Somatotropin physiology – a review

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Received 4 June 1993; accepted 15 November 1993

The continuing search for improved efficiency has led to the introduction of exogenous growth and lactation promotants to enhance traditional animal production systems. Various species-limited somatotropins have been synthesized by means of recombinant gene technology, thereby allowing milk production in dairy cattle, and lean meat production in pigs, sheep and cattle to be manipulated. Growth hormone occurs in plasma in several forms, which overlap considerably with the structures of placental lactogen and prolactin. The peptide is stabilized by two disulphide bridges, and is folded into four α-helix regions. The somatogenic and lactogenic regions have been mapped, and two different binding sites found. Somatotropin is secreted episodically from the anterior pituitary with a striking ultradian rhythm in all mammals investigated thus far. The primary control over this release would appear to be neuroendocrinological, with somatostatin and releasing hormone (GHRH) playing the major roles, with considerable species differences. Starvation primes the system to optimally release somatotropin when feeding recommences. While sexual dimorphism in the release of somatotropin has been clearly demonstrated in the rat, most studies in other species have concentrated on male animals. Although somatotropin exhibits negative feedback on its release, it does not appear to have a direct effect on the somatotroph. In the ruminant at least, somatotropin may also be involved in satiety control. Other systems that have been implicated in the control of somatotropin release in ruminants include cholecystokinin, serotonin, dopamine and α-adrenergic receptors and plasma glucose concentrations, all via regulatory mechanisms in the hypothalamus, which change from pre- to postweaning (glycogenic to gluconeogenic). Recently, GHRH analogues, consisting of a series of short (5–6 amino acids) peptides similar to encephalin, as well as a nonpeptidyl secretagogue have been developed. Although GHRH normally contains 40–44 residues, it is only the 29 amino acids at the amino terminal end that are associated with the releasing activity. By replacing arginine residues with agmatine (decarboxylated arginine), it is possible to create analogues that have an increased potency of more than 50-fold. The short-term response to somatotropin in high-yielding Holstein cows varies between 2 and 5 kg/day. The dose response appears to be curvilinear and is not affected by the pattern of administration. There is no increase in feed intake during short-term administration of somatotropin, although in the long term increases of 10–25% have been found, when measured over the entire lactation. In such cases, feed intake increased gradually to match the increased milk production, owing to an improvement of feed conversion. The lactogenic effect does not appear to be mediated by any direct effect on the mammary gland. The most pronounced effect of somatotropin is the release of the insulin-like growth factors (IGF), chiefly from the liver, and to inhibit lipogenesis. Although somatotropin decreases body fat, non-esterified fatty acids only increase during a negative energy balance. Although studies in sheep generally support these contentions, the effect on growth is controversial. Average daily gain (ADG) and feed conversion efficiency (FCE) generally improve with treatment, accompanied by a fairly consistent 10 to 20% decrease in carcass fat. The major effect in pigs is anabolic, leading in general to a 10–20% increase in ADG, a 15–35% improvement in FCE, a 30–40% decrease in lipid deposition and a 20–30% increase in protein deposition. The optimum dose depends on whether optimal growth or maximal feed efficiency is required. Nile crocodiles (*Crocodylus niloticus*) also respond to treatment with human somatotropin, by increasing feed intake, gaining body weight and increasing body length. Somatotropin released into the blood stream is bound by one of the two specific proteins found in plasma, one with high affinity and one with low affinity. In man, about 50% of circulating somatotropin is complexed to binding proteins, which are low at birth, and increase to reach maximum values in young adults. By sequestering somatotropin, the high affinity binding protein substantially interferes with the interaction between somatotropin and its receptor. The clearance rate of the bound form is 10-fold lower than that of the free form. Although similar binding proteins are found in the rabbit, pig, mouse and rat, there is little in ovine and bovine species. The binding protein appears to be generated by one of two mechanisms: either a proteolytic cleavage of the receptor near the transmembrane domain, or *de novo* synthesis from a truncated mRNA. The former mechanism has been demonstrated in man and rabbit, and the latter in pregnant mouse and rat. The receptor belongs to a superfamily which include those for, *inter alia*, prolactin, the interleukins, erythropoietin and the interferons. Somatotropin receptors have now been demonstrated in many tissues other than the liver, such as kidney and the cardiovascular and respiratory systems. The distribution of the receptor has also been described within the cell, even in the nucleus. It has been suggested that this receptor is synthesized locally to facilitate intracellular transport of somatotropin and/or to regulate transcription of IGF.

Die volgehoue strewe na verbeterde doeltreffendheid het gelei tot die toediening van eksogene groei- en laktasie-stimulante om erkende diereproduksiestelsels te bevorder. Verskeie spesiebeperkte somatotropiene is deur middel van rekombinante geentegnologie gesintetiseer, om melkproduksie in melkbeeste en maervleisproduksie in varke, skape en beeste te manipuleer. Groeihormoon kom voor in plasma in verskillende vorms wat met die strukture van plasentale laktogeen en prolaktien ooreenstem. Die peptied word deur twee disulfiedbande gestabiliseer en in vier α-heliks-

gebiede gevou. Die somatogeniese en laktogeniese gebiede is gekarteer en twee verskillende bindingsplekke is ontdek. In alle soogdiere wat tot dusver ondersoek is word somatotropien episodies vanuit die anterior pituïtêre klier afgeskei met 'n opmerklike ultradiese ritme. Die primêre beheer oor hierdie vrystelling is neuro-endokrinologies, waar somatostatien en vrystellingshormoon (GHRH) die belangrikste rol speel, met aansienlike spesieverskille. Uithongering berei die stelsel voor vir optimale vrystelling van somatotropien sodra voer weer beskikbaar word. Terwyl geslagstweevormigheid in die vrystelling van somatotropien duidelik uitgewys is in die rot, is die meerderheid studies met ander spesies op manlike diere uitgevoer. Alhoewel somatotropien sy vrystelling negatief beheer, het dit nie 'n regstreekse invloed op die somatotroof nie. In die herkouer, tenminste, speel somatotropien ook 'n rol in die beheer van versadiging. Ander stelsels wat ook 'n rol in die beheer van somatotropienvrystelling in die herkouer speel, sluit in cholesistokinien, serotonien, dopamien en α-adrenergiese reseptore sowel as plasmaglukosekonsentrasies, almal deur beherende meganismes in die hipotalamus wat van voor- tot naspeen (glikogenies tot glukoneogenies) verander. Onlangse is GHRH-analoë wat uit 'n reeks kort peptiedes (5–6 aminosure) soortgelyk aan enkefalien bestaan, asook 'n nie-peptiedverbinding ontwikkel. Alhoewel GHRH uit 44 aminosure bestaan, is net die terminale 29 aminosure biologies aktief. Deur arginien met agmatien (gedekarboksileerde arginien) te verplaas, is dit moontlik om analoë met 'n 50-maal verhoogde doeltreffendheid te sintetiseer. Die korttermynreaksie tot somatotropien in hoëproduserende Holsteinkoeie varieer vanaf 2–5 kg/dag. Die reaksie is kromlynig en word nie deur die patroon van toediening beïnvloed nie. Voerinname styg nie tydens hierdie korttermynbehandeling nie, alhoewel dit met tussen 10–25% tydens langtermyntoediening kan toeneem, gemeet oor die volle laktasie. In sulke gevalle sal die voerinname geleidelik toeneem om die verhoogde melkproduksie te volg, as gevolg van 'n verbeterde voeromset. Die laktogeniese invloed word nie deur enige regstreekse effek op die melkklier uitgeoefen nie. Die vernaamste invloed van somatotropien is die vrystelling van somatomediene deur die lewer, en die vermoë om lipogenese te beperk. Alhoewel somatotropien liggaamsvet verminder, neem vryevetsure toe slegs tydens 'n negatiewe energiebalans. Alhoewel navorsing by skape hierdie opmerkings ondersteun, bly die uitwerking op groei onduidelik. Die algemene daaglikse toename (ADT) en die doeltreffendheid van voeromset (VOD) verbeter oor die algemeen met behandeling, gepaardgaande met 'n redelik konstante 10–20% vermindering in karkasvet. Die belangrikste uitwerking in varke is anabolies, wat in die algemeen lei tot 'n verhoging van 10–20% in ADT, 'n verbetering van 15–35% in VOD, 'n afname van 30–40% in vetneerlegging en 'n styging van 20–30% in proteïensintese. Die optimum toediening is van die verlangde effek afhanklik, naamlik optimale groei of maksimum doeltreffendheid van voeromset. Die Nylkrokodil (*Crocodylus niloticus*) reageer ook op toediening van menslike somatotropien deur 'n toename in voerinname, liggaamsmassa en lengte te toon. Somatotropien wat in die bloed vrygestel is, word deur een van twee bindingsproteïene opgeneem, een met hoë en een met lae affiniteit. In die mens word ongeveer 50% van sirkulerende somatotropien só deur bindingsproteïene, wat lae waardes pas na geboorte en maksimum waardes in jong volwassenes bereik, vervoer. Op dié manier belemmer die hoë-affiniteit bindingsproteïen die koppeling tussen somatotropien en sy reseptor. Die suiweringstempo van die gebonde vorm is ongeveer 10-maal laer as dié van die vrye vorm. Alhoewel soortgelyke bindingsproteïene in die konyn, vark, muis en rot gevind is, kom daar min voor in skaap- en beesrasse. Hierdie bindingsproteïen word deur een van twee meganismes vervaardig: óf 'n proteolitiese afskeiding van die reseptor naby die transmembraangedeelte, óf *de novo*-sintese vanaf 'n verkorte mRNA. Die eersgenoemde meganisme is in die mens en konyn aangetoon, en die tweede in die dragtige muis en rot. Die reseptor behoort aan 'n superfamilie wat, onder andere, die reseptore vir prolaktien, interleukiene, eritropoïetien en die interferone insluit. Somatotropienreseptore is nou in talle weefsels buiten die lewer gevind, bv. die nier, kardiovaskulêre en respiratoriese stelsels. Die verspreiding van die reseptor is ook binne-in die sel beskryf, selfs in die kern. Dit is voorgestel dat hierdie reseptor plaaslik vervaardig word om intrasellulêre vervoer van somatotropien te vergemaklik en/of om transkripsie van somatomediene te beheer.

Keywords: Growth hormone, mechanism, release, review, somatotropin, structure, synthesis.

Introduction

The continuing search for improved efficiency has led to the introduction of exogenous growth and lactation promotants to enhance traditional animal production systems. Although the role of somatotropin in regulating growth has been known for more than 50 years (Bauman *et al.*, 1982), and its role in lactogenesis for more than 40 (Young, 1947), it is only over the last 10 years that modern technology has given us the means to apply that knowledge in a practical way. Various species-limited somatotropins have been synthesized by means of recombinant gene technology (Seeberg *et al.*, 1983), thereby allowing us to manipulate milk production in dairy cattle (bovine somatotropin, bST), and lean meat production in pigs (porcine somatotropin, pST), sheep (bST) and cattle (bST). More specifically, somatotropin treatment may alter the body composition of growing lambs (McLaughlin *et al.*, 1993), as well as increase the milk production of primiparous ewes (Stelwagen *et al.*, 1993). Furthermore, somatotropin has also been shown to increase milk production in goats (Knight, 1992) and body mass in chickens (Johnson *et al.*, 1993) and fish (Moriyama *et al.*, 1993).

When administered to dairy cows, bST typically increases milk production by 3 to 5 kg per cow per day, regardless of race or genetic potential, provided that the nutritional status of the cow is adequate. This translates to a proportional increase of about 15–20%, while productive efficiency is also improved (kg milk per kg feed) by about 10% (Peel *et al.*, 1981; Bauman *et al.*, 1985; Eppard *et al.*, 1985; McCutcheon & Bauman, 1985; Richard *et al.*, 1985; Sechen *et al.*, 1985). Administration of pST to pigs typically increases growth rate by 10–12% and improves productive efficiency by about 15%. At the same time, adipose tissue mass decreases by up to 80% and muscle growth increases by about 50% (Campbell *et al.*, 1988; Evock *et al*, 1988). These applications have obvious implications for animal production, leading to not only increased production and improved efficiency, but also to

products that may be more acceptable to consumers in terms of health risk. While there may be some resistance to the use of exogenous hormones in production animals, that aspect will not be dealt with in this review. Instead, I will outline the current information regarding the structure, mechanisms of release and the physiology of somatotropin.

Structure

Growth hormone may exist in plasma in several forms that range from fragments to high molecular weight aggregates (Baumann, 1991), although a peptide monomer of 191 amino acids, with a molecular weight of 22 kDa is the most common form (Lewis, 1992). Another form that is commonly found in humans (but not yet in other animals) has a molecular mass of 20 kDa and is lacking amino acids 32–46. The physiological significance of the two forms is still not clear, although the smaller form has been linked to the immune status of an animal (Smal *et al.*, 1985). Some of the fragments may be produced by proteolytic enzymes present in the plasma such as plasmin, thrombin and chymotrypsin. Such fragments may have enhanced lactogenic and growth-promoting activities in humans (Ingram *et al.*, 1992). The growth-promoting activity may be associated with a slower clearance rate of the fragments from the plasma. There is also considerable structural overlap with placental lactogen and prolactin, which are phylogenetically related hormones (Thomas *et al*, 1987).

The peptide is stabilized by two disulphide bridges, and is folded into four α-helix regions which lie parallel and which run up-up-down-down (instead of the more usual up-down-up-down where the regions are separated by short loop sections, Thomas *et al.*, 1987; Aston *et al.*, 1991). The somatogenic region has now been mapped using antigenic analysis (monoclonal antibodies) and has been shown to be associated with three sites on the loops connecting helices 1 to 2 (residues 35–53) and 3 to 4 (residues 120–140 overlapping with 134–154). The lactogenic site appears to overlap this region, but is more strongly centred on residues 18, 21 and 25 in helix 1 and residues 167, 168, 172, 174 and 176 of helix 4. These are not the only amino acids involved in binding, but appear to be the most important, together with a Zn molecule which is necessary at this site (Cunningham & Wells, 1991). This is, however, not the region which is involved with binding to the receptor site, which was recently defined for human somatotropin, where helices 2, 3 and 7 bind over a contact area of 1 230 $Å^2$ with the one receptor monomer via nine hydrogen bonds while the N-terminus end binds with helices 1 and 6 via four hydrogen bonds over a contact area of 900 Å (de Vos *et al.*, 1992). There is some activity associated with the hydrophobic region between helices 1 and 2 (residues 80– 83) as well as glu33. There would also appear to be a binding region on the loop between helices 3 and 4 (96–133). The fact that there are at least two different binding sites has been suggested by the observation that somatotropin binds to three structurally different receptors (binding to receptor 2 is calcium-independent, whereas binding with the other two is not, Aston *et al.*, 1991).

Synthesis and release

Somatotropin is secreted episodically from the anterior pituitary with a striking ultradian rhythm in all mammals investigated thus far. The primary control over this release would appear to be neuroendocrine in nature, with somatostatin (14 residues, single disulphide bridge) and releasing hormone (GHRH, 44 residues) playing the major roles. Recent studies show that the most important factor controlling the episodic release is the cyclic nature of GHRH release from the hypothalamus (Wehrenberg *et al.*, 1984, Moore *et al.*, 1992). In the rat, somatostatin and GHRH are released in reciprocal 3–4 h cycles (about 180° out of phase) from the median eminence into the hypophyseal portal blood, from where they act upon the pituitary somatotrophs to generate the observed ultradian rhythm (Tannenbaum, 1991). There is a delicate relationship between these two peptides, as exemplified by the results of a trial in which rats were starved for 72 h. In these animals, somatostatin did not inhibit the release but paradoxically enhanced the responsiveness of the somatotrophs to GHRH (Tannenbaum *et al.*, 1989). The mechanism is not clear, but may involve either an enhanced storage capacity, or else the down-regulation of the somatostatin receptor. The net result is that starvation primes the system to optimally release somatotropin when feeding recommences, thereby possibly priming the system for compensatory growth. Please note that considerable species differences probably occur, and that these patterns in all probability do not reflect the situation in ruminants. For example, a pattern of fasting followed by refeeding causes changes in concentration of circulating ST in rats which is the exact opposite of that found in all other species (Lapierre *et al.*, 1992), in particular the cow. In rats (Tannenbaum *et al.*, 1976), fasting inhibits the secretion of growth hormone, whereas elevated glucose, *i.e.* refeeding, has little effect. In cattle (McAtee & Trenkle, 1971), on the other hand, fasting increases the concentration of circulating growth hormone, chiefly by increasing the half-life.

While sexual dimorphism in the release of somatotropin has been clearly demonstrated in the rat, most studies in other species have concentrated on male animals. Male rats exhibit the typical ultradian rhythm, while in females the pulses are more frequent and of a lower amplitude (Tannenbaum, 1991). The mechanism responsible for this difference is unknown, although several possibilities have been proposed. Firstly, the neurons responsible for synthesizing and releasing somatostatin have an intrinsic secretory pattern that is different to that exhibited by the neurons that release GHRH. These patterns differ between the sexes. Secondly, the patterns may result from neuronal interactions between somatostatin and GHRH within the hypothalamus. Thirdly, the gonadal steroid environment influences the release pattern. These suggestions are not mutually exclusive, and probably reflect in some measure the actual control mechanism.

The neuronal control of somatotropin release is diagrammatically represented in Figure 1 (taken from Tannenbaum, 1991). It is currently thought that, in addition to the somatostatin-containing projection to the median eminence originating from the periventricular nucleus, there is probably an additional collateral projection onto GHRH-containing arcuate neurons from either the periventricular nucleus or the local somatostatin neuronal network in the arcuate nucleus. This implies that somatostatin may directly and, therefore, centrally regulate the infundibular release of GHRH into the portal circulation.

An aspect that is currently enjoying attention is the discovery of somatomammotrophic cells in several species (Kineman *et al.*, 1992) as well as a considerable heterogeneity in the function of so-called pure somatotrophs within a species (Takahashi, 1992). The fact that the same cell may produce

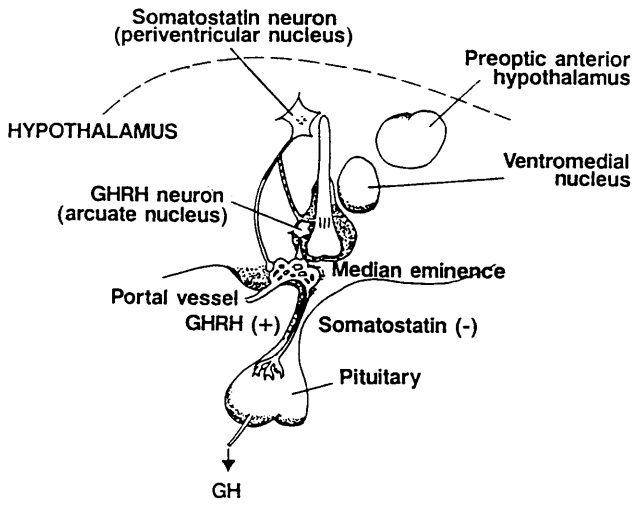

Figure 1 Hypothalamic peptidergic neurons of the somatotropin neuroendocrine axis. Taken from Tannenbaum (1991).

both somatotropin and prolactin aids in understanding some of the apparent contradictions in earlier work. Furthermore, the age of the animal also affects the rate of synthesis and the storage capacity of somatotropin within the somatroph (Console *et al.*, 1993), and is an important component of the immunomodulation of hormone release (Lean *et al.*, 1992), particularly in older animals (Farmer *et al.* 1993), and in animals under stress (Kelly & Dantzer, 1990). However, experimental data obtained from cows do not seem to support this (Cole & Hansen, 1993).

The role of other factors in controlling the release of somatotropin from the pituitary has also been demonstrated. Although somatotropin exhibits negative feedback on its release, it does not appear to have a direct effect on the somatotroph in the pituitary (Kraicer *et al.*, 1988). This observation implies that the mechanism must involve central control, which has subsequently been demonstrated to be mediated by acetylcholinergic receptors (Kelijman & Frohman, 1991). Another likely interpretation may lie in the observation that exogenous pST increases the number of somatotrophs in the pituitary (Andres *et al.*, 1993). Moreover, somatotropin exerts a direct, negative control over the gene expression of GH-releasing hormone in rats, independent of any influence from peripheral IGF-1 (Sato & Frohman, 1993).

In the rat, treatment with pyridostigmine, an acetylcholinesterase inhibitor, enhances the release of somatotropin, suggesting that somatostatin release is inhibited. However, this enhancement effect of pyridostigmine appears to operate via a different mechanism to that responsible for the feedback effect of somatotropin, which involves the increase of somatostatin in the hypothalamus. This effect has not been reported in the ruminant.

Another factor that has recently been implicated in the central control of somatotropin release is cholecystokinin (CCK). This peptide is found not only in the gut, but also in the central nervous system, where it contributes to the control of satiety. It is possible that, in the ruminant at least, somatotropin may also be involved in the response to satiety (Driver & Forbes, 1981; Tindal *et al.*, 1985). Intravenous administration of CCK in man and rat stimulates release of somatotropin, while in sheep CCK was only active when administered intracerebroventricularly (Spencer *et al.*, 1991). Experiments using loxiglumide, an antagonist of CCK, suggested that the effect of CCK was independent of hypothalamic somatostatin. Furthermore, pulses of luteinizing hormone appear to be positively correlated with the release of somatotropin in foetal sheep (Albers *et al.*, 1993). These findings imply that at least one other somatotropin-inhibiting system exists in the hypothalamus of sheep, thereby supporting the conclusions drawn from the pyridostigmine experiments (Kelijman & Frohman, 1991).

Other systems that have also been implicated in ruminants include serotonergic mechanisms that stimulate release, dopamine that inhibits release, and α-adrenergic receptors that potentiate release (Sartin *et al.*, 1991), all via regulatory mechanisms in the hypothalamus. These mechanisms have been shown to change during the development of the animal from pre- to postweaning, when the hepatic metabolism changes from glycogenic to gluconeogenic.

The concentration of glucose in the plasma has also been shown to control the release of somatotropin, which suggests that some kind of glucoreceptor must be present in the central nervous system. Experiments were carried out on rats in which the aqueduct between the lateral ventricles and the fourth ventricle was blocked. An inert glucose analogue, 5-thioglucose, was then injected either into a lateral or the fourth ventricle, and the plasma glucose and somatotropin response was followed. The results showed that receptors mediating the hyperglycaemic response to glucoprivation and the subsequent decrease in the release of somatotropin are located in the hindbrain, and not in the forebrain (Pénicaud *et al.*, 1990).

A field of interest that has quietly grown over the last 10 years, with sporadic bursts of activity from the drug companies, is the development of GHRH analogues. Initial interest was generated by the discovery that certain opiod peptides stimulated the release of somatotropin. This report led to the development of a series of short (5–6 amino acids) peptides based on enkephalin (Momany *et al*, 1981; Swart & Van der Walt, 1986; Bowers *et al.*, 1991; Robinson *et al.*, 1992). Development of these compounds was initiated shortly before the structure of the naturally-occurring GHRH was elucidated in 1982. Interest in these compounds has continued to the present time, although apparently restricted to certain pharmaceutical companies (Bowers *et al.*, 1991; Robinson *et al.*, 1992). Recently, a nonpeptidyl secretagogue has been reported (Smith *et al.*, 1993), which suggests that interest in this line of research has not altogether waned.

Another approach has been to manufacture structural analogues of the naturally-occurring GHRH. Although GHRH normally contains 40–44 residues, it is only the 29 amino acids at the amino terminal end that are associated with the releasing activity (Ling *et al.*, 1984). By replacing arginine residues with agmatine (decarboxylated arginine, or 4-guanidinobutylamine), it is possible to create analogues that have an increased potency of more than 50-fold (Zarandi *et al.*, 1992; Roberge *et al.*, 1992). It has been suggested that this increase in potency may be due to either an increased receptor affinity (Zarandi *et al.*, 1992) or a greater biological stability, i.e. a greater resistance to proteolytic degradation (Roberge *et al.*, 1992).

Physiology of action

Any discussion of the physiology of somatotropin must be based on a species approach, owing to the wide variability of the response to this hormone across different species.

Although many of the mechanisms have been elucidated using the rat, this review will concentrate on the action of this hormone in productive domestic animals.

Cattle

The dairy cow has been used extensively as a model for research as a result of the lactogenic effect of somatotropin in that animal. The effect of somatotropin administration, both short and long term, on the lactation response has been well documented and has been extensively reviewed (Peel & Bauman, 1987). Briefly, the short-term response varies between 2 and 5 kg/day in high-yielding Holstein cows, regardless of the stage of lactation. The dose response appears to be curvilinear although the pattern in which the hormone is administered does not affect the response. There is no increase in feed intake during short-term administration of somatotropin. On the other hand, long-term administration of the hormone may lead to an increase of between 10% and 25% in high-yielding dairy cows, when measured over the entire lactation. In such cases, feed intake increased gradually to match the increased milk production, owing to an improvement of feed conversion. The lactogenic effect does not appear to be mediated by any direct effect on the mammary gland, as no-one has been able to demonstrate the presence of somatotropin receptors in the gland (Akers, 1983). The most pronounced effect of somatotropin is the release of the somatomedins or insulin-like growth factors (IGF) (Cohick *et al*, 1987; Davis *et al.*, 1987), chiefly from the liver, and there is some evidence to suggest that some of the lactogenic effect of bST may be mediated via these hormones (Prosser *et al.*, 1991). While the direct effect of these IGF hormones on the udder is still not clear, they would seem to coordinate the metabolism of a number of tissues in such a way as to provide the substrates necessary for the synthesis of the key constituents of milk, thereby promoting lactation (McCutcheon & Bauman, 1985). For example, cardiac output rises by 10%, while blood flow to the mammary gland increases by 35% (Davis *et al.*, 1988). The coordination of these various metabolic activities has been referred to as homeorhesis (Bauman & Currie, 1980).

However, the most striking effect of somatotropin in cattle is the ability to inhibit lipogenesis and possibly to increase lipolysis (Bauman, 1992), thereby exerting a major effect on lactogenesis. Although a decrease in body fat accompanies the administration of somatotropin, an increase in non-esterified fatty acids is only seen when the cows are in a negative energy balance, which suggests that the major effect is on lipogenesis (Ingle *et al.*, 1972; Eppard *et al.*, 1985; McCutcheon & Bauman, 1986). One of the mechanisms responsible for this effect may be a reversal of the effect of insulin on adipose tissue (Etherton *et al.*, 1987). Insulin promotes lipogenesis through increasing the uptake of glucose via specific transmembrane carriers (Birnbaum, 1992; Robinson & James, 1992), through the inhibition of hormone-sensitive lipase (Scow & Blanchette-Mackie, 1991), and by acting as substrate for glycerol-3-phosphate synthesis (Dominguez & Herrera, 1976). Adipocytes take up and hydrolyse insulin via a non-lysosomal pathway (Olefsky *et al.*, 1982), and somatotropin has been shown to modulate the activity of the protease responsible for this hydrolysis (Marinchenko *et al.*, 1992). However, note that the effect of insulin on lipolysis and protein synthesis in adipose tissue is not altered by the IGFs.

Sheep

Although not of any great economic importance from a milk producing viewpoint, sheep have been used as a model for studies on the effect of somatotropin. Studies on the role of adipose tissue in sheep have supported the contention that somatotropin chiefly affects the lipogenic side of the substrate cycle (Vernon, 1978; Vernon, 1979; Vernon *et al.*, 1981; Vernon, 1982; Vernon & Finley, 1985). The effect on growth is controversial, although in most cases there is an improvement in average daily gain (ADG) and feed conversion efficiency. A fairly consistent 10 to 20% decrease in carcass fat was observed in most studies (Prosser *et al.*, 1991; Heird & Hallford, 1991; Johnsson *et al.*, 1985). One study (Johnsson *et al.*, 1985) showed recombinant bovine hormone to increase wool growth, whereas other studies (Wallace, 1979; Wynn, 1982) using the ovine form purified from the pituitary initially suppresses wool growth, followed by a rebound after cessation of treatment. Whether this is due to a difference between the ovine and bovine forms, which is unlikely due to the single amino acid difference, or whether it is some other factor in a possibly unpure version of the ovine hormone, is not clear. If it is due to a species difference, then recombinant DNA technology may be used to engineer a chimaeric somatotropin molecule with wool growth-promoting activity.

As in cattle, the major anabolic effects of somatotropin appear to be mediated by the IGF family. This somatotropin/IGF axis is dose-dependent and is sensitive to nutritional status (Bass *et al.*, 1987). Although diet affected the amount of somatotropin binding to liver membranes, as well as the concentration of IGF-1 in plasma, that did not reflect in the growth rate or carcass composition of lambs (McLaughlin *et al.*, 1993).

Another potential use for somatotropin may lie in improving the reproductive response of ewe lambs. It is well known that ewe lambs that grow at faster rates and mature earlier, conceive sooner (Southam *et al.*, 1971). Unfortunately, even after four months of treatment, the response of these lambs to treatment was disappointing, with only marginally enhanced reproductive and growth responses (Heird & Hallford, 1991). Similar studies showed that somatotropin does not in fact provide any direct clues for the normal timing of neuroendocrine maturity in female sheep (Suttie *et al.*, 1991).

Pigs

The major effect in pigs is anabolic, leading in general to a 10–20% increase in daily gain, a 15 to 35% improvement in feed conversion efficiency, a 30 to 40% decrease in lipid deposition and a 20 to 30% increase in protein deposition (Chung *et al.*, 1985; Evock *et al.*, 1988; Campbell *et al.*, 1988). The optimum dose depends on which growth variable one wishes to encourage. For example, optimal growth is obtained at a lower dose than required for maximal feed efficiency (Boyd *et al.*, 1986). It is possible, in the light of the effect on body composition, that even greater effects may be obtained by careful rebalancing of the diet, to provide the necessary extra protein. However, a word of caution should be sounded; the action of somatotropin in pigs may also lead to some systemic abnormalities such as liver and kidney degeneration, oedema and an arthritis-like condition (Evock *et al.*, 1988). In the end, the dosage chosen will have to reflect the economic realities of gain balanced against loss. In pigs, it would appear that the anabolic effect of somatotropin is also

mediated by circulating IGF-1, rather than in a paracrine fashion (Grant *et al.*, 1991).

Reptiles

Nile crocodiles (*Crocodylus niloticus*) also responded to treatment (0.325 μg/g twice a week for 4 weeks) with human somatotropin, by increasing feed intake from 2.8 g/kg to 29.8 g/kg, gaining 8.1% body weight (while control animals lost 6.3%) and increasing body length by 3.93% (none in control animals, Kimwele *et al.*, 1992). In this case, the treatment was given to correct for anorexia, but there may be commercial applications resulting from this report.

Mechanism of action

Somatotropin released into the blood stream is bound by one of the two specific proteins found in plasma, the one with a high affinity and the other with a low affinity (Baumann *et al.*, 1988). Either of the binding proteins will bind one molecule of somatotropin, to form complexes of 83 kDa = about 246 residues (high affinity) or 125 kDa (low affinity). The high affinity protein corresponds to the extracellular portion of the hepatic somatotropin receptor (Baumann, 1990). In man, about 50% of circulating somatotropin is complexed to binding proteins, which are low at birth, and increase to reach maximum values in young adults (Fontoura *et al.*, 1991). By sequestering somatotropin, the high affinity binding protein substantially interferes with the interaction between somatotropin and its receptor (Mannor *et al.*, 1991). The clearance rate of the bound form is 10-fold lower than that of the free form of the hormone. The bound form is distributed in almost double the intravascular space compared to the distribution of the free form in a volume equal to the entire extracellular space. Although this complex inhibits the binding of somatotropin to the cellular receptor by competition *in vitro*, it actually enhances the activity of the hormone *in vivo*, owing mainly to its effect on half-life and persistence (Baumann, 1991). These binding proteins also appear to exhibit a diurnal rhythm, in which the nadir occurs at night (Carlsson *et al.*, 1993).

Similar binding proteins are found in many mammals, those closest to man are the rabbit, pig, mouse and rat. On the other hand, ovine and bovine species appear to have little binding protein (Shaw & Baumann, 1988). The binding protein appears to be generated by one of two mechanisms, either a proteolytic cleavage near the transmembrane domain, or *de novo* synthesis from a truncated mRNA. The former mechanism has been demonstrated in man and rabbit, and the latter in pregnant mouse and rat (Baumann, 1990). The low affinity binding protein has a greater affinity for the smaller somatotropin, a finding for which there is as yet no physiological function. The somatotropin receptor consists of a single polypeptide chain of 620 residues, with a single transmembrane domain (see Figure 2). It has been shown to belong to a superfamily of receptors which include those for, *inter alia*, prolactin, the interleukins, erythropoietin and the interferons (Kelly *et al.*, 1991; De Meyts, 1992). The binding of the hormone to its receptor exhibits several unique features. The receptor exists as a dimer, but it binds only one molecule of a non-symmetrical hormone. What is remarkable is that the two identical faces of the receptor bind to two different sites on opposite sides of the four-helical bundle of the hormone. The dimerization of the receptor is probably induced by the binding of the hormone, and it therefore also brings together the intracellular domains that are responsible for the 'second messenger' portion of activation (De Meyts, 1992) (see Figure 2). Although somatotropin receptors were originally thought to be associated mainly with the liver, they have now been demonstrated in many other tissues, such as kidney and the cardiovascular and respiratory systems (Lobie *et al.*, 1992). The distribution of the receptor has also been described within the cell, where it has been found not only on the cell membrane, but also within the cell, even in the nucleus. It has therefore been suggested that this receptor is synthesized locally to facilitate intracellular transport of somatotropin and/or to regulate transcription of presumably the IGF in a variety of tissues (Lobie *et al.*, 1992). An example of this is the demonstration of receptors in the canine renal proximal tubule, where the hormone promotes ammoniagenesis (Chobanian *et al.*, 1992). Since somatotropin promotes rapid growth which is often accompanied by a metabolic acidosis owing to an increase in circulating fatty acids and ketone bodies, an ability to provide ammonia for acid-base balance in the kidney is required.

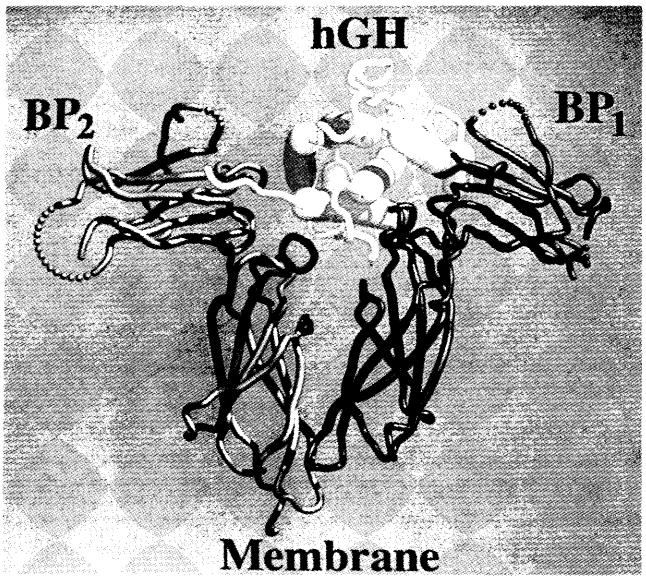

Figure 2 Ribbon diagram of human somatotropin (white) in complex with two molecules of the extracellular domain of its receptor, shown as binding protein 1 (black) and binding protein 2 (grey). Taken from De Meyts (1992).

The ontogeny of these receptors in bovine hepatocytes has been investigated, and it was shown that the number of these receptors increased with age during the first six months of growth, in parallel with plasma concentrations of IGF-1, whereafter both declined slightly (Badinga *et al.*, 1991). It is, therefore, suggested that increasing receptor numbers in bovine liver contribute significantly to postnatal growth in cattle (Breier & Gluckman, 1991). In rats, however, receptor mRNA in the brain declines with age, in contrast to the increase in these receptors in peripheral tissues (Lobie *et al.*, 1993), thereby suggesting that a specific somatotropin-IGF-1 axis influences brain growth and maturity.

Conclusions

While there have been startling advances over the past few years, there is still much to learn about the mechanism and physiology of somatotropin. The fact that there are so many different forms of the hormone in circulation (the so-called dominant form, the 22 kDa molecule, constitutes only about 21% of the total immunoreactivity in the plasma), complicates the interpretation of results, and leads to the confusion surrounding the assay of somatotropin. The puzzle of the mammary gland still needs to be solved, as does the role of the different forms of binding proteins. I have not attempted to describe the role of the IGF hormones, although it is difficult to understand the action of somatotropin without them. It is obvious that any future description of somatotropin action will have to include the role of the IGF proteins. There are many avenues open for exploration, particularly in the domestic animals. Most of what we know is derived from the rat and the human, and, as pointed out above, there are known to be major differences between species. Although most of the research to date has concentrated on the role of somatotropin itself, it is possible that exotic releasing factors, modified forms of binding protein, or genetically engineered receptors may play important roles in the future.

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