Physiological control of antler growth

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Summary

Studies on antlers have become more mechanistic and specific rather than associative and pertaining to the whole antler cycle. The majority of recent publications have described pedicle growth and velvet antler growth. An increase in plasma testosterone concentration has been shown to be the major stimulator of pedicle growth and a decrease in plasma testosterone concentration heralds the transition to antler after the pedicle is sufficiently long (>5 cms in red deer) and a change in ossification type has taken place. Spontaneous antler growth after testosterone-induced pedicle formation in females and castrate males is a novel finding. In vitro studies reveal that testosterone increases IGF 1-stimulated growth. A neural link between the brain and the antlerogenic region is not required for pedicle growth.

IGF 1 has been shown to specifically increase and testosterone to reduce antler cell growth in vitro. Although IGF 1 mRNA is expressed constitutively at a low level in the antler, the Type 1 IGF receptor is expressed abundantly in the epidermis/dermis and the cartilage. Plasma-borne IGF 1 plays an important role in determining antler size. Testosterone is critical for pedicle initiation and growth but has a negative effect on antler growth. Testosterone has a major effect on timing of the antler cycle.

Recent publications on antlers have reflected two trends, one away from association and correlation studies to more mechanistic and experimental approaches (Schnare, 1992; Kierdorf *et al.*, 1993; Kolle *et al*, 1993), and another to investigating specific components of the antler cycle rather than the whole cycle namely, antler casting (Goss *et al.*, 1992), pedicle induction (Goss, 1991; Jaczewski, 1991) and the antler as a putative tumour (Goss, 1990). Bubenik & Bubenik (1990) provided a state-of-the-art review which has stimulated a great deal of research. In the space of this current review it is not possible to summarise the physiological control mechanisms of all parts of the antler, as was done by Fennessy & Suttie (1983), or present a smorgasbord of new ideas (Suttie & Fennessy, 1990). Rather this review reflects the two areas where major research efforts have taken place, pedicle growth and velvet antler growth, and attempts to integrate diverse results in these areas to produce a synthesis from which novel hypotheses can emerge and gaps in the knowledge can be determined. Hitherto the initiation of pedicle growth and the transformation to the first antler have received little attention and velvet antler growth studies have been facilitated by advances in *in vitro* techniques. In the present review the species studied was red deer (*Cervus elaphus*), unless otherwise indicated.

Pedicle growth

Pedicles are the permanent bony protuberances of the frontal bones of the skull from which deciduous antlers grow, clean, cast and then regenerate each year (Chapman, 1975; Goss, 1983). Pedicles are normally only grown by male deer and become visible about the onset of puberty (Lincoln, 1971). When pedicles reach a height of 5-6 cms the morphology changes apparently abruptly to that of the first antler (Fennessy & Suttie, 1983). Pedicle growth is associated with increasing plasma levels of testosterone but in contrast velvet antler growth is associated with decreasing or low plasma testosterone concentrations (Suttie *et al.*, 1991a). Stags castrated before pedicle growth initiation

do not develop pedicles (Wislocki *et al.*, 1947). Testosterone administration to females and prepuberal castrates results typically in pedicle growth only (Jaczewski, 1990). The role of nerves in pedicle growth is equivocal (Bubenik & Bubenik, 1990).

Histological studies

Light microscopy of tissue taken from the antlerogenic periosteum, the developing pedicle and the early first antler has revealed a pattern of development with three critical stages (Fig. 1) (Li & Suttie, 1994a). The first stage is characterised by intramembranous ossification; early, reversible, development of the antlerogenic periosteum to a pedicle height of 2 cm is of this type. The second stage, while the pedicle grows from 2-4 cms, is a transition between intramembranous to endochondral ossification. The third stage from 4 cms of pedicle and throughout the antler growth is endochondral ossification. This means that the pedicle has the antler type of ossification before it has developed the typical velvet hair type. The transition between ossification types is the point where the permanent pedicle ceases to grow and the first antler is initiated (Li & Suttie, 1994b).

The effect of nerves on pedicle growth

Bubenik (1982) proposed that a direct neural link is required between the central nervous system and the antlerogenic periosteum at the time of pedicle initiation for that event to occur. To test this hypothesis (Li *et al.*, 1993) removed the sensory nerves from deer prior to pedicle initiation; normal pedicle growth took place. In a further study sympathetic nerve removal in conjunction with sensory nerve removal was carried out and pedicle development was unaffected (Suttie *et al.*, 1995). It is concluded that nerves do not play a role in pedicle initiation and the transition to the first antler.

The effect of testosterone treatment on pedicle and antler growth

Freemartins and females

Because female deer do not spontaneously grow antlers from testosterone-induced pedicles

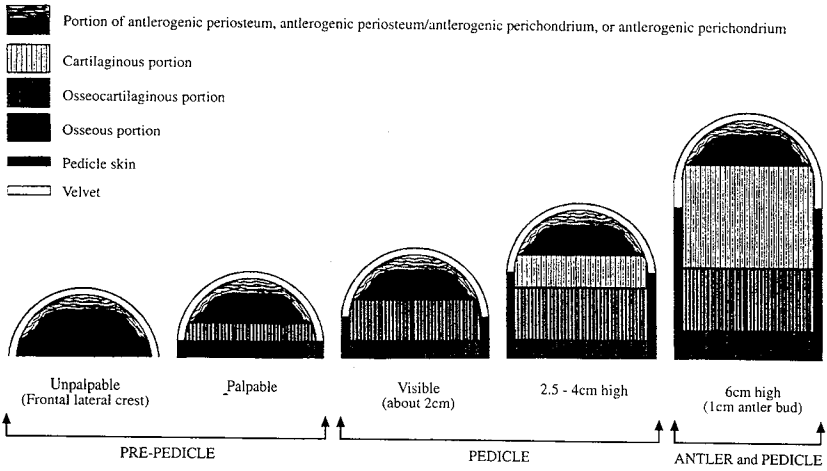

Figure 1. Diagram of histogenesis of the pedicle and early first antler in red deer.

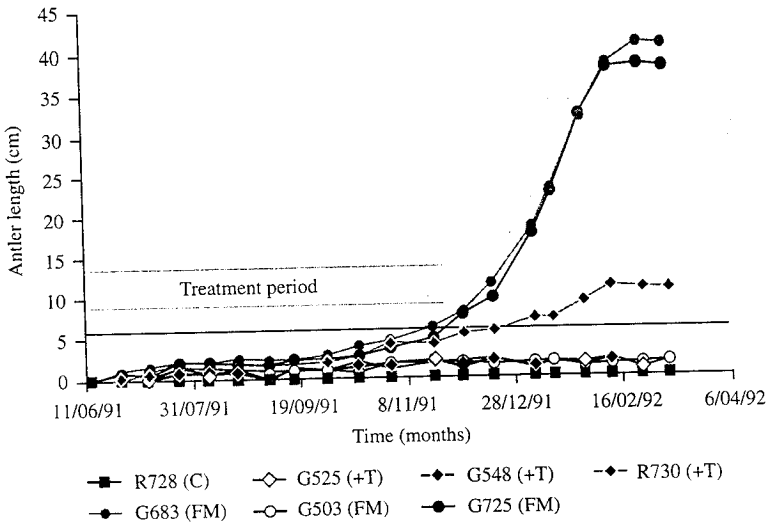

Figure 2. Antler length and plasma testosterone level in freemartins and female deer. C indicates control female, T indicates female treated with testosterone and FM indicates freemartins treated with testosterone. The horizontal line at 6 cms indicates the transition from pedicle to visible antler.

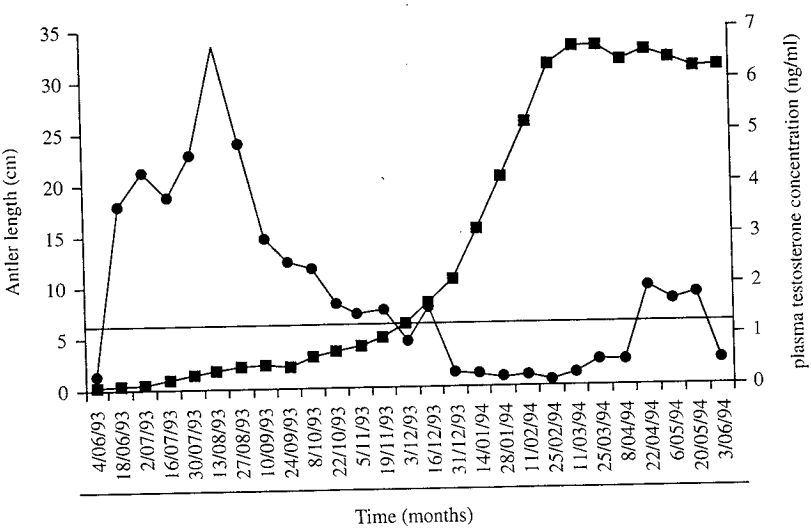

Figure 3. Antler length (—■—) and plasma testosterone concentration (—●—) in castrated male deer treated with testosterone. The horizontal line indicates the transition from pedicle to visible antler.

(Jaczewski 1990), it was hypothesised that intra-uterine conditions could pre-dispose potentially antlerogenic cells to respond differentially to testosterone in post-foetal life. Three freemartins (karyotyped) born co-twin to males and three normal females were treated with silastic testosterone implants and three normal females were untreated as controls (Fig. 2). Two of the three freemartins grew antlers from the pedicles after they had reached a height of 5 cms and after testosterone treatment ceased. Also one of the testosterone-treated females grew an antler from the induced pedicles spontaneously. It is concluded that intra-uterine conditions do not influence antlerogenic responses to testosterone treatment and that the minimum *in vivo* requirements for antler growth from the pedicle are sufficient testosterone to grow a sufficiently large pedicle followed by a decrease in testosterone supply.

Pre-pubertally castrated male deer

Spontaneous antler growth in castrated male deer after testosterone-induced pedicle growth has not been observed (Jaczewski, 1990). In view of the results from the freemartin study, the same experimental approach was adopted in six-month-old castrated males. Eight stag calves were castrated and testosterone injection was given until both pedicles reached 5 cms in length; at this time testosterone treatment ceased. All eight stags developed antlers at the conclusion of treatment (Fig. 3). This confirms the conclusion that testosterone treatment followed by withdrawal is sufficient to cause first pedicle and seasonal antler growth.

Antlerogenic Cell Culture

Antlerogenic periosteal cells contain testosterone binding sites (Li & Suttie, 1994c). Antlerogenic cells in culture do not grow in serum-free media supplemented with testosterone only but do in the presence of insulin-like growth factor 1 (IGF 1). Testosterone enhances the IGF 1-stimulated growth (C. Li, *et al.*, unpublished data).

In summary, testosterone stimulation is required for pedicle growth. It is speculated that testosterone is required to initiate and maintain the change in ossification type, but, after endochondral ossification is established, testosterone is no longer required. Neural links with the central nervous system are not required. Testosterone stimulates pedicle growth synergistically with IGF 1.

Velvet antler growth

The timing of the antler growth cycle is controlled by testosterone but the trophic control of actual growth is controlled by growth factors including IGF 1 (Suttie & Fennessy, 1992). This dichotomy is consistent with published whole animal studies but no *in vitro* confirmation has to date been available.

Effect of IGF 1 on antler cells in culture

Cells from the fibroblast and the cartilage zones from antler tips midway through antler development have been separately grown in culture (Sadighi *et al.*, 1994). Both cell types show dose-dependant increases in growth after IGF 1 and 11 treatment in serum-free media (Fig. 4). Antler cells also grew slowly in serum-free media. Radio-labelled IGF 1 binds to antlers and this binding can be inhibited by cold IGF 1 and IGF 1 anti-serum. This result confirms the findings of Elliott *et al.* (1992; 1993) and extends them to indicate that IGF is mitogenic for antler growth.

Location of IGF 1 and IGF type 1 receptor mRNA in growing antler

Northern blot analysis revealed that IGF Type 1 receptor is found in all antler tip tissues but is lowest during early growth (30 days after casting), peaks during mid-growth (60 days) then decreases (90 days) (Fig. 5). The pattern of expression within the tissues, where expression is highest in the epidermis/dermis and the cartilage, confirm the findings of Elliott *et al.* (1993; 1994) based on autoradiography. In contrast IGF 1 mRNA is found constitutively at a very low level of expression in all tissues. These data indicate that, although the antler does produce a small amount of IGF 1 itself, the high expression of Type 1 receptor means that plasma IGF 1 is important for antler growth.

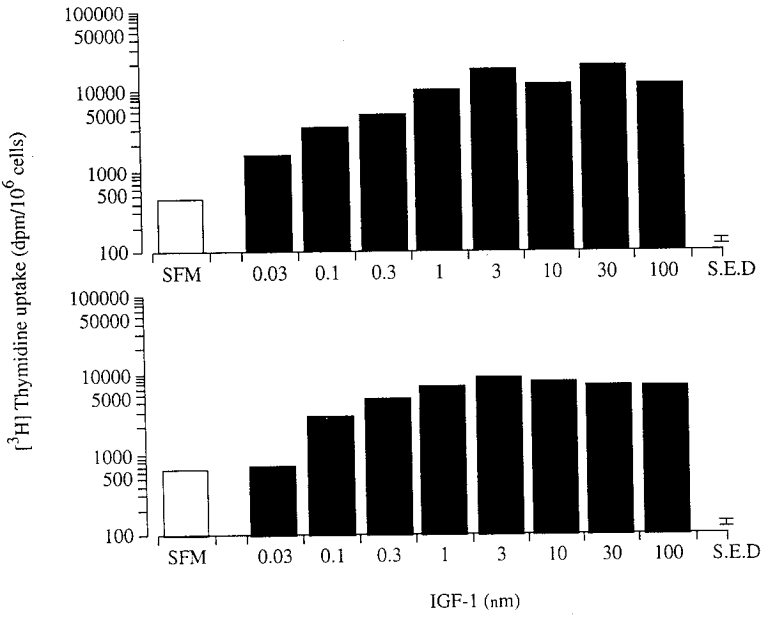

Figure 4. The effect of IGF 1 on antler cells in culture. Data shown is for the fibroblast (upper) and cartilage (lower) zone cells.

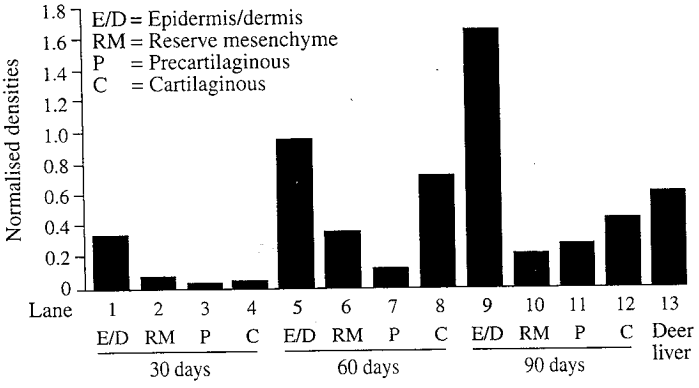

Figure 5. IGF Type 1 receptor Northern Analysis normalised by the 5kb ribosomal band in antler tissues. Antlers were removed at 30, 60 or 90 days after casting of the preceding hard antler and dissected into epidermal/dermal, reserve mesenchyme, pre-cartilaginous and cartilaginous zones. Deer liver is a positive control.

Effect of anti-androgen on antler growth

Anti-androgen (cyproterone acetate [CPA] or medroxy progesterone acetate) treatment, while inducing antler casting, did not prevent antler growth or the attainment of species-specific antler shape (Kolle *et al.*, 1993; Kierdorf *et al.*, 1993; Suttie *et al.*, 1994). Indeed antlers grown by red deer treated with CPA were larger than those of control deer (Suttie *et al*, 1994). This indicates that *in vivo* testosterone is not required for antler growth.

Effect of testosterone on antler cells in culture

Cells from the fibroblast and cartilage zones of the antler tip were cultured in serum- free media with and without testosterone and IGF 1. Testosterone alone did not stimulate growth in contrast to IGF 1 which was stimulatory (Fig. 6). Testosterone in combination with IGF 1 reduced growth compared with IGF 1 alone. Elliott (1994) treated fallow deer bucks with testosterone in mid-antler growth and measured labelled IGF 1 uptake *in vitro* in antler tip sections after antler removal. Testosterone significantly reduced IGF 1-binding particularly in cartilage. Taken together, the above data indicate that testosterone reduces IGF 1-stimulated antler growth probably by reducing IGF 1-binding to the Type 1 receptor.

In summary, IGF 1 is a potent stimulator of antler cell proliferation and probably reaches the antler via the blood stream, i.e. it is a true hormonal action. IGF 1 also plays a role in protein synthesis in the antler because receptors for IGF 1 are found in cartilage where less cell division takes place. The fact that antlers grow in serum-free media indicates that IGF 1 or any exogenous growth factor is not a critical requirement for antler growth but rather IGF 1 is a potent growth rate-determining factor. Indeed antler length correlates significantly and positively with IGF 1 plasma level (Suttie *et al.*, 1991b). Testosterone reduces antler growth by influencing IGF 1 binding. The role of IGF 1 in controlling velvet antler growth has been confirmed and likely mechanisms determined.

Future directions

It has been speculated that there is a change in sensitivity to testosterone during the transition between the stages of ossification types. Testing this hypothesis will require an in-depth study of androgen receptors and androgen binding. This work is hampered by the fact that androgen receptor antibodies do not recognise ruminant androgen receptors. Future research should be

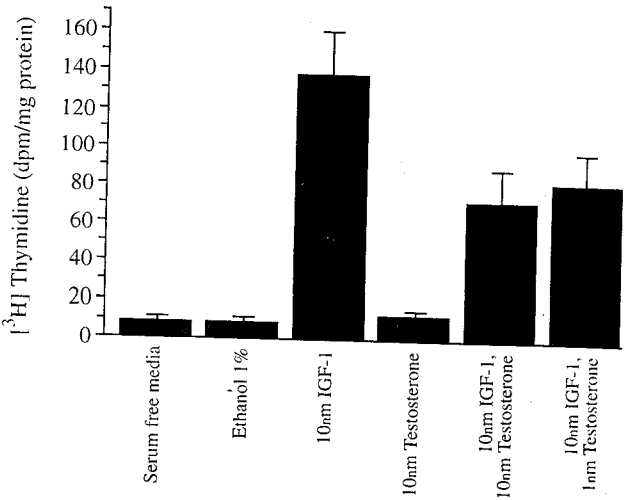

Figure 6. The effect of testosterone on IGF-stimulated antler cell growth.

directed to determining ways of measuring this receptor and its dynamics in pedicles and antlers.

The role of IGF 1 and 11 have received a great deal of attention. Apart from one study on EGF (Ko *et al.*, 1986) and one on a novel IGF 1 (Gutierrez *et al.*, 1993), there are no other published studies on other growth factors. Several growth factors are likely candidates for antler regulation (Suttie & Fennessy, 1992).

The vast majority of antler research has been carried out on two species of deer, *C. elpahus* and *Odocileus virginianus*. Clearly there are variations among other species, for example, antlered female *Rangifer tarandus*, and mechanisms for these variations need further study. Antler control mechanisms in tropical deer are unstudied. Comparative studies would surely yield a great deal of information.

The rapid growth rate of the antler makes it an ideal candidate to study gene expression and the sequence of developmentally regulated (and regulating) genes. The antler is a relatively simple column of developing bone but it requires support tissues of hair follicles, sweat glands, epidermis, dermis, blood vessels and nerves. All of these grow and develop very rapidly. The antler is a multi-potent model for the growth of a more diverse range of tissues than is immediately apparent.

Antler research has become specialised into specific studies of control mechanisms for part of the cycle. However the spread of study is not uniform. The calcification process and antler cleaning have received little recent study and antler casting and early regeneration has received little study. Future research should aim to broaden the base of specialised knowledge by taking advantage of novel techniques.

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