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J Anim Sci 2000. 78:371-379.

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Investigation of the primary cause of hypoadrenocorticism in South African Angora goats (*Capra aegagrus*): A comparison with Boer goats (*Capra hircus*) and Merino sheep (*Ovis aries*)¹

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ABSTRACT: Our objective was to identify the primary site of the reduced adrenal function in South African Angora goats (*Capra aegagrus*) that causes a decrease in cortisol production and leads to severe losses of Angora goats during cold spells. Angora goats, Boer goats (*Capra hircus*), and Merino sheep (*Ovis aries*) were assigned to three intravenous treatments: 1) insulin, 2) corticotropin-releasing factor (CRF), and 3) ACTH. Blood cortisol concentrations were determined over a 90-min period to determine any differences in the response of the experimental animals to these treatments. For both the insulin and ACTH treatments, cortisol concentrations were less in Angora goats than in the other experimental animals. The adrenal gland was subsequently investigated as a possible cause for the observed hypoadrenocorticism. Primary adrenal cell cultures were prepared from these species, subjected to different treatments, and the cortisol pro-

duction determined. Upon pregnenolone (PREG) addition, all the experimental animals' cortisol production increased significantly, with the production in Boer goats higher ($P < .01$) when compared with that in the other species. The stimulation of cortisol biosynthesis by ACTH was only obtained for Boer goats and Merino sheep. The stimulation of cortisol production by forskolin and cholera toxin were compared with ACTH, and, for Angora goats, only cholera toxin caused a significant increase in cortisol production. For Boer goats, no difference ($P > .05$) between the PREG, ACTH, forskolin, or cholera toxin treatments were observed. The Merino adrenal cells were increasingly stimulated in the following order: PREG, ACTH, forskolin, and cholera toxin (forskolin and cholera toxin stimulated cortisol production to the same extent). This investigation of the hypothalamic-pituitary-adrenocortical axis, therefore, identified the adrenal gland as the primary site of the Angora's hypoadrenocorticism.

Key Words: Adrenal Glands, Angora, Goats, Heat Regulation, Sheep, Stress

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J. Anim. Sci. 2000. 78:371–379

Introduction

South African Angora goats (*Capra aegagrus*) are perhaps the most efficient fiber producing, but least hardy, small stock breed. South Africa currently produces approximately 15×10^6 kg of mohair annually, and more than 95% of this is exported. South African mohair represents almost half of the global crop. Thus, the South African Angora industry represents an important farming activity, but frequent and severe losses of young and newly shorn Angora goats during cold spells hamper the industry.

An abrupt decrease in blood glucose concentration seems to be the crucial factor leading to the failure of the mechanism responsible for metabolic heat production (Wentzel et al., 1974). This failure could be the result of hypoadrenocorticism, because Van Rensburg (1971) reported that selection for mohair production resulted in reduced adrenal function. Stress stimulates the release of corticotropin-releasing factor (CRF) from the hypothalamus, and CRF stimulates ACTH secretion from the anterior pituitary (Gemma et al., 1994). This hormone promotes the secretion of glucocorticoids from the adrenal cortex, which favor glucose production at the expense of glycolysis (Munch, 1971).

Adrenocortical insufficiency, therefore, results in a vulnerability to stress, because survival during cold conditions depends on the ability to produce metabolic heat (Vallerand et al., 1995; Buckingham, 1996). In this study, Angora goats were compared with South African-bred Boer goats (*Capra hircus*) and Merino sheep (*Ovis aries*), two breeds generally accepted as hardy. The ad-

¹This study was supported by the FRD and the University of Stellenbosch.

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Received December 22, 1998.

Accepted July 13, 1999.

renal response of Angora goats, Boer goats, and Merino sheep to insulin-induced stress as well as ACTH and CRF stimulation was determined *in vivo*. Primary adrenal cell cultures were subsequently stimulated with ACTH, forskolin, and cholera toxin. The production of cortisol was measured and compared.

Materials and Methods

Animals. Eight Angora goat does, eight Boer goat does, and seven Merino sheep ewes were used for the insulin, CRF and ACTH stimulation tests, respectively. The animals were bred and kept at the Grootfontein Agricultural Institute at Middelburg in the Northern Cape. They were 6 mo of age and were given *ad libitum* access to ground alfalfa hay. For the primary cell culture preparations, material was collected either from the abattoir (Merino sheep) or from local farmers (Angora and Boer does). The donor animals were between 2 and 6 yrs old and of both sexes.

Insulin, CRF, and ACTH Stimulation Test. Biosynthetic human insulin (Novo Nordisk, Johannesburg, South Africa) was diluted to 1 IU/mL in 1% NaCl solution and given as a single injection intravenously into the left external jugular vein at a dose of .1 IU/kg of body weight. Blood samples were collected from the right external jugular vein into heparinized vacuum tubes and placed on ice at 0, 15, 30, 60, and 90 min. The plasma were harvested by centrifugation ($2,500 \times g$; 4°C ; 10 min). Twenty-four hours later, sheep synthetic CRF (Sigma Chemical Co., St. Louis, MO) was diluted to 10 $\mu\text{g}/\text{mL}$ in 1% NaCl and injected intravenously at a dose of 10 $\mu\text{g}/\text{mL}$. The blood samples were collected and treated as described above. On the third day, human synthetic ACTH (Sigma Chemical Co.), diluted to 100 $\mu\text{g}/\text{mL}$ in 1% NaCl, was given at a dose of 10 mg/mL, after which blood samples were collected and treated as previously described. All plasma samples were stored at -20°C until assayed.

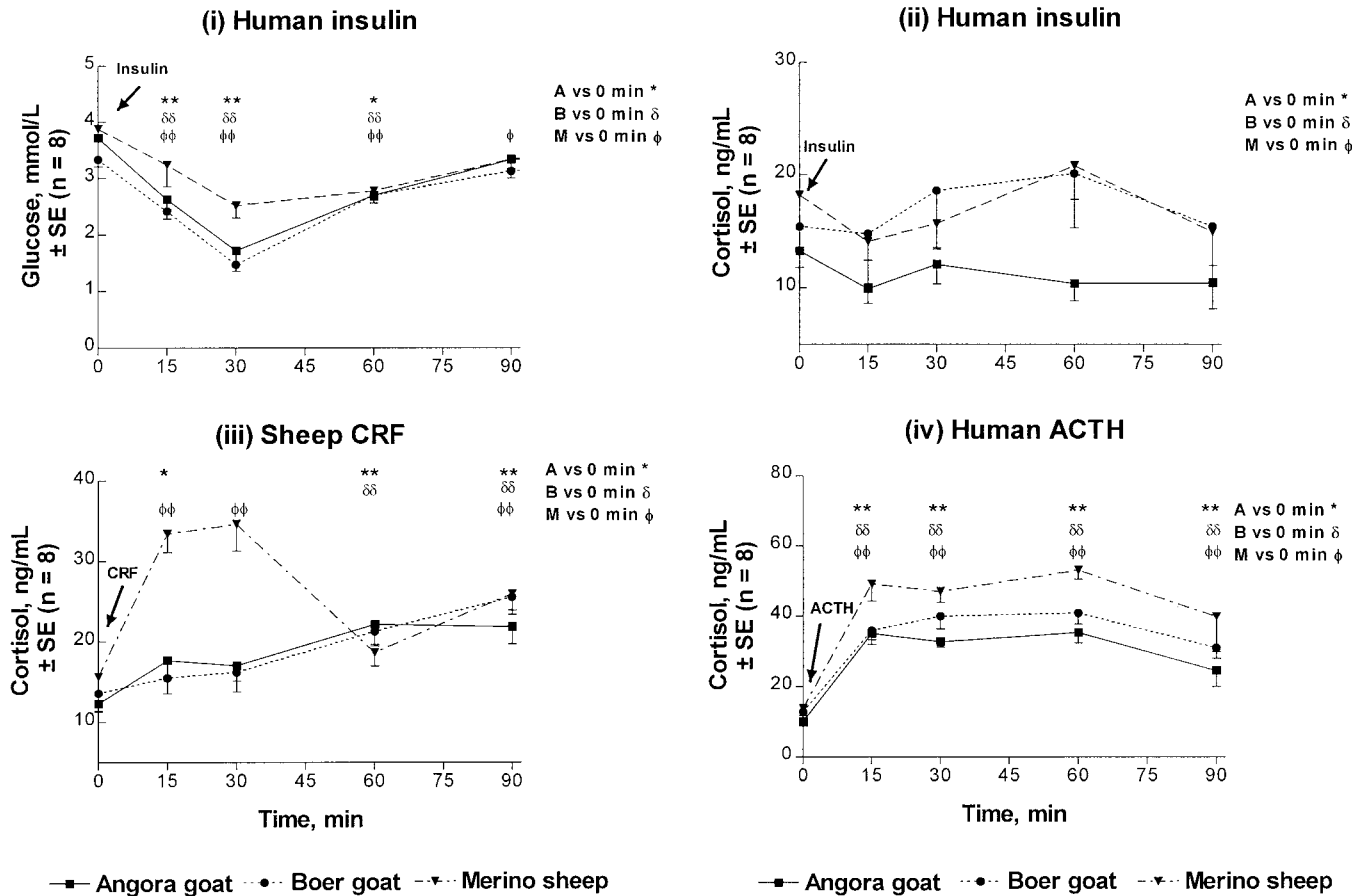
Preparation and Maintenance of Adrenal Cell Cultures. Primary adrenal cell cultures were prepared using a method previously described by Williams et al. (1989). Angora goat, Boer goat, and Merino sheep adrenals were collected immediately after decapitation and placed on ice in Earl's balanced salt solution. All subsequent steps were carried out under sterile conditions. After trimming away the fatty tissue, the glands were rinsed with an Earl's balanced salt solution (Sigma Chemical Company) that contained .2% BSA (EBSS) (Boehringer Mannheim GmbH, Mannheim, Germany). The glands were placed in a Stadie-Riggs microtome, and slices of tissue of approximately 100- μm thickness were cut using a skin graft blade. The first slice, which contained the entire zona glomerulosa and the outer part of the zona fasciculata, was discarded. The next slice, which contained the zona fasciculata and zona reticularis, was finely chopped and digested by incubation with collagenase (Worthington Biochemical Corp., Lakewood, NJ) dissolved in EBSS. Incubation was car-

ried out in tightly capped 50-mL disposable centrifuge tubes in a 37°C incubator for 150 min with vigorous shakes at 30-min intervals throughout the entire incubation period.

Dispersed cells were separated from undigested tissue by filtration through a 250- μm and then a 100- μm mesh nylon gauze (Lockertex, Cheshire, UK). The cells were then harvested by centrifugation at $400 \times g$ for 20 min. The pelleted cells were gently resuspended in EBSS, and each suspension was passed through a 30- μm mesh nylon gauze. The cell suspension (12 mL) was then applied to a Sephadex column that consisted of 5-mL Sephadex G-50 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) supported on 15-mL Sephadex G-10 (Sigma Chemical Company). The column was equilibrated with 15 mL of EBSS at room temperature prior to addition of the cell suspension. The column was then washed with 25 mL of EBSS, and the cells harvested by resuspending the Sephadex and filtering through a 30- μm nylon gauze. The resulting cell suspension was centrifuged at $400 \times g$ for 30 min and resuspended in Ham's F10 (Sigma Chemical Company) containing 10% fetal calf serum (Boehringer Mannheim) and 1 mL each of penicillin (1,000 IU/mL), streptomycin (1,000 mg/mL), and amphotericin B (250 mg/mL) (Sigma Chemical Company). The 20-mL cell suspension was plated out at 2×10^5 cells per well of a 12-well plate. Routinely, 6 g of adrenal glands yielded 72×10^6 purified cells. The cells were cultured at 37°C in an atmosphere of 10% CO_2 with medium changes after 24 h. The cells were monitored under a light microscope on a daily basis to confirm normal cell growth.

Incubation of Adrenal Cells with Pregnenolone. The medium was replaced with antibiotic-free medium 36 h after preparation of the primary cell cultures and 24 h before the experiments were commenced (Funkenstein et al., 1983). The cells were incubated with 100 μM pregnenolone (PREG) alone and with 100 μM PREG and 1 μM ACTH (Sigma Chemical Company) (Purvis et al., 1973; Tangalakis et al., 1992), or 10 μM forskolin (Sigma Chemical Company) (Scarceriaux et al., 1995; Cobb et al., 1996; Dessauer et al., 1997) or 1 $\mu\text{g}/\text{mL}$ cholera toxin (a kind gift from I. Mason, University of Edinburgh) (Ransjo et al., 1995). A control experiment was included that constituted the addition of growth medium that contained no PREG. The cells were incubated for 72 h and medium was removed at regular time intervals. The reaction was terminated by addition of ice-cold ethanol (Tuckey and Cameron, 1993) to the medium and stored at -20°C until assayed for cortisol production with a RIA (Tuckey and Cameron, 1993).

Assay Procedures. Blood cortisol concentrations were determined using a commercially available RIA kit (BioMaker bm, Rehovot, Israel). Plasma was diluted 1:3.5 (vol/vol) with .05 M Tris-HCl buffer (pH 8.0) containing .1 M NaCl, .1% NaN_3 and .1% gelatin. Cortisol was extracted from plasma with petroleum ether prior to the assay. Intra- and interassay CV for the cortisol analyses, were 14.7 and 15%, respectively. The produc-



Breed effect:	Insulin	Insulin	CRF	ACTH
Serum:	Glucose	Cortisol	Cortisol	Cortisol
P-value summary:	**	ns	ns	***
A vs B	ns	***	ns	ns
A vs M	ns	***	ns	***
B vs M	**	***	ns	**

Interaction between time and breed.				
Serum:	Insulin	Insulin	CRF	ACTH
	Glucose	Cortisol	Cortisol	Cortisol
Interaction:	ns	ns	***	ns
Breed:	***	**	***	***
Time:	***	ns	***	***

Figure 1. The effect of an intravenous injection of insulin (.1 IU/kg), β -corticotropin-releasing factor (CRF, 1 μ g/kg), and ACTH (10 μ g/kg) on mean \pm SE plasma glucose and cortisol concentrations in Angora goats (A), Boer goats (B), and Merino sheep (M). The data were analyzed comparing cortisol levels for each species to that at 0 min and are indicated on the respective graphs (repeated measures ANOVA). The cortisol levels of each species were also compared with the response of the other animals (repeated measures ANOVA). The time \times breed interaction was also analyzed (two-way ANOVA) ($P > .05$, ns; $*P < .05$; $**P < .01$, $***P < .001$).

tion of cortisol by the primary adrenal cell cultures were determined using a RIA kit purchased from Sigma Chemical Company; [1,2,6,7-³H]cortisol was purchased from Amersham International (Buckinghamshire, UK).

Statistical Analysis. The *in vivo* results are presented as mean \pm SE and represent groups of at least seven experimental animals. The *in vitro* results are presented as mean \pm SE and represent groups of at least three experimental animals. The data were evaluated with analysis of variance procedures for repeated measures. A *P*-value smaller than .05 was considered as significant.

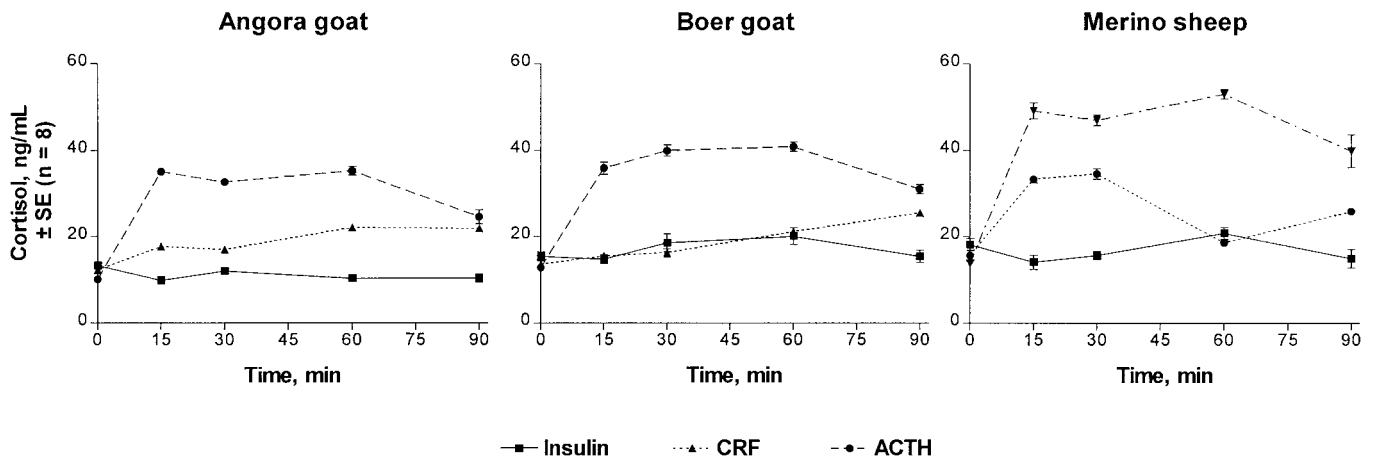
GraphPad Software Version 2 (San Diego, CA) was used to analyze the data. For the *in vivo* experiments: in Figure 1, the model used to analyze the data contained terms for time (means compared with Dunnett's test), breed (means compared with Bonferroni's), time \times breed (Model 1, two-way ANOVA), and residual, and, in Figure 2, it contained hormone treatment (Bonferroni's) and residual. For the *in vitro* experiments: in Figure 3, the model contained time (Dunnett's), breed (Bonferroni's), time \times breed (Model 1, two-way ANOVA), and residual; in Figure 4, it contained time (Dunnett's), time \times breed (Model 1, two-way ANOVA), and residual; and, in Figure 5, it contained hormone (Dunnett's) and residual.

Results

Insulin, CRF, and ACTH Stimulation Test (*In Vivo*).

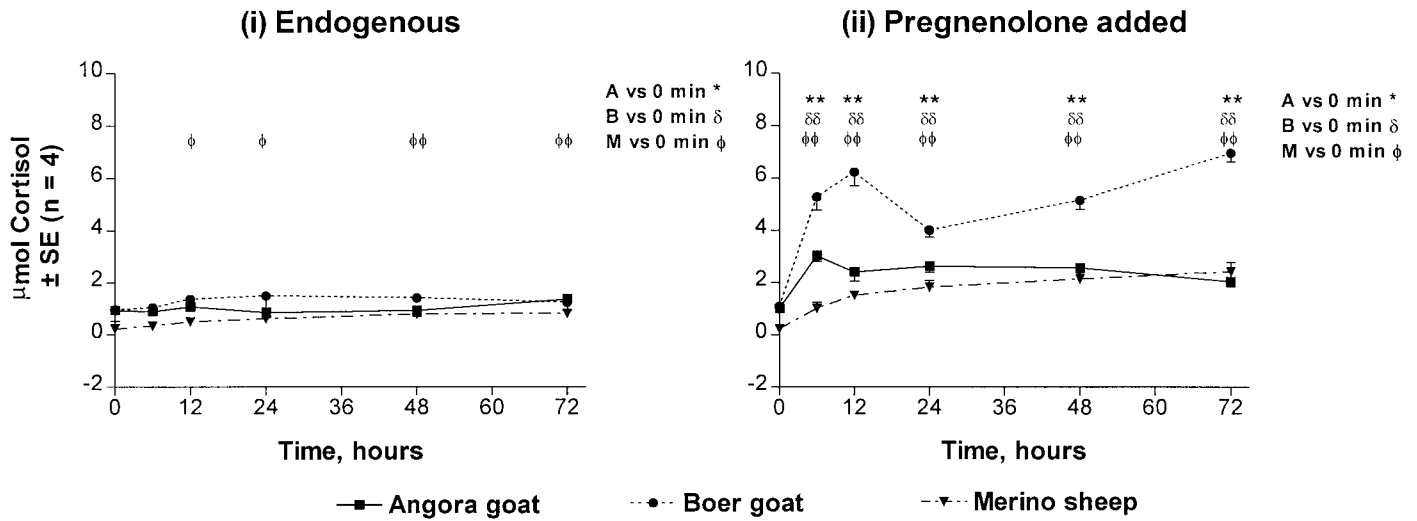
The effect of an intravenous injection of human insulin

on blood glucose levels in Angora goats, Boer goats, and Merino sheep is illustrated in Figure 1(i). The glucose concentrations in the experimental animals decreased on average from approximately $3.6 \pm .24$ (n = 8) mmol/L to $1.9 \pm .19$ mmol/L after 30 min. After 90 min, the blood glucose concentrations were again normal. The effect of this artificially induced stress on cortisol production is presented in Figure 1(ii). The maximum plasma cortisol concentration was 20.8 ± 3 ng/mL in the Merino sheep and 20.1 ± 4.8 ng/mL in the Boer goats after 60 min (n = 8). In contrast to these results, the Angora's maximum plasma cortisol concentration was only 10.5 ± 2.3 ng/mL. Figure 1(iii) illustrates the effect of an intravenous injection of sheep CRF on the blood cortisol concentration in the three species tested. In this experiment, the cortisol levels of the Merino increased from 15.7 ± 3.1 ng/mL to 34.6 ± 3.3 ng/mL after 30 min. Although both goat species did not respond so drastically, their cortisol concentrations increased gradually over the time period from 12.3 ± 1 and 13.6 ± 2.2 ng/mL to 21.9 ± 2.2 and 25.6 ± 2.2 ng/mL for Angora and Boer goats, respectively. Figure 1(iv) presents the adrenal response, in terms of cortisol production, after ACTH administration. Although the cortisol concentration in all three species increased in a similar way (*P* < .01, n = 7), the increase in the Merinos' cortisol concentration (47.1 ± 3.14 ng/mL) was the highest, followed by the Boer goats' (40 ± 3.14 ng/mL) and the Angora goats' (35.4 ± 2.9 ng/mL). The Merinos' response was significantly higher than that of the Angora and Boer goats. A comparison of the effect of insulin, CRF, and



Hormone effect within a breed:	Angora goat	Boer goat	Merino sheep
Insulin vs CRF:	ns	ns	ns
Insulin vs ACTH:	*	*	*
CRF vs ACTH:	ns	*	ns

Figure 2. The effect of an intravenous injection of insulin (.1 IU/kg), β -corticotropin-releasing factor (CRF, 1 μ g/kg), and ACTH (10 μ g/kg) on mean \pm SE plasma cortisol levels in the different species, as indicated. The data were analyzed with repeated measures ANOVA, comparing the stimulation of the different hormones in each species (*P* > .05, ns; **P* < .05; ***P* < .01; ****P* < .001).



Breed effect:	Endogenous	Pregnenolone added
A vs B	***	**
A vs M	***	ns
B vs M	***	***
Interaction between time and breed:		
Interaction	ns	***
Breed	***	***
Time	ns	***

Figure 3. The production of cortisol (mean ± SE) by Angora goat (A), Boer goat (B), and Merino sheep (M) adrenal cell cultures. The cells were incubated with (ii) or without (i) 100 µM pregnenolone (PREG). The results were analyzed by comparing cortisol levels for each species with that at 0 min and are indicated on the respective graphs (repeated measures ANOVA). The cortisol levels of each species were also compared with the response of the other animals (repeated measures ANOVA), and the interaction between time and breed were analyzed (two-way ANOVA) ($P > .05$, ns; * $P < .05$; ** $P < .01$; *** $P < .001$).

ACTH on cortisol release in the three species is given in Figure 2. In all the species, ACTH had the most pronounced effect on cortisol release, with no difference in the effect of CRF and insulin. In the Angora goats and Merino sheep, there was no difference in the effect of CRF and ACTH.

Incubation of Adrenal Cell Cultures with PREG, ACTH, Forskolin, and Cholera Toxin. Primary Angora goat, Boer goat, and Merino sheep adrenal cell cultures were incubated with or without (endogenous) 100 µM PREG. The production of cortisol was compared at regular intervals (Figure 3). The maximum endogenous cortisol production for Angora goats, Boer goats, and Merino sheep were $1.371 \pm .432$, $1.51 \pm .507$, and $.827 \pm .097$ µmol of cortisol, respectively. However, when PREG was added, the maximum production of cortisol for Angora goats was $3.05 \pm .24$ µmol ($P < .01$) and for Merino sheep was $2.43 \pm .34$ ($P < .01$). The Boer goats produced $6.97 \pm .35$ µmol cortisol ($P < .01$) after 72 h, which was higher ($P < .01$) than in the other two species. The influence of ACTH stimulation on cortisol produc-

tion was subsequently studied. This was achieved by comparing the cortisol production when PREG alone or PREG together with ACTH were added to the cell cultures (Figure 4). In the Angora goat adrenal cells, there was no difference in the production of cortisol with or without ACTH stimulation. After 24 h, the ACTH-stimulated Boer goat adrenal cells produced more ($P < .01$, $n = 4$) cortisol than the cells without ACTH, and the ACTH-stimulated Merino sheep adrenal cells produced more ($P < .05$, $n = 6$) cortisol after 24 h. In Figure 4b, the cortisol production under both these treatments is expressed as the percentage of endogenous cortisol production.

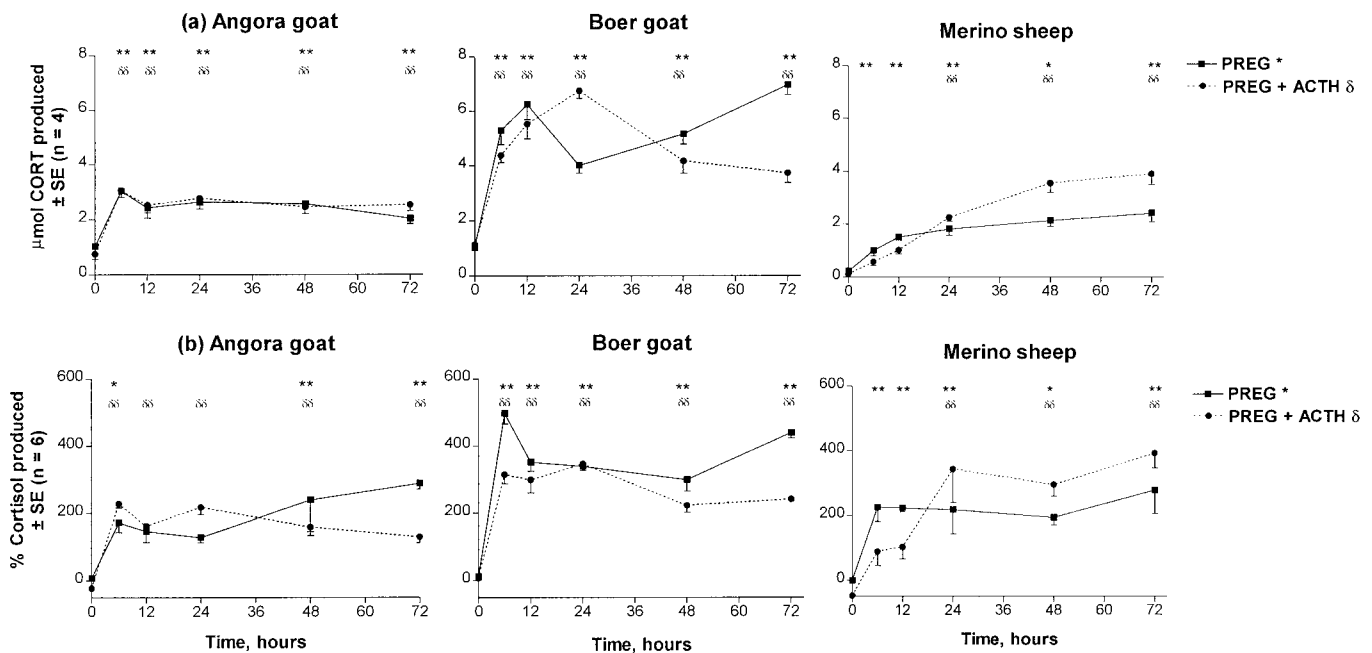
The differences in the effects of forskolin, cholera toxin, and ACTH on the stimulation of cortisol production are illustrated in Figure 5. For Angora goats, cholera toxin increased cortisol production more ($P < .01$, $n = 4$) than ACTH, with no difference ($P > .05$, $n = 4$) in the stimulating effects of ACTH and forskolin. There was no difference in the effect of the stimulants on the cortisol production for Boer goat adrenal cells. For

Merino sheep, forskolin and cholera toxin both increased ($P < .01$, $n = 4$) cortisol production to a greater extent than ACTH.

Discussion

The secretion of glucocorticoids by the adrenal cortex is a central feature of the stress response in mammals, and a functional hypothalamic-pituitary-adrenocortical axis is essential for glucose homeostasis (Gemma et al., 1994). Many of the stress-related problems of Angora goats are linked to the inability of the animal to maintain blood glucose levels under stress (Wentzel et al., 1974; Wentzel and Botha, 1976). Several studies confirmed that reduced adrenal function could be the cause of the Angora goat's susceptibility to minor stress conditions (Wentzel et al., 1979; Herselman and Pieterse, 1992). The role of thyroid malfunctioning in abortions

resulting from nutritional stress in pregnant Angora does was excluded by previous research (Judge et al., 1968). The response of the Angora's hypothalamic-pituitary-adrenocortical axis to artificially induced stress has received little attention. The intravenous administration of insulin caused blood glucose levels to drop in all three species, indicating that induction of stress by insulin administration was successful. When compared with the other experimental animals, Angora goats did not have increased cortisol after insulin stimulation. This lack of response to the drop encountered in blood glucose supported the view of Van Rensburg (1971) and Herselman (1990) of hypoadrenocorticism in high fiber producing Angora goats. The hypothalamic-pituitary-adrenocortical axis was subsequently investigated in more detail to further elucidate this phenomenon. The anterior pituitaries of the experimental animals were stimulated by CRF administration, and the blood corti-



Interaction between time and species:

	Angora goat			Boer goat			Merino sheep		
	Interaction	Hormone	Time	Interaction	Hormone	Time	Interaction	Hormone	Time
(a)	ns	ns	***	***	*	***	***	**	***
(b)	***	ns	***	***	***	***	*	ns	***

Figure 4. A comparison of the influence of substrate (100 μ M pregnenolone [PREG]) and 1 μ M ACTH on the production of cortisol (mean \pm SE) by Angora goat, Boer goat, and Merino sheep adrenal cell cultures respectively. In (a), the cortisol (CORT) production is expressed as micromoles produced, and in (b) the cortisol production is expressed as percentage of endogenous cortisol production. The results were statistically analyzed by comparing cortisol levels for each species with that at 0 min and are indicated on the respective graphs (repeated measures ANOVA). In the table, the interaction between time and species is given (two-way ANOVA) ($P > .05$, ns; $*P < .05$; $**P < .01$; $***P < .001$).

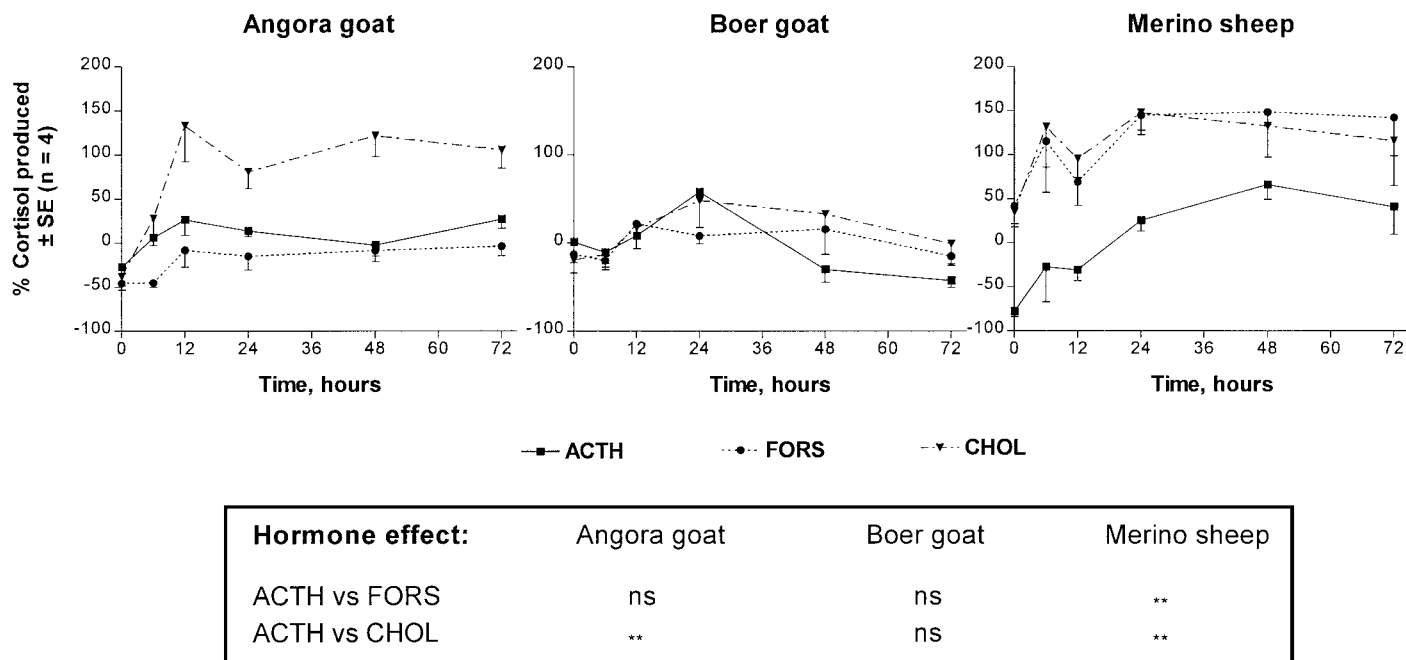


Figure 5. A comparison of the influence of 1 μM ACTH, 10 μM forskolin (FORS), and 1 $\mu\text{g}/\text{mL}$ cholera toxin (CHOL) on cortisol production (mean \pm SE) by Angora goat, Boer goat, and Merino sheep adrenal cell cultures. The cells were incubated with 100 μM pregnenolone (PREG) and stimulated with either ACTH, forskolin (FORS), or cholera toxin (CHOL), as indicated. A repeated measures ANOVA test was performed, comparing FORS and CHOL with ACTH ($P > .05$, ns; $*P < .05$; $**P < .01$; $***P < .001$).

sol concentrations were determined. Although the response of the Merino sheep was the most profound, the two goat species also responded with elevated cortisol levels. The *in vivo* experiments were concluded by investigation of the adrenal gland. Upon ACTH stimulation, cortisol production in all the experimental animals increased in a similar way. The Angoras' overall response was, however, significantly less than that of the Merino sheep. Although not significantly different, cortisol release in Angora goats was lower than that in Boer goats. Escobar et al. (1998) recently found a difference in the cortisol production of Angora and non-Angora goats. These differences were observed in the goats during anestrus and pregnancy, but only marginally so at midestrus. In addition, a chronic stimulation test for 7 d resulted in peak cortisol concentrations on the third day in both groups, but, despite continued ACTH treatment, only the non-Angora group exhibited a second peak. The animals used in the present study (*in vivo*) were only 6 mo of age, and a single stimulation test was performed. These factors could explain why, in the present study, no significant difference in response between the Angora and Boer goats upon ACTH stimulation was observed. The Angoras' overall response upon insulin stimulation was, however, significantly lower than that of the other experimental animals and confirms its inability to elevate cortisol levels upon stress.

Increased plasma levels of glucocorticoids are generally considered to be the classical response to stress

(Niezgoda et al., 1993), and, based on these findings, we decided to further investigate the regulation of the adrenal gland as a possible cause for the observed hypoadrenocorticism in Angora goats. Three compounds, ACTH, forskolin, and cholera toxin were used in these experiments. The hormone ACTH stimulates steroidogenesis by binding to the ACTH receptor on the outer membrane of the adrenal gland. This binding activates adenylate cyclase, which in turn, via cAMP, increases glucocorticoid production and secretion from the adrenal cortex. Forskolin, a natural herbal product, stimulates adenylate cyclase by direct binding to the enzyme (Seamon et al., 1981; Nelson and Seamon, 1985). A G-protein is necessary for the full expression of the effect of forskolin, and forskolin synergistically increases the stimulation of adenylate cyclase by hormone-receptor agonists (Juska and de Foresta, 1995). The toxin of *Vibrio cholerae*, cholera toxin, activates adenylate cyclase irreversibly by binding to the α -subunit of the G_s protein to maintain an active adenylate cyclase binding conformation (Gilman, 1987).

The viability of the primary adrenal cell cultures was confirmed by the addition of PREG as substrate. In all the experimental cultures, cortisol production was increased. The cortisol release from Boer goat adrenal cells was greater than from the other two species. The stimulation of cortisol synthesis by ACTH was studied by comparing its production when PREG alone and PREG together with ACTH were added to the cell cultures. It is well known that ACTH stimulates adrenal

steroidogenesis by increasing the amount of available cholesterol to P450_{scc}, the first enzyme in glucocorticoid biosynthesis (Jefcoate et al., 1992). It was, however, decided to use pregnenolone as a substrate in this study, because addition of cholesterol would not indicate if this first and rate-limiting step, cholesterol release from its esters, would be affected. In addition, the extreme insolubility of cholesterol severely complicates the use of this steroid as a substrate. Recent research has also clearly indicated that cytochrome P450_{c17} activity is mainly regulated by ACTH in a cAMP-dependent manner (Briere et al., 1997). In a parallel study to this one, we have shown that the activity of Angora cytochrome P450_{c17} differs considerably from that of Boer goats and Merino sheep (our unpublished observations). In the Angora goat adrenal cell cultures, ACTH did not stimulate cortisol production, but, for Boer goats and Merino sheep, stimulation was observed. There was no difference in the cortisol production of Boer goat adrenal cell cultures in the presence of ACTH, cholera toxin, and forskolin when compared with that of pregnenolone. This result can be attributed to the fact that the PREG concentration used was limiting in the case of the Boer goat cell cultures. In the Merino adrenal cells, both forskolin and cholera toxin stimulated cortisol production to the same extent. The greater stimulation of these two compounds, when compared with ACTH, can be attributed to the fact that they act directly on the G_s-protein complexes of all species, and their interactions are not limited by specific hormone-receptor interaction like ACTH.

In the Angora goat adrenal cell cultures, only cholera toxin, and not forskolin, stimulated cortisol production. Cholera toxin binds directly and irreversibly to the α -subunit of the G_s-protein complex. This interaction leads to a dissociation of the β - and γ -subunits, allowing adenylate cyclase binding and permanent activation. In contrast, forskolin can only exert maximum stimulation once the hormone receptor complex induced a dissociation of the β - and γ -subunits from the α -subunit of the G_s-protein complex. The results obtained with the primary adrenal cell cultures, therefore, indicate that the ACTH-receptor interaction in the Angora goat adrenal cell membrane cannot dissociate and, therefore, activate the G_s-protein complex and that the lower cortisol production by Angora adrenals could be attributed to this phenomenon.

Implications

This study confirms the observed reduced adrenal function as the cause of hypoadrenocorticism in South African Angora goats, on a molecular basis. Results obtained with established stimulators of G_s-protein complex function, forskolin and cholera toxin, indicate that the adrenocorticotrophic hormone receptor on the adrenocortical cell membrane of Angora goats cannot adequately stimulate the cAMP signaling mechanism

required for enhanced glucocorticoid production under stress.

Literature Cited

- Briere, N., D. Martel, M. Cloutuar, and J. G. Le Houx. 1997. Immunolocalization and biochemical determination of cytochrome P450 in adrenals of hamsters treated with ACTH. *J. Histochem. Cytochem.* 45:1409–1416.
- Buckingham, J. C. 1996. Stress and the neuroendocrine-immune axis: The pivotal role of glucocorticoids and lipocortin. *Br. J. Pharmacol.* 1(118):1–19.
- Cobb, V. J., B. C. Williams, J. I. Mason, and S. W. Walker. 1996. Forskolin treatment directs steroid production towards the androgen pathway in the NCI-H295R adrenocortical tumour cell line. *Endocr. Res.* 22:545–550.
- Dessauer, C. W., T. T. Scully, and A. G. Gilman. 1997. Interactions of forskolin and ATP with the cytosolic domains of mammalian adenylate cyclase. *J. Biol. Chem.* 272:22272–22277.
- Escobar, C. J., P. K. Basrur, C. Gartley, and R. M. Lipton. 1998. A comparison of the adrenal cortical response to ACTH stimulation in Angora and non-Angora goats. *Vet. Res. Commun.* 22:119–129.
- Funkenstein, B., J. L. McCarthy, K. M. Dus, E. R. Simpson, and M. R. Waterman. 1983. Effect of adrenocorticotropin on steroid 21-hydroxylase synthesis and activity in cultured bovine adrenocortical cells. *J. Biol. Chem.* 258:9398–20608.
- Gemma, C., A. De Luigi, and M. G. De Simoni. 1994. Permissive role of glucocorticoids on interleukin-1 activation of the hypothalamic serotonergic system. *Brain Res.* 651:169–173.
- Gilman, A. G. 1987. G proteins: Transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56:615–649.
- Herselman, M. J. 1990. The energy requirements of Angora goats. M.S. Thesis. University of Stellenbosch, Stellenbosch, South Africa.
- Herselman, M. J., and D.M.E. Pieterse. 1992. The effect of cortisol treatment on Angora goats. *Proc. 31st Congress S.A. Society of Animal Production, South Africa.*
- Jefcoate, C. R., B. C. McNamara, I. Artemenko, and T. Yamazaki. 1992. Regulation of cholesterol movement to mitochondrial cytochrome P450_{scc} in steroid hormone synthesis. *J. Steroid Biochem. Mol. Biol.* 43:751–767.
- Judge, M. D., E. J. Brisley, R. G. Cassens, J. C. Forrest, and R. K. Meyer. 1968. Adrenal and thyroid function in stress susceptible pigs (*Sus domesticus*). *Am. J. Physiol.* 214:146–151.
- Juska, A., and B. de Foresta. 1995. Analysis of effects of corticotrophin, forskolin and fluoride on activity of adenylate cyclase of bovine adrenal cortex. *Biochim. Biophys. Acta* 1236:289–298.
- Munch, A. 1971. Glucocorticoid inhibition of glucose uptake by peripheral tissue: Old and new evidence, molecular mechanisms, and physiological significance. *Perspect. Biol. Med.* 14:265.
- Nelson, C. A., and K. B. Seamon. 1985. Regulation of [³H]forskolin binding to human platelet membranes by GppNHp, NaF, and prostaglandin E₁. *FEBS Lett.* 183:349–352.
- Niezgoda, J., S. Bobek, D. Wronska-Fortuna, and E. Wierzechos. 1993. Response of sympatho-adrenal axis and adrenal cortex to short-term restraint stress in sheep. *J. Vet. Med.* 40:631–638.
- Purvis, J. L., J. A. Canick, J. I. Mason, R. W. Estabrook, and J. L. McCarthy. 1973. Lifetime of adrenal cytochrome P-450 as influenced by ACTH. *Ann. N. Y. Acad. Sci.* 212:319–343.
- Ransjo, M., A. Lie, E. I. Mackie, I. Feyen, and U. H. Lerner. 1995. The adenylate cyclase stimulators forskolin and cholera toxin stimulate formation of osteoclast-like cells in mouse marrow cultures and cultured mouse calvariae. *Bone Miner. Res.* 10:S220 (Abstr.).
- Scarceriaux, V., P. Pelaprat, P. Forgez, A. M. Lhiaubet, and W. Rostene. 1995. Effects of dexamethasone and forskolin on neurotensin production in rat hypothalamic cultures. *Endocrinology* 136:2554–2560.
- Seamon, K. B., W. Padgett, and J. W. Daly. 1981. Forskolin: Unique diterpene activator of adenylate cyclase in membranes and in intact cells. *Proc. Natl. Acad. Sci. USA* 78:3363–3367.

- Tangalakis, K., F. E. Roberts, and E. M. Wintour. 1992. The time-course of ACTH stimulation of cortisol synthesis by the immature ovine foetal adrenal gland. *J. Steroid Biochem. Mol. Biol.* 42:527–532.
- Tuckey, R. C., and K. J. Cameron. 1993. Side-chain specificities of human and bovine cytochrome P-450_{SCC}. *Eur. J. Biochem.* 217:209–215.
- Vallerand, A. L., J. Zamecnik, and I. Jacobs. 1995. Plasma glucose turnover during cold stress in humans. *J. Appl. Physiol.* 78:1296–1302.
- Van Rensburg, S. J. 1971. Reproductive physiology and endocrinology of normal and aborting Angora goats. *Onderstepoort J. Vet. Res.* 38:1.
- Wentzel, D., and L.J.J. Botha. 1976. Thyroid function in nutritionally-stressed pregnant Angora goat does. *Agroanimalia* 8:163–164.
- Wentzel, D., J. C. Morgenthal, C. H. Van Niekerk, and C. S. Roelofse. 1974. The habitually aborting Angora doe II. Effect of an energy deficiency on the incidence of abortion. *Agroanimalia* 8:59.
- Wentzel, D., K. S. Viljoen, and L.J.J. Botha. 1979. Physiological and endocrinological reactions to cold stress in the Angora goat. *Agroanimalia* 11:19.
- Williams, B. C., E.R.T. Lightly, A. R. Ross, I. M. Bird, and S. W. Walker. 1989. Characterization of the steroidogenic responsiveness and ultrastructure of purified zona fasciculata/reticularis cells from bovine adrenal cortex before and after primary culture. *J. Endocrinol.* 121:317–324.