisol were found in sheep with extensive myiasis, as compared with unstruck sheep or those with only localized myiasis. In five susceptible sheep with extensive cutaneous myiasis, mean plasma levels of β-EP, ACTH and cortisol were 307 ± 71 pg ml^{-1}, 953 ± 58 pg ml^{-1} and 232 ± 46 nmol l^{-1} respectively, compared with 818 ± 89 pg ml^{-1}, 641 ± 41 pg ml^{-1} and 107 ± 17 nmol l^{-1} in six unstruck sheep handled similarly. Whereas significant increases in plasma ACTH and cortisol can result from pituitary–adrenal responses to acute emotional or surgical stress, and are usually accompanied by a concomitant release of β-EP from the pituitary, the present findings indicate a marked reduction in β-EP levels and a significant increase in ACTH and cortisol in sheep following blowfly strike and acute handling restraint. This result suggests that cutaneous myiasis in susceptible sheep can alter the pituitary–adrenal response to acute restraint stress, and this could occur either by an alteration of precursor processing in the pituitary or by the selective release of ACTH.

Introduction

In Australia, the larvae of the blowfly *Lucilia cuprina* are largely responsible for a cutaneous myiasis of sheep that can range from slight to extensive, and can result in the death of the more susceptible sheep (Seddon 1967). The activity of the larvae is associated with an inflammatory response, fever, and other systemic changes resulting from the absorption of toxic products from the larvae and from *Pseudomonas aeruginosa* and other bacteria on the skin surface (Broadmeadow *et al.* 1984*a*, 1984*b*; Dimmock, 1984; Gibson *et al.* 1984). Endotoxins have been shown to stimulate opioid peptide hormone secretion into the blood circulation of the sheep (Carr *et al.* 1982). Thus, the present investigation was aimed at evaluating the stressful effect of myiasis on the release of proopiomelanocortin-derived (POMC-derived) peptides, immunoreactive β-endorphin (β-EP) and adrenocorticotrophin (ACTH) from the anterior pituitary, and cortisol from the adrenal of the sheep, as part of a continuing study of hormonal indicators of stress affecting the sheep (Fell *et al.* 1985; Shutt *et al.* 1987). These hormones were selected as they have been shown to be secreted in increased amounts into the blood circulation in response to certain stressors (Guillemin *et al.* 1977).

Materials and Methods

Jugular blood samples (10 ml) were collected into heparinized vacutainers from 16 one-year-old Merino wethers that were being examined and treated for fly strike, and thus exposed to acute handling restraint stress. The sheep were located at the New South Wales Department of Agriculture's Research Centre at Trangie. The sheep had been exposed to an artificial wetting system and the blowfly *L. cuprina* to induce fleece-rot and fly strike in susceptible sheep as described by Merritt and Watts (1978). Four days after

0004-9417/88/030297$03.00

being exposed to *L. cuprina*, six of the sheep were found to have no fly strike, five to have slight myiasis and five to have moderate to severe myiasis. The sheep designated as having slight myiasis had larvae in small patches in the wool; those designated as having moderate to severe myiasis had extensive overt body strike and abrasion of the skin surface.

Blood samples were centrifuged at 2000 g for 45 min to obtain plasma, and then the plasma was frozen in aliquots until assayed for β-EP, ACTH and cortisol. The vycor glass extraction of plasma for β-EP and ACTH, and radioimmunoassay (RIA) procedures for β-EP and cortisol have been described previously (Shutt *et al.* 1987; Fell *et al.* 1985). The RIA method for ACTH was that of Lim *et al.* (1982). Sensitivity of the RIAs for β-EP and ACTH was 4 pg per tube and the within-assay coefficient of variation (CV) for these peptides was 8–12%. Sensitivity of the cortisol assay was 1 nmol l^{-1} with a within-assay CV of 2%. The antiserum (Rl-3) used in the RIA for ACTH was highly specific (cross-reacts less than 0·2% with αMSH or CLIP), but the antiserum (R56) used in the RIA for β-EP cross-reacts on an equimolar basis with β-lipotrophin (β-LPH) (Lim *et al.* 1982). Thus, in this paper, immuno-reactive β-EP represents a mixture of β-EP and β-LPH. The extraction and RIA procedures for β-EP and ACTH have been applied before to the assay of these hormones in sheep plasma (Clarke *et al.* 1986).

A one-way analysis of variance was used to compare the concentrations of the three hormones in unstruck sheep, in sheep with slight myiasis, and in sheep with moderate to severe myiasis. Differences were evaluated using the test of least significant differences.

Results

As shown in Fig. 1, a slight myiasis in one group (*b*) of five sheep had no significant effect

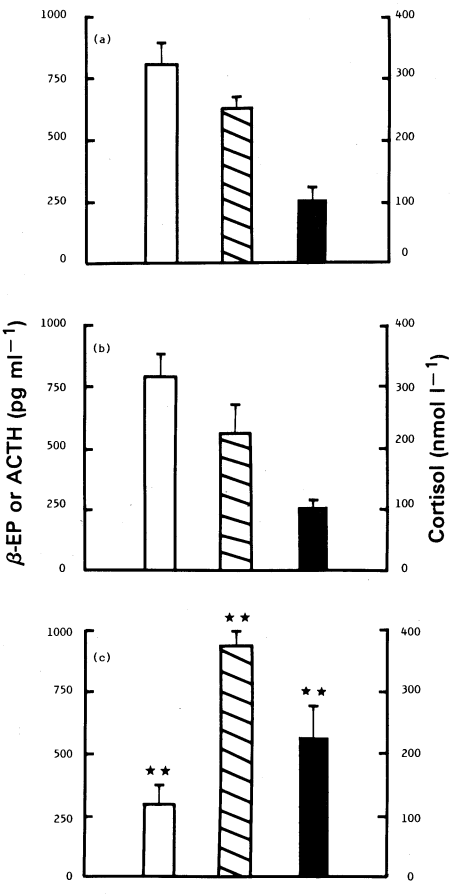

Fig. 1. Effects of myiasis on concentrations (mean ± s.e.m.) of immunoreactive β-endorphin (β-EP) (open bars), adrenocorticotrophin (ACTH) (hatched bars), and cortisol (solid bars) in ovine venous plasma: (*a*) unstruck sheep, $n = 6$; (*b*) sheep with slight myiasis, $n = 5$; and (*c*) sheep with moderate to severe myiasis, $n = 5$. Using one-way analysis of variance and least significance differences, $^{**}P < 0{\cdot}01$.

on the mean plasma levels of β-Ep, ACTH or cortisol when compared with six sheep with no fly strike (*a*), when sampled during examination and treatment for fly strike. Relative to

unstruck sheep, however, the five sheep exhibiting moderate to severe myiasis (*c*) had significantly lower ($P < 0{\cdot}01$) levels of plasma β-EP but significantly higher ($P < 0{\cdot}01$) levels of ACTH. Mean plasma levels (± s.e.m.) of β-EP and ACTH in the unstruck sheep were 818 ± 89 pg ml^{-1} and 641 ± 41 pg ml^{-1}, respectively; in the sheep with extensive myiasis, there was a threefold reduction in the ratio of β-EP to ACTH in the plasma, mean plasma levels being 307 ± 71 pg ml^{-1} and 953 ± 58 pg ml^{-1}, respectively. Mean plasma cortisol levels were also significantly ($P < 0{\cdot}01$) elevated in the badly flystruck group (*c*), and this represented a twofold increase from 107 ± 17 nmol l^{-1} in the unstruck sheep to 232 ± 46 nmol l^{-1} in the group with extensive strike.

All sheep recovered after removal of the affected area of wool and associated larvae, followed by application of a dressing to the skin, with the exception of one sheep which died within a few hours of being examined. Plasma levels for β-EP, ACTH and cortisol were 124 pg ml^{-1}, 1152 pg ml^{-1}, and 400 nmol l^{-1}, respectively, in this sheep.

Discussion

In blood samples collected from untrained sheep responding to acute restraint stress, and suffering from various degrees of cutaneous myiasis, a marked increase in the ratio of ACTH to β-EP was found in the plasma of those sheep with extensive blowfly strike, when compared with the response in unstruck sheep or in less susceptible sheep with localized strike. A significant increase in ACTH during handling restraint is a characteristic response to stress (Selye 1973). However, what was unexpected in sheep with extensive myiasis responding to restraint stress was the significantly reduced levels of plasma β-EP. This apparently can not be explained by the presence of toxic products in the plasma interfering with the β-EP assay, for, when standard amounts of β-EP were added to plasma samples from sheep with extensive myiasis before vycor glass extraction and RIA, no reduction in recovery was obtained. We obtained similar results in a recent controlled experiment, carried out in collaboration with Dr. Gerry Murphy of the Queensland Department of Primary Industries, in which 4500 *L. cuprina* larvae were implanted on each of eight sheep. When jugular blood samples were taken 3 days after the implants from these sheep, prior to euthanasia for post-mortem examination, the mean ratio of plasma ACTH : β-EP was 3·6 (2·5–5·0) as compared with 1·6 (1·4–1·7) in two controls (unpublished data).

The standard response to stressors is the concomitant release of β-EP and ACTH from the pituitary with subsequent adrenal corticoid release (Guillemin *et al.* 1977). Since Guillemin's report, other researchers have shown that the composition of immunoreactive β-EP in the pituitary and plasma is usually a mixture of β-EP and its precursor molecule, β-lipotrophin (β-LPH), whose relative proportions may depend on the degree of stress and the timing of sampling. For example, Carr *et al.* (1982) and De Souza and Van Loon (1985) have shown that the initial response to endotoxin or restraint stress respectively is a release of β-EP with very little β-LPH. This is then followed by increasing amounts of the unprocessed precursor β-LPH. Concomitant increases of ACTH with β-EP/β-LPH have been found in plasma in response to restraint stress (De Souza and Van Loon 1985) or in the anterior pituitary in response to daily footshock (Shiomi *et al.* 1986). Various other treatments that can alter both the pituitary content and the plasma levels of ACTH and β-EP/β-LPH in the rat have not shown ACTH to be increased at the expense of β-EP/β-LPH (Lim *et al.* 1982; Young and Akil 1985). One major difference, however, between cited research on chronic stress/acute stress (Young and Akil, 1985; Shimoi *et al.* 1986) and the present investigation is that the regulation of ACTH and β-EP/β-LPH in the above groups was studied in rats, in response to a standard footshock applied for about 30 min daily. This would allow a longer recovery period between episodes of stress than in the case of a continuous physiological stress from cutaneous myiasis.

One consequence of a sustained stress may be the further processing of β-EP in the pituitary to smaller peptides. For example, Smith *et al.* (1986) found a processing change in *N*-acetyl forms of β-endorphin, in extracts of anterior pituitaries from some abattoir sheep and from

sheep after chronic dexamethasone (glucocorticoid) treatment, in comparison with pituitaries from sheep taken directly from the field. In abattoir sheep, there was an increase in the shorter congeners of *N*-acetyl-β-endorphin (31 amino acids), *N*-acetyl-α-endorphin (16 amino acids) being the major component. The results of Smith *et al.* (1986) also suggest that the processing change is mediated by glucocorticoids, and this supports the proposal of Munck *et al.* (1984) that glucocorticoids are released to modulate or reduce the activity of numerous intercellular mediators and, thus, to dampen down those reactions which themselves may threaten homeostasis.

It is concluded that extensive cutaneous myiasis in susceptible sheep can alter the response of the pituitary–adrenal axis to acute restraint stress, and this could occur either by an alteration in peptide processing or by the selective release of ACTH. Further work will be required to clarify the relationship of cutaneous myiasis to these possible changes in the pituitary.

Acknowledgments

We wish to thank Mr Herman Raadsma of the N. S. W. Department of Agriculture's Agricultural Research Centre at Trangie and Dr Gary Le Vot of the N. S. W. Department of Agriculture's Biological and Chemical Institute at Rydalmere for expert assistance and advice on these investigations.

References

Broadmeadow, M., Connell, J. A., and O'Sullivan, B. M. (1984*a*). Fever after larval challenge. *Proc. Aust. Soc. Anim. Prod.* **15**, 173–5.

Broadmeadow, M., Cotton, A., and Hopkins, P. S. (1984*b*). Physiological aspects of challenge by *Lucilia cuprina*. *Proc. Aust. Soc. Anim. Prod.* **15**, 171–3.

Carr, D. B., Bergland, R., Hamilton, A., Blume, H., Kasting, N., Arnold, M., Martin, J. B., and Rosenblatt, M. (1982). Endotoxin-stimulated opioid peptide secretion: two secretory pools and feedback control *in vivo*. *Science (Wash. D.C.)* **217**, 845–8.

Clarke, I. J., Clements, J. A., Cummins, J. T., Dench, F., Smith, A. I., Robinson, P. M., and Funder, J. W. (1986). Elevated plasma levels of pro-opiomelanocortin-derived peptides in sheep following hypothalamo-pituitary disconnection. *Neuroendocrinology* **44**, 508–14.

De Souza, E. B., and Van Loon, G. R. (1985). Differential plasma β-endorphin, β-lipotropin, and adrenocorticotropin responses to stress in rats. *Endocrinology* **116**, 1577–86.

Dimmock, C. K. (1984). Haematological changes in sheep suffering from blowfly strike. *Proc. Aust. Soc. Anim. Prod.* **15**, 175–7.

Fell, L. R., Shutt, D. A., and Bentley, C. J. (1985). Development of a salivary cortisol method for detecting changes in plasma 'free' cortisol arising from acute stress in sheep. *Aust. Vet. J.* **62**, 403–6.

Gibson, J. A., Thomas, R. J., O'Sullivan, B. M. (1984). Pathological consequences of myiasis in sheep. *Proc. Aust. Soc. Anim. Prod.* **15**, 178–81.

Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W., and Bloom, F. (1977). β-Endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science (Wash. D.C.)* **197**, 1367–9.

Lim, A. T., Khalid, B. A.K., Clements, J., and Funder, J. W. (1982). Glucocorticoid and mineralocorticoid effects on adrenocorticotropin and β-endorphin in the adrenalectomized rat. *J. Clin. Invest.* **69**, 1191–8.

Merritt, G. C., and Watts, J. E. (1978). The changes in protein concentration and bacteria of fleece and skin during the development of fleece-rot and body strike in sheep. *Aust. Vet. J.* **54**, 517–20.

Munck, A., Guyre, P. M., and Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.* **5**, 25–44.

Seddon, H. R. (1967). 'Diseases of Domestic Animals in Australia, 2. Arthropod Infestations.' (Commonwealth of Australia Dept Health: Canberra.)

Selye, H. (1973). The evolution of the stress concept. *Am. Sci.* **61**, 692–9.

Shiomi, H., Watson, S. J., Kelsey, J. E., and Akil, H. (1986). Pretranslational and posttranslational mechanisms for regulating β-endorphin-adrenocorticotropin of the anterior pituitary lobe. *Endocrinology* **119**, 1793–9.

Shutt, D. A., Fell, L. R., Connell, R., Bell, A. K., Wallace, C. A., and Smith, A. I. (1987). Stress-induced changes in plasma concentrations of immunoreactive β-endorphin and cortisol in response to routine surgical procedures in lambs. *Aust. J. Biol. Sci.* **40**, 97–103.

Smith, A. I., Wallace, C. A., Clarke, I. J., and Funder, J. W. (1986). β-Endorphin processing: differences between slaughterhouse and unstressed sheep. Proc. 1st Internatl Cong. Neuroendocrinol. San Francisco, U.S.A. Abstr. 199.

Young, E. A., and Akil, H. (1985). Corticotrophin-releasing factor stimulation of adrenocorticotropin and β-endorphin release: effects of acute and chronic stress. *Endocrinology* **117**, 23–30.

Manuscript received 7 August 1987, accepted 5 February 1988