Salivary and plasma cortisol as an index of stress in goats

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SUMMARY: Total assayable cortisol in plasma was highly correlated (r = 0.97) with physiologically active free cortisol in plasma after routine management procedures in 1- to 3-weeks-old goats. Transport of adult goats caused significant increases (P < 0.001) in free cortisol in saliva and in free and total cortisol in plasma. No difference (P > 0.05) between concentrations of free cortisol in saliva and in plasma was apparent before or after transport. The results demonstrated that the salivary cortisol method is a useful measure of stress in adult goats, and that the relationship between free and total cortisol in plasma, and the adrenocortical response to transport, appear to be similar in sheep and goats.

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Introduction

Salivary and plasma cortisol are useful measures of adrenocortical response to stress in sheep and cattle (Fell and Shutt 1986a, b). Plasma cortisol is also a useful measure of this response in goats (Sanhouri *et al* 1989; Greenwood and Shutt 1990). However, the salivary cortisol technique of Fell *et al* (1985) has limitations when used in 1- to 4-weeks-old goats, because of difficulties in collecting timed samples in some experiments without contamination with blood plasma (Greenwood and Shutt 1990).

The present investigation was conducted to assess free cortisol concentrations in saliva, and total and free cortisol concentrations in plasma, before and after transport. Additional results, from a previously reported experiment (Greenwood and Shutt 1990) on the relationship between concentrations of physiologically active free cortisol and total cortisol are provided also. The results are discussed in relation to the usefulness of the salivary cortisol technique as a measure of stress in goats.

Materials and Methods

In experiment 1, blood samples were obtained from the jugular vein of 1- to 3- weeks-old Saanen kids 15 min after disbudding (n=5) or rubber-ring castration (n=5) and from control animals (n=4), as previously reported by Greenwood and Shutt (1990).

In experiment 2, 6 mature, non-pregnant, non-lactating Saanen does were yarded on the evening before the investigation. The does were from a large commercial dairy operation and were accustomed to routine handling. Before transport, each doe was led from the holding pen up a ramp into a pen mounted on a trailer. The pen had a woven mesh floor, which measured 2.5 m x 1.3 m, and had sides constructed of steel mesh with 15 cm x 10 cm spacings. The pen contained a holding crate (1.2 m x 0.5 m) in which goats were sampled. The goats were transported for 30 min on a gravel road, during which time manoeuvres including U-turns, stopping and starting, and turns to left and right were carried out. After transport the goats were returned to the holding pen for one hour.

Blood and saliva samples were obtained before and immediately after transport, and 1 h later. Saliva was collected by aspiration as described by Fell *et al* (1985), and blood samples were collected from the jugular vein into containers with heparin*. Saliva and plasma free cortisol and total plasma cortisol were determined using the methods described by Fell *et al* (1985).

* Vacutainer® Becton Dickson and Company, Rutherford, New Jersey

Results

When total (free and protein bound) assayable cortisol in plasma was compared with the physiologically active free cortisol in plasma obtained from kids 15 min after routine management practices, an excellent correlation (r = 0.97) was obtained, as shown in Figure 1. The physiologically active free cortisol represented about 25% of the total cortisol, as measured. The concentrations (mean ± SEM) for total and free cortisol, respectively, in plasma were 179.2 ± 29.5 and 44.0 ± 19.7 nmol/l for disbudded kids, 186.0 ± 25.3 and 46.6 ± 11.5 nmol/l for castrated kids and 114.9 ± 37.9 and 21.7 ± 10.4 nmol/l for kids in the control group.

Comparative values for free cortisol in plasma and saliva samples, and free and total cortisol in plasma, obtained before, immediately after 30 min transport, and 1 h after transport are shown in Figure 2.

Significant differences (P < 0.001) were apparent for the concentrations of total and free cortisol in plasma, and free cortisol in saliva, between samples obtained immediately after transport in comparison with those obtained before and 1 h after transport. There was no significant difference (P > 0.05) between free cortisol concentrations in plasma and saliva at any of the sampling times. Differences were not apparent (P > 0.05) between samplings before, and 1 h after transport for any of the three measures of cortisol concentration.

Concentrations (mean ± SEM) immediately after transport, were 212.0 ± 18.6 nmol/l for total plasma cortisol, 22.7 ± 2.3 nmol/l for

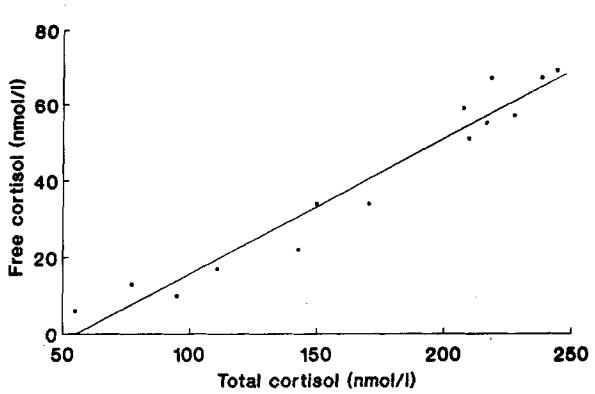

Figure 1. Correlation between plasma free cortisol (assayed after centrifugal ultrafiltration), and total assayable plasma cortisol concentrations in blood samples from 14 kids obtained 15 min after routine management practices. The regression equation is y = -20 + 0.35 x, r = 0.97.

free plasma cortisol and 22.2 ± 2.4 nmol/l for free salivary cortisol, while basal concentrations measured before transport were 37.7 ± 11.0 nmol/l, <1.0 nmol/l, and 3.0 ± 1.3 nmol/l, respectively, for these measures. Concentrations of free cortisol in plasma and saliva immediately after transport were about 11% of the concentration of total cortisol in plasma.

During transport, does huddled towards the rear of the trailer and two of the does remained in a recumbent position throughout the trip, although there was space (0.54 m^2/doe) for goats to move around the trailer. Upon release into the holding pen, two other does lay down and within 50 min all does were lying down.

Discussion

The marked increase after routine management practices in the concentration of physiologically active free cortisol in kids, which was highly correlated with total assayable cortisol, was very similar to results in lambs presented by Shutt *et al* (1987). These authors compared free and total cortisol in 15 min post-operative blood samples of 3- to 5-weeks-old lambs. In both studies free cortisol concentration, as determined after centrifugal ultrafiltration, was about 25% of the total cortisol.

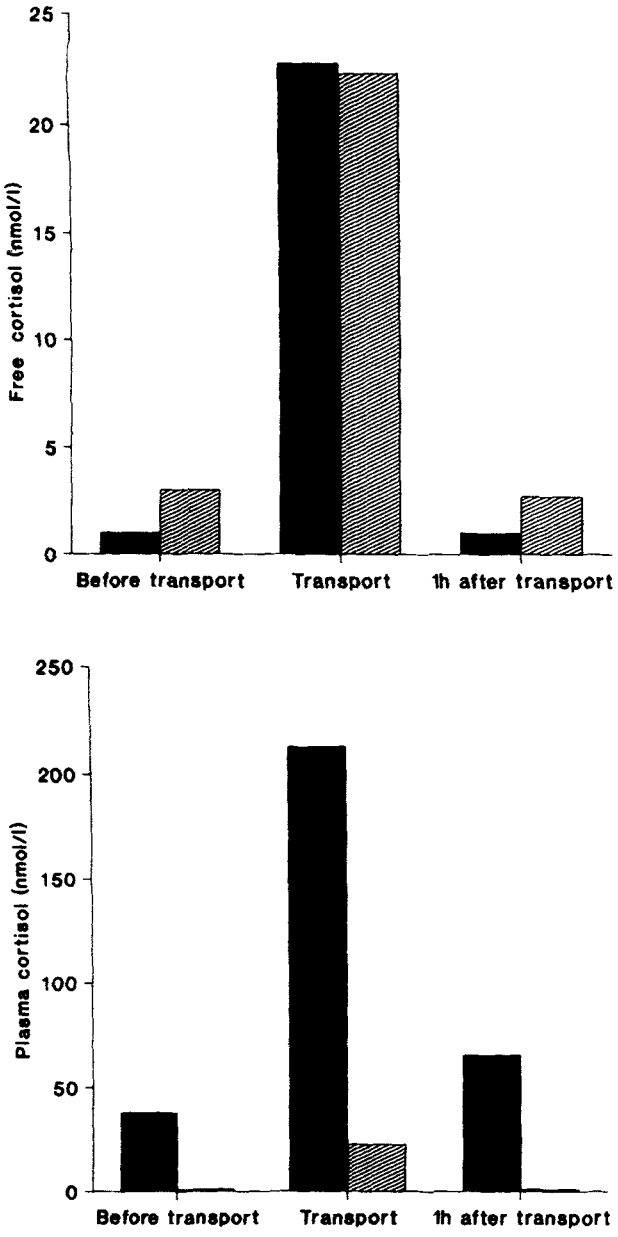

Figure 2. Comparison of mean concentrations of free cortisol in paired samples of saliva ▩ and blood plasma ■ (upper figure) and free ▩ and total ■ cortisol in plasma (lower figure) from 6 does sampled before and immediately after transport on a gravel road and 60 min after transport.

The results presented here demonstrate also that the concentrations of free cortisol are similar in saliva and plasma samples obtained from adult goats. Furthermore, in adult goats no difficulties were experienced in obtaining an adequate amount of saliva uncontaminated with blood plasma at a prescribed time. This was not possible with 1- to 4-weeks-old kids (Greenwood and Shutt 1990), probably because of the secretion of saliva at low rates from the parotid gland of young kids compared with goats 3 months of age or older (Kay 1960), and to damage by the plastic suction tube to the membrane lining of the mouth, which caused bleeding. In the previous study, an inadequate volume of saliva was collected from 5% of kids, and 20% of saliva samples appeared to be contaminated with blood and caused grossly elevated readings (PL Greenwood and DA Shutt, unpublished data).

Transportation resulted in a marked increase in cortisol concentrations in mature goats. Maximum mean concentrations of salivary and plasma free cortisol and total cortisol measured in samples obtained immediately after transport were similar to those presented by Fell *et al* (1985), and for salivary cortisol by Fell and Shutt (1986b), after similar treatment of sheep. The ratio of salivary and plasma free cortisol to total cortisol immediately after transport in the present study was about 11%, within the range of values (10-25%) at maximum cortisol concentrations found by Fell *et al* (1985) in sheep after injection of synthetic adrenocorticotrophic hormone, but lower than for kids (about 25%) in the present study. The total:free cortisol ratio was also higher in ewes (Fell *et al* 1985) when compared with lambs (Shutt *et al* 1987) and indicates that the plasma of older ruminants contains more cortisol binding globulin. A gradual increase in cortisol binding globulin activity in growing rats was demonstrated by Gala and Westphal (1965) and our findings in goats appear consistent with these observations in the rat.

Total cortisol concentrations after transport were, however, about three times higher in the current study than maximum concentrations measured in male goats subjected to a noisy trolley trip or a motorised van trip by Sanhouri *et al* (1989), who concluded that noise had a greater effect on the adrenocortical response than motion. In the present study, factors including noise, motion, the longer duration of transport, wind chill and sex may have contributed to the magnitude of the response. Certainly, the animals were subjected to a degree of rough, although not atypical, transport over a pot-holed and furrowed road and were transported over cattle grids. Furthermore, although the pen was built to fit the trailer, there was considerable rattling noise during the trip.

Peak concentrations of total cortisol in plasma consistently occurs 30 to 40 min after the onset of a stressful episode in sheep (Kilgour and de Langen 1970). Our results, and those of Sanhouri *et al* (1989, 1991) suggest that this may also be the case for goats, although Greenwood and Shutt (1990) and Sanhouri *et al* (1991) showed that rate of decline from peak cortisol concentrations in goats will vary according to the type and duration of stressor. Despite differences in maximum mean concentrations of cortisol measured in the present study and those of Sanhouri *et al* (1989, 1991), the rates of decline in cortisol concentration 1 h after transport appeared similar to those previously reported by these researchers.

In conclusion, it is apparent from the results presented here that the salivary cortisol technique of Fell *et al* (1985) is a useful measure of the adrenocortical response to stress in adult goats. Furthermore, the relationship between free and total cortisol in plasma, and the response to transport, appear to be similar in sheep and goats.

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Locomotor effects in sheep of alkaloids identified in Australian *Tribulus terrestris*

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SUMMARY: Fresh, mature, ungrazed *Tribulus terrestris* plant material was subjected to a standard alkaloid extraction procedure. The extract was fractionated by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Two major alkaloid fractions were demonstrated. These fractions were identified by means of TLC, ultraviolet spectrofluorimetry (UVS) and HPLC, as the beta-carboline indoleamines harmane and norharmane. The extractable alkaloid content was determined to be 44 mg/kg dry matter. Synthetic harmane and norharmane were administered subcutaneously to sheep at a dose rate of 54 mg/kg. Both compounds caused similar nervous effects. The main effect observed was limb paresis, which in some sheep was body side biased. The clinical signs observed in the experimental sheep were consistent with those described for naturally occurring cases of *Tribulus terrestris* staggers. It was proposed that harmane and norharmane accumulate in tryptamine-associated neurones of the central nervous system, during months of tribulus ingestion, and gradually interact irreversibly with a specific neuronal gene DNA sequence.

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Introduction

Tribulus terrestris is a drought-tolerant, summer growing, prostrate herb. It has long stems, small pinnate leaves, small yellow flowers and large spined fruits. It has a world wide distribution, but only in Australia has its ingestion by sheep resulted in outbreaks of a locomotor disorder (Bourke 1984). The beta-carboline alkaloids, harmane and harmine, have been identified in the aerial parts of Polish *T terrestris* (Borkowski and Lutomski 1960). There have been no analytical studies reported for plant material from other countries. The administration of simple beta-carbolines to rats, rabbits and sheep have produced nervous signs consistent with functional abnormalities of the central nervous system (Fuentes and Longo 1971; Bourke *et al* 1990). *Tribulus terrestris* staggers is an asymmetric locomotor disorder in sheep, which develops as a result of a functional abnormality in the central nervous system (Bourke 1987). In this investigation the alkaloid content of Australian *T terrestris* was determined by the use of TLC, UVS and HPLC. The locomotor effects of synthetic samples of each of the major alkaloids found in the plant were ascertained by administration to normal sheep. The clinical effects of these compounds, in sheep, have been reported in a previous study (Bourke *et al* 1990). However, asymmetric locomotor effects were not observed in the pair of sheep used. Therefore, in this investigation a larger treatment group was selected to increase the chance of demonstrating this phenomenon.

Materials and Methods

Extraction and Identification of Alkaloids

Fresh, mature, aerial parts of *T terrestris* were harvested in midsummer at Coonamble and Cudal, New South Wales. Twenty-four plants were collected at random from each site then bulked and refrigerated in a sealed container for 48 hours. The plant material was coarsely chopped and 2.5 kg (wet weight) was immediately taken and macerated in 20 l of methanol for 10 days. An additional 2.5 kg was taken, oven-dried, and the dry matter content determined. The methanol extract was rotary evaporated, the residue acidified (pH 1), washed with chloroform and petroleum solvent, alkalised (pH 10), and washed with chloroform again. This chloroform extract was evaporated, and the above procedure repeated on the residue. TLC was undertaken with Merck 5553 silica gel 60 plates, 0.2 mm layer on aluminium sheets. The plates were developed with freshly mixed 85:10:5, chloroform, methanol and acetic acid, then viewed under an ultraviolet lamp (wavelength 366 nm). Authentic harmane, norharmane, harmine, harmaline, harmol, harmalol and tetrahydronorharmane were chromatographed as controls.

The fractions separated by TLC were suspended in a mixture of 1 ml methanol and 200 µl 28% ammonium hydroxide, and centrifuged. The supernatant recovered was mixed with 170 µl acetic acid and 2.17 ml acetate buffer (0.2N, pH 4.0). The excitation and emission peaks for each fraction and for authentic harmane, norharmane, harmine and harmol were determined using a 204-A Perkin Elmer Fluorescence Spectrophotometer.