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The reduction of cell division rate in wool follicles by noradrenaline

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ABSTRACT

Local sympathectomy was achieved by injecting 6-hydroxydopamine into the dermis at two sites on the right midside of each of three Merino wethers. Saline was injected into matching sites on the left midside. After one week, noradrenaline was injected into one site on each side and saline into the other. Colchicine was administered into all sites immediately after noradrenaline, skin biopsies removed from each site two hours later and histological preparations assessed for the accumulation of mitoses in wool follicles.

Compared with saline treated sites, noradrenaline significantly depressed the number of accumulated mitoses at control and sympathectomised sites by 34 and 36% respectively ($P = 0.002$). A pharmacological dose of noradrenaline therefore has the potential to reduce wool production. Sympathectomy increased mitotic rate at saline and noradrenaline treated sites by 16 and 18% respectively, although this increase was not significant at the 5% level ($P = 0.094$). Elevation of mitotic activity via elimination of endogenous sources of noradrenaline may help to explain the mechanism by which surgical sympathectomy has previously been reported to increase wool production.

Keywords Cell division, colchicine, sheep, noradrenaline, mitotic rate, wool follicles.

INTRODUCTION

Unilateral sympathectomy has been shown to increase wool production, primarily by abolition of the seasonal rhythm (Ferguson, 1949; Williams and Willis, 1987). Changes in the relative rates of length growth to diameter have frequently been observed under the influence of cold (Doney and Griffiths, 1967; Slee and Ryder, 1967; Downes and Hutchinson, 1969; Jolly and Lyne, 1970; Lyne *et al.*, 1970) and sympathectomy (Folde and Maxwell, 1981). However, the influence of catecholamines from the adrenal medulla is unclear with little or no effect in some cases (Ferguson *et al.*, 1965) and a positive correlation between blood catecholamine levels and wool growth in others (Reklewska, 1975).

It has been concluded that the reduction in wool growth is an indirect effect of vasoconstriction and suppression of blood flow to the follicles (Ferguson, 1949; Wallace, 1979). Alternatively, Cunningham *et al.*, (1979) have suggested that noradrenaline acts directly on wool follicles through extravascular sites, since pure vasoconstricting agents had little effect on uptake of [³⁵S] cysteine into the fibre. The shortest periods of wool growth measurement used in the experiments outlined above were four days (Cunningham *et al.*, 1979). Given the short half-life of catecholamines in the sheep (Pearson, 1979), it became of interest to determine whether the effects on wool growth might occur within relatively short periods of time. The following experiment was designed to determine whether exogenous and locally produced noradrenaline can influence cell division in wool follicles over a period of two hours, using a modification of the mitotic arrest technique of Hynd *et al.*, (1986).

Alexander and Stevens (1980) treated foetal lambs with 6-hydroxydopamine (6-OH-dopamine) to produce sympathectomised new born animals. Dopamine is a precursor noradrenaline, and is rapidly taken up into nerve terminals. The extra hydroxyl group of 6-OH-dopamine creates instability within the molecule, and decomposition causes the degeneration of the

nerve terminals in which it is concentrated by the uptake process (Thoenen and Tranzer, 1973). During the present experiment small doses of 6-OH-dopamine were injected into the skin of sheep in an attempt to establish local sympathectomy and provide an alternative to surgical methods.

MATERIALS AND METHODS

All chemicals were obtained from the Sigma Chemical Co, St Louis, Missouri, USA. Solutions of noradrenaline and 6-OH-dopamine were prepared immediately prior to use, and were dissolved in 0.9% NaCl, which served as the control injection for both. Colchicine, 6-OH-dopamine and saline were administered by gently inserting a 27-gauge hypodermic needle into the dermis and slowly injecting the solution. The concentrations of the solutions were 1 mg/ml for 6-OH dopamine, and 0.5 mg/ml for both noradrenaline and colchicine.

On day 1, 6-OH-dopamine (1 mg) was injected into two sites on the right midside of three Merino wethers (50 kg) of the South Australian strongwool strain, maintained indoors in individual pens and fed 800g of a commercial pelleted ration per day. Two sites in a similar position on the left midside were injected with an equal volume (1 ml) of the vehicle. The sites of injection were marked with a felt tip pen.

On day 7, noradrenaline (50 µg) was injected into one site on each midside, and the vehicle (0.1 ml) into the other. Immediately following the injection of noradrenaline and saline, colchicine (50 µg) was injected into all four sites. Two hours later skin biopsies were collected and treated as per the method of Hynd *et al.*, (1986).

The average number of mitoses accumulated per random follicle bulb section (10 µm) was determined for at least two hundred bulbs for each site. Analysis of variance was carried out using GENSTAT 5.

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RESULTS

The average number of mitoses per follicle bulb section accumulated during two hours of mitotic arrest at each site is displayed in Table 1. The overall average for each treatment is given in the last line of Table 1.

Treatment with noradrenaline during the period of colchicine arrest resulted in a significant reduction in the accumulation of mitoses per follicle ($P = 0.002$). Pre-treatment with 6-OH-dopamine on day 1 led to a slight increase in the number of mitoses accumulated during two hours of colchicine arrest on day 7 ($P = 0.094$). The response to 6-OH-dopamine was variable, which is most clearly seen using sheep number three as an example. Unlike the other two animals, sheep three exhibited no evidence of a response between sites treated with saline on day 7, yet the sympathectomised site showed an elevated number of cells accumulated in comparison with the noradrenaline treated site on day 7.

TABLE 1 Average number of colchicine accumulated mitoses per random follicle bulb section in skin biopsies collected one week after treatment with saline or a chemical sympathectomising agent (6-OH-dopamine) and two hours after treatment with noradrenaline or saline (cells/bulb section/2 hours)

Day 1	Treatment			
	6-OH-dopamine	Saline	6-OH-dopamine	Saline
Day 7	Saline	Saline	Noradrenaline	Noradrenaline
Sheep 1	5.46	4.62	3.95	3.40
Sheep 2	5.60	4.16	3.03	3.35
Sheep 3	6.15	6.15	4.36	2.78
Average	5.77	4.98	3.78	3.18

DISCUSSION

Clearly, sites treated with noradrenaline over the period of mitotic arrest exhibited a decline in cell proliferation with respect to saline treated sites. Since mitotic rate per follicle is strongly correlated with wool growth rate (Hynd *et al.*, 1986) the evidence presented here suggests that this hormone has the potential to reduce wool production as shown by earlier work (Cunningham *et al.*, 1979). The fact that this response can occur within two hours was previously unknown, and given the rate at which noradrenaline is released and degraded it would be interesting to determine how long mitosis would remain suppressed after its removal. Local chemical sympathectomy appears to have exerted a variable effect, and on average the accumulation of mitoses was increased by 16 and 18% at saline and noradrenaline treated sites respectively. Noradrenaline secreted by nerve terminals may therefore be sufficient to produce a reduction in mitotic rate and therefore wool growth. Thus the results pre-

sented here for mitotic arrest concur with evidence obtained for wool growth following surgical sympathectomy (Ferguson, 1949; Williams and Willis, 1987; Foldes and Maxwell, 1981).

Noradrenaline from an exogenous source clearly has the potential to rapidly reduce cell division, yet the relationship between the dose used and normal physiological levels is unknown. Further experiments using both surgical and chemical sympathectomy to abolish endogenous release are currently being analysed and will be reported elsewhere.

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