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Role of Steroids in Antler Growth of Red Deer Stags

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ABSTRACT A series of six studies were carried out in red deer stags to test hypotheses concerning the importance of steroid control of velvet antler growth and to investigate mechanisms by which these hormones exert their effects. Medroxyprogesterone acetate (MPA) an LH inhibitor administered to stags during hard antler caused premature antler casting, reduced subsequent antler weight and caused a reduction in the LH and testosterone responses to GnRH. In two separate studies blockade of testosterone receptors with cyproterone acetate (CPA) administered to stags, either during early velvet antler growth or during the hard antler stage, significantly reduced LH and testosterone responses to GnRH. In both studies antler length, but not weight, was increased by CPA treatment. In another study testosterone implants were used to prevent the gradual decline in plasma testosterone levels normally observed during winter. Implants were removed 3 weeks before the anticipated date of antler casting. The implants significantly increased plasma testosterone levels and subsequent antler growth (expressed as a proportional increase compared with the previous year) compared with untreated controls. To determine whether the annual cycle of plasma testosterone response following GnRH stimulation was due simply to a lack of LH stimulation, ovine LH was injected on six occasions at defined stages of the antler cycle to red deer stags and the testosterone response measured. The testosterone responses were low at antler casting and during velvet antler growth compared with antler cleaning and peak rut. It appears low testosterone levels are due, in part, to a loss of responsiveness by the testes to LH as well as a low level of secretion of LH during the antler growing season. Finally synthetic ACTH was injected at the same defined stages of antler growth as in the previous study to determine whether cortisol and adrenal androgen production altered with the stage of the antler cycle. No significant differences were found in the dehydroepiandrosterone (DHEA) response, but cortisol responses were higher from late velvet antler growth to peak rut, compared with the times of antler casting and early velvet growth. Overall it was concluded that velvet antler growth can occur without testosterone stimulation during the period of velvet growth, but the data reinforce the concept that the timing of antler growth is linked to the annual cycle of testosterone.

Antlers are cranial organs of bone which are grown and lost (cast) annually by male deer. In temperature species this cycle is strongly seasonal. In red deer, hard antlers are cast in late winter and immediately regrow in spring and summer; during this time they are soft, covered with a hairy skin, and are described as velvet antlers. In late summer, when velvet antler growth is complete, the blood supply to the antler stops, the velvet dies, and peels off (cleans) to reveal hard dead bone which is carried throughout autumn and winter (Bubenik, '66; Chapman, '75; Goss, '83; Bubenik and Bubenik, '90). The timing of the antler cycle is linked to the annual reproductive rhythm of plasma levels of gonadal androgens, particularly testosterone, such that antler casting and velvet antler growth occur when plasma levels are either undetectable or very low. When testosterone is increasing antler cleaning occurs and testosterone levels are high when the stags carry hard antler (Wislocki et al., '47; Lincoln et al., '70; Suttie et al., '84). The effects of testosterone on the antler tissues are believed to be due to peripheral aromatisation to estradiol or reduction to 5-α-dihydrotestosterone (5αDHT) (Morris and Bubenik, '83; Fennessy and Suttie, '85).

The precise mechanisms of action of testosterone during antler growth have not been investigated although medroxyprogesterone acetate

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(MPA), a potent inhibitor of LH and thus testosterone release (Muir et al., '82), and an androgen receptor blocker, cyproterone acetate (CPA) (Bubenik et al., '75a, '87), have been used to study the steroid regulation of the timing of the antler cycle. Although testosterone appears to be inimical for velvet antler growth, high levels of testosterone in stags in hard antler could act in a priming role by preparing cells for subsequent growth factor stimulation and thereby increase subsequent antler size (Suttie and Fennessy, '90), but this has not been investigated in adult stags.

Intravenous injections of gonadotrophin releasing hormone (GnRH) have been used to study the responsiveness of the pituitary gland in terms of LH secretion and hence testicular testosterone release during the antler cycle (Lincoln and Kay, '79; Suttie et al., '84; Fennessy et al., '88). This approach has shown that the pituitary of the deer is largely insensitive to GnRH during early velvet antler growth. Testosterone release is also insignificant during early velvet antler growth, but whether this is simply due to the lack of LH stimulation or whether the testes are also insensitive to LH during this time is unknown.

Gonadal androgens have been well studied during the antler cycle but the role of adrenal androgens is not known. The adrenal androgen secreted in the highest quantities in humans is dehydroepiandrosterone (DHEA) (Ducharme et al., '76), which can be metabolised to 5αDHT. DHEA release from the adrenal can be stimulated by adrenocorticotrophic hormone (ACTH) (Rosenfeld et al., '75).

We report here six studies designed to test hypotheses concerning the importance of steroid control of velvet antler growth and to investigate the mechanisms by which these hormones exert their effects.

MATERIALS AND METHODS

Experiment 1

The effects of MPA on LH, testosterone, and antler growth were investigated to determine whether antler casting could be stimulated to occur out of season, whether this would be accompanied by a reduction in reproductive hormone secretion and whether subsequent velvet antler growth would be reduced.

Animals and treatment

Eight adult red deer stags, which had previously been used for the study of seasonal responses to GnRH (Fennessy et al., '88), were randomly allocated to receive either a single intramuscular injection of 150 mg MPA (Deprovera, Upjohn Co., Ltd., Kalamazoo, MI) (n=4) or no treatment (n=4), on June 14. This is early winter in the Southern Hemisphere and the stags were in hard antler. The stags were kept indoors under natural lighting conditions and fed a pelleted concentrate diet ad libitum.

The stags were all sampled on five occasions either at defined times of the antler cycle or in relation to the timing of MPA treatments. The MPA treated stags were sampled 1) immediately prior to treatment, 2) 14 days later, 3) the day the previous hard antlers were cast, 4) 65 days after antler casting, at which time the velvet antler was removed, and 5) at the same time as the control stags had velvet antler removed (i.e., 65 days after controls had cast their antlers). The control stags were sampled 1) 14 days after the MPA treatment began, 2) the day the MPA treated stags cast their antlers, 3) the day their own antlers were cast, 4) the day the MPA treated stags had velvet antlers removed, and 5) the day they had their own velvet antlers removed, 65 days after antler casting. Stags from each group were paired so that each day a stag in one group required to be sampled because it had reached a defined point in its sampling regimen, the corresponding animal in the other group also was sampled.

On each sampling day the stags were injected intravenously with 10 μg GnRH (Sigma Chemical Co., St Louis, MO). Individual doses were made from one batch of GnRH; the doses were stored at –20°C until required. They were reconstituted in 5 ml sterile saline 1 hr before injection. Blood samples were taken immediately before the GnRH injection and at 10, 40, 75, and 120 min after GnRH (Fennessy et al., '88). The GnRH was administered at around 9:00 am on each occasion.

The velvet antlers were weighed after removal, using standard humane procedures.

Blood sampling

On each sampling occasion the stags were sedated with intramuscular xylazine hydrochloride (Rompun, Bayer [NZ], Ltd. Petone, New Zealand; 100 mg per stag). Stags became recumbent within 15 min and remained so throughout the blood sampling period. Recovery from sedation was uneventful. Blood samples (10 ml) were withdrawn from the jugular vein using evacuated pre-heparinised tubes. Samples were centrifuged and the

plasma removed and stored at –20°C until required for analysis.

Experiment 2

The effect of CPA on LH, testosterone, and antler growth was investigated to determine whether androgen receptor blockade influenced hormone secretion and velvet antler growth in red deer stags, when administered during the actual period of antler growth.

Animals and treatments

Nine 2-year-old red deer stags were randomly allocated to treatment with either weekly injections of 150 mg CPA (n=3), 350 mg CPA (n=3), or the vehicle solution only (n=3) for a 5 week period after antler casting. The CPA, which was made up in a mixture of castor oil, benzyl benzoate, and propandiol (5:1:3), was delivered as a 7 ml intramuscular injection in the hind limb. The stags were sedated with xylazine hydrochloride as for Experiment 1 for each injection. The stags were fed a pelleted concentrate diet ad libitum indoors on a manipulated photoperiod such that successive 2 month periods of 16 hr of light followed by 8 hr of dark (16L:8D) were followed by 2 months of 8L:16D (Suttie et al., '84). The purpose of this photoperiod regimen was to induce 3 cycles of antler growth each calendar year, thus increasing the number of velvet antler growth cycles for study.

Blood sampling and measurements

On six occasions, at four weekly intervals from the onset of CPA treatment, the stags were sedated with xylazine hydrochloride and 10 μg GnRH was injected into the jugular vein. Blood samples were withdrawn from the jugular vein prior to and 10 and 60 min after the GnRH.

Antler status (whether hard or velvet antler) was recorded weekly and when the antlers were clean of velvet they were removed, measured, and weighed. Testis diameter was measured with Vernier callipers at the time of antler removal.

Experiment 3

The effect of CPA on LH, testosterone and antler growth was investigated to determine whether androgen receptor blockade induced hard antler casting and influenced subsequent velvet antler growth, if administration began when the stags were in hard antler prior to antler casting.

Animals and treatments

Nine 3-year-old red deer stags were randomly allocated to treatment. Six of the stags had been used in Experiment 2 (the previous antler cycle) and treatments were re-randomised so that no animal received the same treatment in both experiments and all previous treatments were equally represented. The stags received weekly intra-muscular injections of either 150 mg CPA (n=3), 350 mg CPA (n=3), or the vehicle solution only (n=3) from 2 weeks after the previous hard antlers were clean of velvet until the CPA treated stags had cast their antlers. The CPA solution was made up and injected in the same way as described for Experiment 2. The stags were fed and maintained under the same photoperiod regimen as in Experiment 2.

Blood sampling and measurements

On seven occasions at 3 weekly intervals from the commencement of CPA treatment the stags were sedated with xylazine hydrochloride and 10 μg GnRH was injected into the jugular vein. Blood samples were withdrawn from the jugular vein prior to and 10 and 60 min after the GnRH.

Experiment 4

From the time of the breeding season in autumn until antler casting in the late winter testosterone levels of male red deer gradually decrease to very low levels. In this experiment subcutaneous silastic testosterone implants were used to maintain high plasma testosterone levels to study their effects on LH and testosterone basal levels and responses to GnRH and LH as well as the effects on subsequent velvet antler growth.

Animals and treatment

Seven 4-year-old red deer stags, which had not previously been used for endocrine studies, were randomly allocated to receive either testosterone treatment (n=4) or no treatment (n=3) for an 8 week period in mid-winter. Treatment ceased 3 weeks prior to the anticipated date (from previous records) of antler casting. The testosterone treatments consisted of eight 30 cm silastic tubes (Dow Corning, MI) of outer diameter 0.46 cm and internal diameter 0.34 cm packed with crystalline testosterone (Sigma Chemical Co., St. Louis, MO). These were implanted subcutaneously in the groin region of each stag under xylazine sedation with local anaesthesia (Xylocaine, Asta Pharmaceuticals, Ltd., NZ). Control stags received no im-

plants. The implants were removed under xylazine and local anaesthesia at the close of treatment. The stags tolerated the implants well and healing and treatment were without incident.

Blood sampling and measurement

One week after the start of testosterone treatment all stags were injected intravenously with 10 μg GnRH under xylazine sedation and blood samples were taken at 45, 30, 15, and 0 min before injection and 10, 40, and 75 min after injection. Three days later blood sampling was repeated except that 100 μg ovine LH (NIH-LH-S19) made up in sterile saline was injected and the same blood sampling regimen was followed. One week before testosterone treatment ended the above GnRH and LH treatments were repeated but with the ovine LH given 3 days before the GnRH. Antler casting date was recorded and velvet antler weight was measured after its removal 65 days after antler casting.

Experiment 5

Stags were injected with ovine LH on six occasions during the annual antler cycle to determine whether the low testosterone responses detected previously following GnRH administration during velvet growth were simply a consequence of low LH responses, or whether they also were partly a reflection of low testicular responsiveness.

Animals, treatment, and sampling

Three 3-year-old red deer stags were injected intravenously with 100 μg ovine LH (NIH-LH-S19) under xylazine sedation 1) at antler casting, 2) after 40 days of velvet antler growth, 3) after 65 days of velvet antler growth, 4) when the velvet antler was completely grown but not yet cleaned of velvet, 5) at antler cleaning, and 6) during peak rut. Blood samples were taken before ovine LH injection and 10, 40, 75, and 120 min after injection.

Experiment 6

Stags were injected with ACTH on six occasions to determine whether adrenal androgen responses varied with the stage of antler cycle. Cortisol was also measured as an indicator of the effectiveness of the ACTH injection, in inducing corticoid secretion.

Animals, treatment, and sampling

Three 3-year-old red deer stags were injected intramuscularly with 75 I.U. ACTH (Synacten, Ciba Geigy) under xylazine sedation at the same stages of the antler cycle as in Experiment 5. Blood samples were taken prior to and 30, 60, 90, and 120 min after ACTH injection.

Hormone analysis

LH and testosterone were measured by radioimmunoassay as previously described (Fennessy et al., '88). The inter- and intra-assay coefficients of variation for the LH determinations were 7.2%, 8.8%, and 17.0% and 3.5%, 3.9%, and 5.5% for plasma pools analysed in all assays and which contained 3.35 ± 0.24 (mean ± s.d.), 1.38 ± 0.12, and 0.46 ± 0.08 ng/ml, respectively. Mean assay sensitivity was 0.08 ng/ml (n=6). For the testosterone assays the inter- and intra-assay coefficients of variation were 10.7%, 15%, and 25% and 7.7%, 14.6%, and 26.3% for pools analysed in all assays which contained 6.43 ± 0.69, 3.19 ± 0.48, and 0.84 ± 0.21 ng/ml, respectively. Mean assay sensitivity was 0.06 ng/ml (n=20). Cortisol was measured by ELISA (Lewis et al., '92). The intra-assay coefficient of variation was 13%, 4.4%, and 3.4% for pools containing 108 ± 14, 446 ± 28, and 804 ± 27 nmol/L, respectively. Sensitivity was 55 nmol/L. All cortisol values were measured in the same assay.

DHEA was measured by RIA as follows. A 200 μl plasma aliquot was extracted with 3 ml ether: hexane 4:1 v/v. The dried extracts were reconstituted with 1.0 ml phosphate buffered saline containing 0.1% gelatin; 100 μl portions of ether extract or standards were added to tubes together with 100 μl [1,2,6,7,tritiated DHEA (Amersham UK, Ltd.)] approximately 20,000 dpm/tube and 100 μl of rabbit anti DHEA (M Ralph, University of Adelaide). Following overnight incubation at 4°C separation was by dextran-coated charcoal and the resulting supernatant, after centrifugation, was decanted into toluene based scintillant for counting. The sensitivity of the assay is 1 nmol/L (n=10 assays). The within and between assay coefficients of variation for pools containing 2.1, 3.9, 8.8, and 19.0 nmol/L were 9.5%, 5.1%, 4.5%, and 10.5% and 33.3%, 10.3%, 9.1%, and 12.1%, respectively.

Biometrics

The hormonal responses to the GnRH, LH, and ACTH injections have been expressed as the areas under the curves, calculated as follows: for each stag at each sampling day the initial concentration was taken as the baseline (mean of initial pre-injection samples in Experiment 6) and then extrapolated through to the last sample on

each day; the hormone concentrations for the remaining samples were then joined and the area under the curve above the extrapolated baseline derived.

ANOVA was used to test the effects of treatment, time and stage of antler cycle as appropriate for hormonal responses. Interactions between treatment and time/stage of antler cycle were also examined. In Experiment 4 there were no significant differences due to time and therefore data following both LH injections and both GnRH injections were pooled separately within each hormone for analysis.

RESULTS

Experiment 1: MPA effects on LH, testosterone, and velvet antler growth

The MPA treated stags cast their hard antlers 21 ± 3.8 days ($\bar{x}$ ± standard deviation) after treatment began, on July 5, while the control stags cast their antlers on September 17 ± 9.3 days ($\bar{x}$ ± standard deviation). Fourteen days after MPA treatment, plasma levels of both LH and testosterone in response to exogenous GnRH were very low (Fig. 1) and remained low for the remainder of the study. In contrast the control stags always had an LH response to GnRH, which was similar in magnitude to that shown by the MPA stags before treatment (Table 1). After antler casting the control stags had minimal testosterone responses (Fig. 1). LH responses were significantly lowered by MPA compared both with control stags and with pre-treatment values for the treated stags (Table 1). Testosterone responses were also lower in the MPA treated stags than in the controls prior to control antler casting and also after the controls had reached 65 days of velvet antler growth (Table 1). There were no significant differences in LH or testosterone (S.E.D. 613 and 298, respectively) between the control stags 14 days post MPA treatment and the MPA treated stags pre-treatment. LH, but not testosterone, was significantly higher ($P<0.01$) at antler casting in the control stags compared with the MPA treated stags (S.E.D. 148 and 16, respectively) despite the fact mean casting was 2 months apart. LH but not testosterone was significantly higher ($P<0.05$) 65 days after antler casting in the control stags compared with the MPA treated stags (S.E.D. 321 and 10, respectively). The velvet antlers of the control stags weighed 1,049 ± 84 g, whereas those of the MPA treated stags weighed 640 ± 51 g ($P<0.01$). The MPA stags all grew a second set of velvet antlers from the cut stumps and these grew to a mean weight of 1,214 ± 370 g in February, but none of the control stags regenerated an antler from the cut surface. In March all animals had normal clean hard antlers.

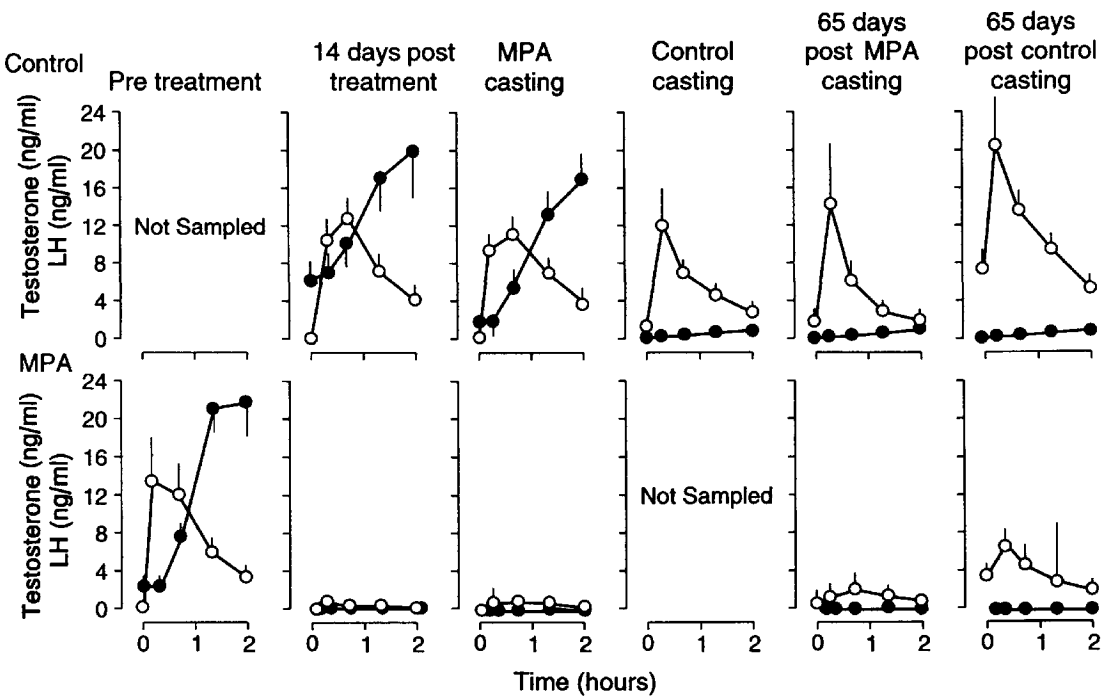

Fig. 1. Mean LH (○) and testosterone (●) responses to a single injection of GnRH in control or MPA treated stags at defined stages of the experiment.

TABLE 1. Experiment 1: Total LH and testosterone (areas under the curves over 2 h) in response to GnRH[1]

	LH			Testosterone		
	Control	MPA	S.E.D.	Control	MPA	S.E.D.
Pre MPA	ND	958		ND	1,625	
S.E.D.		(250)**			(286)***	
14d post MPA	971	144	165***	1615	107	543**
S.E.D.		(219)*	(42)*	(584)*	(27)*	
MPA casting	930	107	240***	1150	56	325***
S.E.D.	(143)*			(106)****		
Control casting	738	ND		56	ND	
S.E.D.	(285)*			(8)*		
MPA casting + 65 d	1,177	174	375**	44	42	12*
S.E.D.	(485)*	(114)**		(6)*	(10)***	
Control casting + 65 d	1,352	456	335**	37	10	9**

[1]Data are the means from four stags per treatment. The S.E.D. (standard error of the difference) columns refer to the comparison between the control and medroxyprogesterone acetate (MPA) treatments. The S.E.D. in parentheses refer to the comparisons between the sampling times they are positioned between within the same treatment.
N.D. indicates sampling not done at that time.
*Indicates no significant differences ($P>0.05$).
**$P<0.05$.
***$P<0.01$.
****$P<0.001$.

Experiment 2: The effect of CPA treatment on LH and testosterone during velvet antler growth

CPA significantly reduced plasma LH responses during and 3 weeks after treatment (i.e., at 4 and 8 weeks after the start) (Table 2). It also reduced the testosterone response significantly 3 weeks after treatment ceased (i.e., 8 weeks after the start) (Table 2). There was no dose dependent effect of CPA on either hormone.

There were no significant effects of CPA treatment on antler weight or peak testis diameter at antler cleaning (Table 3), but duration of antler growth and antler length were significantly longer in the CPA treated groups.

Experiment 3: The effects of CPA treatment used to induce antler casting on LH, testosterone and subsequent velvet antler growth

Treatment with CPA significantly advanced antler casting (Table 4) and it significantly lengthened the duration of antler growth (the time from casting to cleaning of the next antler). The CPA_{350} group had significantly longer antlers than either the control or the CPA_{150} groups, but there was a trend that these longer antlers were lighter. CPA treatment reduced the LH responses to GnRH significantly at the samplings around the time of previous antler casting (Table 5). CPA reduced significantly the testosterone responses at antler

TABLE 2. Experiment 2: Total plasma LH and testosterone responses (areas under the curves; mean of three stags per group) following a single GnRH challenge at four weekly intervals in control or cyproterone acetate (CPA) treated stags[1]

	LH				Testosterone			
Time (weeks)	Control	$CPA^{(150\ mg)}$	$CPA^{(350\ mg)}$	S.E.D. (dose)	Control	$CPA^{(150\ mg)}$	$CPA^{(350\ mg)}$	S.E.D. (dose)
0	346	516	214	129	51	80	40	19
4	218	71	50	44*	24	4	5	9
8	348	176	163	58*	362	54	19	58*
12	286	312	229	83	704	644	452	214
16	163	218	174	20	583	316	279	445
20	189	82	92	104	50	47	28	58
S.E.D. (time)		47				125		

[1]The stags were treated with CPA or the vehicle alone at weekly intervals for 5 weeks.
S.E.D. is the standard error of the difference.
*Significant difference ($P<0.05$) between control and CPA treated groups within a sampling time.

TABLE 3. Experiment 2: Antler size, duration of growth, and peak testis size in control and CPA treated stags[1]

	Treatment			
	Control	$CPA^{(150\ mg)}$	$CPA^{(350\ mg)}$	S.E.D. (dose)
Duration of antler growth (days)	61	63	70	2.1*
Antler weight (g)	245	230	281	54
Antler length (cm)	27	37	42	3.4*
Peak testis diameter (cm)	2.6	2.5	2.4	0.2

[1]Duration of antler growth was the number of days between casting of the previous hard antler and cleaning of the antler which grew during the study. When the antler was clean of velvet it was removed, weighed and measured. On that day testis diameter was also measured. S.E.D. is the standard error of the difference.
*$P<0.05$.

casting (Table 5) and at the higher dose level (CPA_{350}) at the time of antler cleaning as well, although there were no significant effects on testis diameter.

Experiment 4: The effect of maintaining elevated winter testosterone on LH and testosterone and on subsequent velvet antler weight

Testosterone treatment significantly raised basal testosterone concentrations but did not influence the testosterone response to LH, either directly or via GnRH (Table 6). Testosterone treatment reduced basal LH levels and the LH responses to GnRH (Table 6). The mean antler casting dates for the testosterone treated and control stags were 29th September and 8th September, respectively, S.E.D. = 9.0 days. Mean velvet antler weights were 1,240 g and 1,280 g (pooled S.E.D. = 0.18) for the testosterone treated and control stags, respectively, but these weights represented a 44% and a 10% (S.E.D. 12.64, $P<0.05$) increase in weight, respectively, compared to those recorded the previous year.

Experiment 5: The effect of stage of the antler cycle on the testosterone response to LH injection

LH levels in plasma after LH injection did not vary significantly during the antler growth period (Table 7). In contrast, during velvet antler growth the testosterone response was significantly reduced compared with periods when the stags had hard antlers ($P<0.001$). The peak response was during the rut (Table 7).

Experiment 6: Effect of stage of antler cycle on the DHEA and the cortisol response to ACTH

All animals responded to ACTH by releasing DHEA and cortisol at all stages of antler growth. There were no significant differences in DHEA due to stage of antler growth, but the cortisol responses were significantly smaller during the rut than recorded at the time of late velvet antler growth and antler cleaning ($P<0.05$) (Table 8). Testosterone did not show a response to ACTH at any stage of the antler cycle.

TABLE 4. Experiment 3: Time from onset of CPA treatment to hard antler casting, duration of antler growth (casting to cleaning), antler size, and peak testis size at antler cleaning in control and CPA treated stags

	Treatment			
	Control	$CPA^{(150\ mg)}$	$CPA^{(350\ mg)}$	S.E.D. (dose)[1]
Time from onset of treatment 2 weeks after cleaning to hard antler casting (days)	4.4	23	29	1.2**
Duration of antler growth (days)	69	86	93	3.4**
Subsequent hard antler weight (g)	417	468	320	93
Subsequent hard antler length (cm)	39	41	46	1.7*
Peak testis diameter (cm)	3.0	2.8	2.9	0.1

[1]S.E.D. is the standard error of the difference.
*$P<0.05$.
**$P<0.01$.

TABLE 5. Experiment 3: Total plasma LH and testosterone responses (areas under the curves: Mean of three stags per group) following a single GnRH challenge at three weekly intervals in control or CPA treated stags[1]

	LH				Testosterone			
Time (weeks)	Control	CPA(150 mg)	CPA(350 mg)	S.E.D. (dose)	Control	CPA(150 mg)	CPA(350 mg)	S.E.D. (dose)
0	187	203	192	41	782	151	244	357
3	236	99	38	68*	113	8	5	21*
6	187	111	184	88	62	6	7	38
9	232	203	114	42	7	5	3	2
12	445	336	260	79	151	182	73	53
15	521	213	211	159	593	514	278	76*
18	220	128	75	42	1,678	1,228	985	340*
S.E.D. (time)		39				160		

[1]The stags were treated with CPA from 2 weeks after antler cleaning until hard antler casting.
S.E.D. is the standard error of the difference. The broken horizontal line indicates that antler casting occurred between the sampling times on either side of the line.
*Indicates that there is a significant difference ($P<0.05$) between control and CPA treated groups within a sampling time.

DISCUSSION

Within two weeks after treatment with MPA the LH responses to GnRH and consequently the release of testosterone were abolished. Thus the effect of MPA on the pituitary-gonadal axis of stags is the same as in rams (Bolt, '71). Antler casting took place about 3 weeks after MPA administration. As antler casting after castration during hard antler also takes about 3 weeks (Fennessy and Suttie, '85) it seems that the complete removal of gonadal steroids has a similar effect on bone resorption in the pedicle whichever way it is caused. The antlers grown by the MPA treated stags were smaller than those of the control stags, possibly because they were grown earlier in the year prior to the spring rise in insulin-like growth factor 1 (IGF1), which is believed to be trophic for velvet antler growth (Suttie and Fennessy, '91). The effects of MPA appeared to be long lasting; 5–6 months after treatment the LH response to GnRH was still partly suppressed. This suppression of LH which led also to a significant suppression of testosterone, permitted the complete regrowth of a second set of velvet antlers in mid-summer in the MPA treated group. The fact that complete suppression of testosterone caused antler casting and did not prevent velvet antler growth lends support to the hypothesis that testosterone is unnecessary for early velvet antler regeneration.

Although CPA acts in a different way from MPA in that it blocks androgen receptors, it also reduced the LH response to GnRH and testosterone levels in each of two studies (Experiments 2 and 3). However, antler length but not weight was increased compared with vehicle-only treated controls. It seems that the suppression of testosterone during velvet antler growth or immediately before velvet antler growth does not influence the propensity to regenerate and may actually increase antler length. Bubenik ('82, '90), on the basis of studies with CPA and also measurement of testosterone in castrate white-tailed deer, considered that small amounts of testosterone were necessary not only for antler regeneration but also increased subsequent antler size. This finding

TABLE 6. Experiment 4: LH and testosterone basal levels and responses to a single injection of either GnRH or LH in control and testosterone treated stags[1]

	LH				Testosterone			
	Basal ng/ml	Area under curve following GnRH	Area under curve following LH	S.E.D.	Basal ng/ml	Area under curve following GnRH	Area under curve following LH	S.E.D.
Control	0.29	1247	999	187	1.4	301	475	41
Testosterone implanted	0.15	434	1039	216	3.6	364	346	36*
S.E.D.	0.04*	236*	204		0.7*	76	66	

[1]As there were no interactions with time data from two separate challenges 8 weeks part have been combined.
S.E.D. is the standard error of the difference.
*$P<0.01$.

TABLE 7. Experiment 5: Total plasma LH and testosterone responses (mean of three stags) after a single intravenous injection of 100 μg oLH at each sampling period

Stage of antler cycle	LH (total area under curve)	Testosterone (total area under curve)
Antler casting	997	21
Early velvet (40 days after casting)	1,384	21
Mid velvet (65 days after casting)	1,925	36
Late velvet	1,086	165
Antler cleaning	1,293	655
Peak rut	1,267	2,508
S.E.D.[1]	341	416

[1]S.E.D. is the standard error of the difference.

clearly differs from the present studies; whether this is due to a difference in the precise role of testosterone between species or not remains to be determined. In castrated red deer stags pretreated with testosterone, regenerated antlers were larger than those regenerated without prior testosterone treatment, but this finding was confounded by the fact that antler cleaning and casting occurred after testosterone treatment (Suttie and Fennessy, '90). Thus the "trophic" effects of testosterone and antler casting could not be distinguished. Therefore we conclude that red deer can regrow normal antlers without testosterone stimulation.

Testosterone implants applied during winter inhibited LH secretion and LH responses to GnRH, presumably by amplifying the steroid negative feedback signal at the pituitary or hypothalamic levels (Bolt, '71). Plasma testosterone levels were significantly raised during the winter period and this was associated with an increase in antler size relative to the size of the growth the previous year. Although it is not possible to causally relate increased antler size with the increased levels of testosterone during winter it is possible that testosterone might have a delayed trophic role. It is known for example that steroids sensitise MCF-7 breast cancer cell lines to the mitogenic effects of IGF1 (Stewart et al., '90). In this respect IGF1 is known to be associated with antler growth when circulating testosterone concentrations are low (Suttie et al., '89; Elliott et al., '92). A trophic effect of testosterone could be explained if it were to exert a priming effect on cells destined to develop into antlers which then became more responsive to IGF1 during velvet antler growth.

In an earlier study (Fennessy et al., '88) well-defined increases in testosterone secretion were reported to follow exogenous GnRH-induced LH peaks. Additionally the increases in testosterone were restricted to times of the year when the antlers were clean of velvet. However, the relevance of that pattern of testosterone secretion in relation to LH was unclear because the LH response to GnRH also varied with antler status; the pattern of testosterone could have been solely due to the pattern of LH secretion at different stages of the antler cycle rather than to any variation in testicular responsiveness. In the present study we have shown that following constant hormonal challenges with LH, the testosterone responses varied with antler status. Specifically the testes were unresponsive to LH during the velvet antler growing phase of the antler cycle. The results mirror closely those obtained when the stag is challenged with GnRH and indicate that the lack of testosterone responsiveness in previous studies was not due solely to a lack of LH stimulation. The almost total abolition of testicular secretory capacity is unusual and it is reasonable to speculate that some other substance (hormone) actually prevented steroid release. The fact that the testes appear unresponsive to LH during velvet antler growth adds weight to the theory that testosterone levels must be very low to facilitate antler growth in red deer.

After intra-muscular administration of ACTH,

TABLE 8. Experiment 6: Total plasma DHEA and cortisol responses after a single intra-muscular injection of 75 I.U. Synacten (ACTH) at each sampling period

Stage of antler cycle	DHEA (Total area under curve)	Cortisol (Total area under curve)
Antler casting	355	276
Early velvet (40 days after casting)	410	284
Mid velvet (65 days after casting)	382	373
Late velvet	282	354
Antler cleaning	360	362
Peak rut	346	197
S.E.D.	128	52

plasma levels of cortisol and DHEA, but not testosterone, increased rapidly to a peak and then fell. Analysis of the total areas under the cortisol response curves revealed that the secretory responses were elevated in late summer when velvet antler mineralisation was taking place, but was low during velvet antler growth. In a study with white-tailed deer in which cortisol was measured in single blood samples, Bubenik and Leatherland ('84) found that such a pattern was shown in "calm" but not "excitable" bucks. However, in earlier studies Bubenik et al. ('75b) found no seasonal or antler growth related rise in cortisol and Bubenik et al. ('83) found cortisol levels elevated during winter. No study has associated elevated cortisol levels with velvet antler growth. In contrast to cortisol, the areas under the DHEA response curves did not vary with the stage of the antler cycle. This is the first study of DHEA in deer, but androstendione, another androgen of adrenal and testicular origin, has been studied previously. In red deer Lincoln ('71) showed that androstendione levels paralleled those of the more potent androgen, testosterone. In intact white-tailed deer androstendione levels increased throughout velvet antler growth and peaked during the breeding season before falling during winter (Bubenik et al., '87), but the origin of the androstendione was unknown. In castrated male white-tailed deer, in which presumably the androstendione must be of adrenal origin, levels are very low throughout the year except for a significant elevation in late autumn coinciding with the breeding season (Bubenik et al., '87). Consequently no clear association with androstendione and velvet antler growth was shown. Taking together the above androstendione data with the DHEA data from the present study, it can be concluded that adrenal androgens probably do not play a major role in velvet antler growth. In support of this concept, when three adult, castrated red deer stags were treated with sufficient dexamethasone for 3 months to abolish the ACTH stimulated rise in DHEA and cortisol, they nevertheless grew apparently normal velvet antlers (J.M. Suttie, unpublished observations). No increase in testosterone response to ACTH was detected in the present study. This indicates that the testes were not responsive to ACTH and any effect of ACTH on adrenal testosterone was obliterated by the prevailing gonadal secretion.

Bubenik ('90) has proposed a biphasic role for testosterone in antler growth. He suggested low levels of testosterone are associated with the stimulation of vigorous species-specific antler growth through a direct action at the antler tissue level, and an effect operating indirectly through a specific central nervous system centre. In contrast, high levels of testosterone are associated with progressively intensified ossification and mineralisation. The precise definition of "low" or "high" is tenuous, but Bubenik ('82) indicates that castrate white-tailed bucks had "low" levels of around 0.4 ng/ml. In the present study velvet antler growth took place even when testosterone secretion was abolished by MPA induced inhibition of LH release and when androgen receptors were blocked by CPA. In addition the capacity of the testes to secrete during velvet antler growth was negligible as judged by the low response to a high dose of LH. The possibility that low amounts of adrenal androgens play a role in velvet antler growth can also be discounted by the present study. There is thus no support for a hypothesis that low levels of androgens are required for velvet antler growth in red deer stags.

In contrast the possibility that low levels of androgen are required for the complete expression of "normal" velvet antler growth must still be considered, for example, castrate antlers are always small (Suttie and Fennessy, '90). Bubenik ('82) suggested on the basis of a weak correlation between velvet antler size and plasma testosterone levels in three castrate white-tailed bucks that increased testosterone levels were responsible for the larger antlers. Lincoln ('75) castrated six adult stags but left the epididymides intact in one of them. That particular individual regenerated antlers with more points than the other five, which Lincoln described as "a more perfect set of antlers." He concluded that minor amounts of testosterone released by the epididymides could have been responsible for this effect. In the present study elevated testosterone levels during winter were associated with higher velvet antler weights in the subsequent spring, indicating that previous testosterone exposure could have a delayed stimulatory effect on velvet antler growth. In both the studies of Bubenik ('82) and Lincoln ('75) it is possible that the reported effects might have been due to previous higher levels of testosterone.

In conclusion velvet antler growth, at least in red deer, does not appear to require testosterone but there may be a priming effect of testosterone on subsequent antler growth. In contrast the timing of the antler cycle is dependent on changes in plasma levels of testosterone. There is a need to

study the effects of very low dose testosterone administration during velvet antler growth in order to test this hypothesis.

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