Physiology of the Somatotropic Axis with Particular Reference to the Ruminant

P. D. GLUCKMAN and B. H. BREIER
Department of Paediatrics
University of Auckland
Auckland, New Zealand

S. R. DAVIS
Ruakura Animal Research Station
Ministry of Agriculture and Fisheries
Hamilton, New Zealand

ABSTRACT

The physiology of growth hormone and the insulin-like growth factors are reviewed with particular reference to the dairy industry. Growth hormone secretion in the ruminant is pulsatile in nature and nutritional factors have a major impact on its secretion. Isolation of growth hormone-releasing factor has allowed further progress in understanding the mechanisms underlying growth hormone release. The receptors appear to be under active endocrine and metabolic control, and nutritional influences on the somatotropic axis are in large part mediated through changes in somatotropic receptors. The mode of action of growth hormone to induce acute metabolic effects and lipolysis remains to be resolved, but there is increasing evidence that its anabolic actions are mediated by the insulin-like growth factors.

Recent studies of measurement of insulin-like growth factor-1 and -2 in the ruminant and the role of growth hormone, nutrition, insulin, and sex steroids in their regulation are reviewed. The relative role of the two factors and the multiple forms of their receptors remain to be resolved.

It is well-documented that growth hormone is galactopoietic. The evidence that this effect is largely due to enhanced nutrient supply to the mammary gland is not convincing. Effects of growth hormone are indirect and may be mediated by the insulin-like growth factors. The potential is considerable for manipulating the growth hormone insulin-like growth factor axis to enhance lactation.

INTRODUCTION

There is a rapidly increasing interest in the potential for manipulation of the somatotropic axis to increase productivity in the ruminant. Attention has focused primarily on the possible role of enhancing circulating growth hormone (GH) concentrations to increase milk production, to increase lean body mass, or to alter carcass composition. A variety of techniques have been developed to manipulate concentration of circulating GH. These techniques include administration of GH produced by recombinant deoxyribonucleic acid (DNA) techniques, introduction of additional copies of the GH gene into the zygote, or administration of GH-releasing factor (GRF). In addition, the possibility that the desired effects of GH are mediated by the insulin-like growth factors (IGF) or somatomedins has stimulated interest in their biochemistry and physiology. There has been a rapid and recent increase in the understanding of the biochemistry of the IGF. The IGF are now known to have structural and evolutionary relationships to proinsulin, and there have been two major forms IGF-1 and IGF-2 isolated. Recent developments of specific assays for these two peptides have allowed rapid progress in resolving the physiology of the somatotropic axis.

This review will focus on the physiology of the somatotropic axis with some brief comments on its relevance to the dairy industry. Where possible, the review will concentrate on data obtained from studies on the ruminant. However, large gaps remain in our knowledge of the somatotropic axis in the ruminant, and examples...
have had to be taken from the more extensive rodent and primate literature. Such extrapolation between species must be interpreted with considerable caution. Most of the studies, particularly in the ruminant, have been based on few animals. They have often been inadequately controlled in terms of nutritional influences on the axis. Further, little consideration has been given to the pulsatile nature of GH release, which means that frequent and prolonged blood sampling is essential. Except where there are compelling reasons for inclusion, such studies have not been detailed in depth in this review.

GROWTH HORMONE

Plasma Concentrations of Growth Hormone

The secretion of GH in adult mammals is pulsatile in nature, but the physiological significance of such pulsatility remains to be elucidated. In man there is evidence that growth disorders may be associated with altered pulsatility of GH (22). In addition, this marked pulsatility means that many studies of GH secretion are of limited value because assessment of GH release was based either on infrequent sampling or on sampling over very short periods.

In the adult rat, the most extensively investigated endocrine model, GH secretion is characterized by a synchronous endogenous ultradian rhythm with high amplitude GH secretory peaks occurring at 3 to 4-h intervals throughout 24 h. In the intervening trough periods, plasma GH concentrations are undetectable (210).

In contrast, GH secretion in cattle appears asynchronous and episodic (5, 221). Our data obtained in Angus steers shows episodic GH pulses that occur throughout the 24-h cycle. These appear to have no consistent relationship to time of feeding or to time of day. There appears to be considerable variation in the GH profiles among individual animals (Figure 1). These different patterns are consistent over many months in individual animals and are seen at all feed intakes, suggesting that they may be genetically determined. There is other evidence of genetic differences in the somatotropic axis (66), and this area merits more formal investigation. The functional significance, if any, of such differences is not known.

Particularly under conditions of high nutritional intake, there is a suggestion of a diurnal rhythm in plasma GH concentrations in the steer with higher interpulse values and a trend to increased pulse frequency at night (Figure 1). However, insufficient data are available to assess whether this is an intrinsic diurnal rhythm or the interaction of feeding and other environmental stimuli.

Release of GH is associated with slow wave sleep in humans (212) and baboon (162) but not in the rhesus monkey (118) or the rat (229). In ruminants, no relationship was observed between sleep phases and GH release (99, 214). Thus, the phenomenon of sleep-related GH release appears restricted to higher primates.

Sexually differentiated patterns of GH secretion are documented in the rat where pulse amplitude is higher and baseline values are lower in the male than in the female (121). Similar sexual dimorphism is observed in the ruminant. Higher GH pulses are observed in rams than wethers (59). Bulls exhibit GH episodes of greater amplitude than steers with
no significant differences in baseline values (5). Average GH concentrations are reported to be higher in bull calves than in heifer calves at 5 mo of age (126).

Plasma GH in the rat shows age-related variations. There is an obvious increase in plasma GH during fetal life and a subsequent decline during the postnatal period (182). During late prepubertal life, GH increases again and gives way to the adult secretory patterns observed after the onset of puberty (120).

The ontogeny of GH secretion in the perinatal period has been most extensively studied in the sheep. In the fetal lamb, GH concentrations are markedly elevated compared with postnatal concentrations (80, 81). Fetal GH release is pulsatile in nature; the male fetus has higher mean GH concentrations and a greater pulse height (9). Within 24 h of birth, GH concentrations have fallen 10-fold with both a reduction in pulse amplitude and interpulse values to a secretory pattern not markedly different from the adult. It has been postulated that the high perinatal GH concentrations are a consequence of immature hypothalamic regulatory mechanisms (80), and prematurely delivered lambs and humans have relatively elevated GH concentrations (85).

Sex steroids increase GH release, and thus, it is expected that at puberty, GH secretion will increase. In prepubertal man exogenous estrogens or androgens enhance the GH response to many stimuli. Testosterone therapy is reported to increase GH pulsatile release (163). In the ruminant there is evidence that sex steroids enhance GH release (93), but detailed study of changing GH release during puberty is needed. Changes in GH secretion during pregnancy are reported, but the effects of nutrition are probably dominant in determining GH secretion during this period. It is not known whether placental lactogens (PL) affect GH secretion in the ruminant as they do in humans (125). Recent studies in the red deer stag (Cervus elaphus) suggest seasonal changes in pulsatility of GH release. Pulsatile GH release was enhanced markedly in spring; onset of this increased GH release is at about the time that the gonadotropic axis is activated, and it may be that androgens mediate the seasonal change in this species (208). Nutritional factors seem an unlikely explanation, as GH secretion generally decreases with improved feed intake. Possible seasonal changes have not been studied extensively in other species. However, in heifers, serum GH concentrations do not change markedly with photoperiodic or ambient temperature changes during fall and winter or spring and summer seasons (161, 168).

Control of Growth Hormone Secretion

Secretion of GH is regulated by a dual system of hypothalamic hypophysiotrophic hormones; GRF stimulates GH release and somatostatin (SRIF) inhibits GH release. The release of these hypothalamic hormones are, in turn, influenced by a network of monoaminergic neurones. Thus, a variety of neurotransmitters such as norepinephrine, dopamine, serotonin, and many neuropeptides play a role in the neuroendocrine regulation of GH secretion. However, these neurotransmitters have been investigated only slightly in the ruminant. Studies in other species are reviewed extensively elsewhere (96, 141). The GRF and SRIF are secreted in the hypothalamus and transported to their site of action, the pituitary, through the hypothalamic-hypophyseal portal vascular system.

Growth Hormone-Releasing Factor

Rivier et al. (187) isolated and characterized a 40 amino acid GRF (hGRF-40) from a pancreatic islet tumor, that had caused clinical symptoms of acromegaly. This peptide was highly potent in stimulating GH secretion in vitro and in vivo. Independently Guillemin et al. (95) reported the structure for a 44 amino acid GRF (hGRF-44) isolated from a pancreatic islet tumor in another acromegalic patient. The amino acid sequence for hGRF-40 was identical to the first 40 amino acids of hGRF-44, and it is now generally assumed that the larger form is the physiological form. Analogues as small as 29 amino acids are biologically active. Synthetic preparations of both hGRF-40 and hGRF-44 peptides possess full biological activity and specifically stimulate the release of GH in vitro in rat pituitary cell culture (24) and in vivo in rats (119, 187) and in cattle (156, 157). Both preparations of hGRF stimulate a GH pulse within 5 to 15 min after injection in Holstein steers, and a dose-response relationship
has been established for hGRF-44 over a range of 10 to 1000 μg (156). Different preparations of GRF induce a similar maximum post-injection concentration of GH in sheep (104).

Moseley et al. (156) examined the effects of extended treatment with hGRF-44, given as frequent microinjections on the release of GH in steers and reported increased serum GH concentrations throughout the 5-d treatment period. Administration of 3.6 mg GRF/day for 5 d increased baseline GH concentrations, the amplitude of GH pulses, and the area under the GH curve but did not change the number of GH pulses per 12 h compared with controls. The GH response to GRF infusion was not diminished during the 5-d treatment period, which suggest that desensitization of the pituitary or down regulation of GRF receptors does not occur, at least with this dosage regimen. Furthermore, the episodic nature of the GH secretory pattern was maintained during GRF treatment.

In rats, pituitary stores of GH were depleted by continuous 24 h infusion of hGRF-44 (15 μg/h) (225). Within 2 h of infusion, plasma GH rose continuously to a concentration 10-fold above the normal range of a pulse. By 6 h, plasma GH concentrations began to decrease slowly and reached a nadir by 12 h. After continuous 24-h infusion, a 2 μg bolus injection of hGRF failed to increase plasma GH concentrations. This may reflect either depletion of pituitary stores of GH or down regulation of GRF. Further evidence for desensitization by GRF is reported for in vitro studies of rat pituitaries (31). Thus, there remains uncertainty as to the factors influencing the effects of GRF on pituitary GH release. Somatostatin (134) and the IGF (19) can noncompetitively modulate the GH response to GRF (92). In addition, considerable effort will be needed to evaluate optimal regimens for administering exogenous GRF.

Somatostatin

Somatostatin, a tetradecapeptide, has been isolated from ovine hypothalami on the basis of its ability to inhibit GH release from anterior pituitary cells in culture (25). It has been identified also in a number of other tissues, notably the central nervous system (167), pancreas (88) and alimentary tract (176). The SRIF has widespread inhibitory effects on different endocrine systems and gastrointestinal and nervous functions (for review see (217)).

The inhibitory actions of SRIF on GH secretion are now well-established (217). The onset of its effect is rapid and duration of action is brief. After cessation of SRIF infusion, GH concentrations tend to rebound only when spontaneous surges of secretion are evident. Administration of SRIF during trough periods of low GH secretion does not result in post-inhibitory rebound (141). Administration of SRIF to mature ewes inhibited the arginine-stimulated GH response but failed to exert a significant effect on basal plasma GH concentrations (54).

Generation of the Growth Hormone Pulse and Negative Feedback

Until recently it has been assumed that the GH pulse was induced by a pulse of GRF. Certainly passive immunization against GRF abolishes the GH pulse (226). It was reported that SRIF affected either basal GH secretion (213) or pulse height (207). It was thought that SRIF release was relatively constant and did not play a role in generating the pulsatile pattern of GH release; however, recent experiments with rats suggest that this model is simplistic and that both GRF and SRIF release is variable (209). Indeed, it may be that the initial event in the generation of a GH pulse is a diminished release of SRIF (45), thus allowing a greater response to and possibly greater release of GRF. The pulse may then be terminated by increased SRIF release. Direct experimental evidence of this model has been obtained recently (174).

There is increasing evidence that negative feedback operates within the somatotropic axis. Both IGF-I and GH when administered centrally inhibit pituitary GH release (1). Somatostatin inhibits the response to GRF (134). Growth hormone stimulates hypothalamic SRIF release (19) and the IGF have been shown to inhibit GH release at both the hypothalamic and pituitary (1, 19).

Influence of Nutritional Status on Growth Hormone Release

There is increasing evidence that nutritional status plays a major role in determining circulating GH concentrations particularly in the
ruminant. This must be taken into account in designing or interpreting studies of GH secretion in the ruminant. During periods of nutritional deficit, elevated concentrations of circulating GH have been reported for several species including pigs (6), man (148), and sheep (63). Plasma GH concentrations are higher in underfed compared with adequately fed lactating cows (101, 113).

In recent studies, we have observed a marked effect of nutritional status on the GH-IGF axis in Angus steers (Figure 2). There was a threefold increase in GH pulse amplitude in steers fed at 1% dry matter (DM) of live weight in comparison with steers fed at 3% DM of live weight of a high concentrate diet. Neither the GH pulse peak frequency nor baseline concentrations were changed with these nutritional manipulations (26). Whether this increase in GH secretion at underfeeding is related to a caloric or a protein effect has not been investigated fully in the ruminant. Kung et al. (133) have reported higher plasma GH concentrations in cows fed a low protein diet than in cows fed a high protein diet, but the former animals had decreased total intakes, and blood samples were not taken frequently enough to compare hormone profiles. Of greater interest, in our study of Angus steers GH secretion was reduced at 3% DM compared with that at 1.8% DM. Because 1.8% DM intake is comparable with that of steers on high quality pasture, it suggests that even under good pasture conditions, circulating GH concentrations may be modulated by nutritional factors. Further, IGF-1 concentrations were similar in the steers on 1.8% DM and 3% DM intake but reduced in the 1% DM group. We conclude that the relationship between GH and IGF-1 release is more efficient in the 3% DM intake group than in the 1.8% DM intake group, which in turn is more efficient than in the 1% DM intake group. These observations led us to postulate that the pasture-fed ruminant may be in a state of partial GH resistance (as defined by the efficiency of GH to stimulate IGF release).

In sheep the GH response to GRF is enhanced during restricted feeding compared with ad libitum feeding (104). This suggests an explanation for the enhanced GH secretion during diminished nutrition; reduced intake may lead to reduced number of GH receptors both peripherally and perhaps centrally and thus diminish negative feedback within the somatotropic axis mediated in part via SRIF.

The increase in GH release associated with limited nutrient availability is postulated (10, 12) to mobilize energy from adipose tissue to satisfy the needs for metabolism and facilitate the transfer of metabolites from adipose to lean tissue when intake of dietary nutrients is not adequate.

It has been pointed out in a previous review (40) that the understanding and interpretation of circulating hormone concentrations has to be seen in light of hormone metabolism. The situation for GH metabolism in the ruminant has been elucidated in part in a study by Trenkle (215). He reported an increase in half-life of injected GH from 20.3 to 31.9 min in fasted calves. In addition, turnover and the metabolic clearance rate of GH were reduced significantly by starvation. This indicates that the high circulating GH concentrations ob-

![Figure 2. Plasma growth hormone profiles of a young steer, fed with a dry matter intake of 3% of body weight/d of liveweight (top) and 4 wk later during negative energy balance while being fed with a dry matter intake of 1% of body weight/d of liveweight (bottom). Blood samples were collected at 15-min intervals over a 25-h period.](attachment:image)
served in the underfed ruminant are not utilized fully and points to a partial refractoriness of target tissue. This again supports the concept of malnutrition-induced, GH resistance.

Although elevated concentrations of GH during peak lactation could be expected to reflect the nutritional status of the animal, we suggest that elevated concentrations of GH are of functional significance in this situation and not a consequence of decreased utilization. In the lactating dairy cow, Vasilatos and Wangsness (221) have observed higher concentrations of plasma GH (increase in magnitude of secretory episodes) at peak lactation than during later lactation. Although there is a distinct possibility that nutritional requirements are not fully met during early lactation, an investigation of kinetic parameters of GH metabolism suggests that turnover of GH is highest at peak lactation and decreases toward the dry period (100). These findings imply that GH is utilized most intensively at peak lactation when GH secretion is at its highest in the dairy cow. We therefore conclude that some important physiological aspects of high GH observed in starved nonlactating ruminants are different to the high circulating GH concentrations in dairy cows at peak lactation; this points toward the physiological significance of GH in stimulating galactopoiesis.

Influence of Sex Steroids on Growth Hormone Secretion

The pattern of GH secretion is sexually dimorphic in many species. Baseline GH concentrations are increased by neonatal or prepubertal gonadectomy in adult male rats (120). Replacement therapy with testosterone completely abolishes this effect, indicating that continuous presence of testosterone is necessary for maintenance of the characteristically low baseline GH concentration of the adult male rat. In contrast, GH pulse height is decreased in male rats during adult life by neonatal, but not by prepubertal gonadectomy, suggesting that neonatal androgen secretion predetermines GH pulse height in adult male rats (121). This appears to be determined by imprinting effects of neonatal testosterone on the hypothalamus rather than due to circulating testosterone itself.

The effect of sex steroids on GH secretion in farm animals has been considered extensively (59, 106). Treatment of wethers with either testosterone or diethylstilbestrol results in increased mean GH concentrations compared with untreated controls. Furthermore, plasma androgen concentrations are significantly correlated with mean plasma GH within intact rams, indicating that rams with higher androgen secretion have also higher plasma GH concentration (59). Implantation of estrogenic anabolic compounds in steers increases the secretion rate of GH from the pituitary gland but does not change its half-life (93).

Metabolic and Environmental Factors Affecting Growth Hormone Secretion

Growth hormone influences carbohydrate metabolism and insulin responsiveness of various tissues (75). Plasma concentrations of glucose affect GH plasma in man, and a drop in plasma free fatty acids (FFA) causes a rise in plasma GH concentration. An increase of FFA, however, almost completely inhibits the response of GH to arginine (149) and GRF (115). Data from our laboratory suggests that the action of FFA is at the pituitary (Bassett and Gluckman, unpublished data).

In contrast to the results obtained for man, intravenous glucose administration does not suppress plasma GH in cows. Reynaert et al. (180) observed a significant increase in plasma GH concentrations after glucose infusion. It is thought that decreasing plasma FFA in cows after glucose infusion causes an increase in plasma GH concentrations. Changes in FFA concentrations are proposed as the prime metabolic controller of GH release in ruminants (107).

Infusion of amino acids increases plasma GH in several species and arginine is the most effective amino acid in sheep (53). The secretory response of GH to daily arginine infusion is maintained over long periods in cows (33). The mechanism of action of arginine-induced GH secretion probably involves stimulation of GRF at the hypothalamus.

Various forms of stress provoke an increase in plasma GH in humans (79) but cause a decrease of plasma GH concentration in rats (211). Reynaert et al. (181) studied the effect
of transport stress in cattle and observed an increase in plasma FFA and a decrease in GH after extended stress. The stress-induced rise in FFA and the decrease in GH in cattle is postulated to be mediated by adrenergic mechanisms (181).

Growth Hormone Receptors

Growth hormone receptors have been described for liver cell membranes, adipocytes, lymphoblastoid cells, fibroblasts, and pancreatic B-cells (20). In light of the physiological functions of GH, the binding of GH to hepatocytes and adipocytes is of particular interest and will be discussed.

The GH, PL, and prolactins form a family of phylogenetically related hormones. The individual hormones have been characterized as "lactogenic" or "somatotropic" based upon their behavior in bioassay. There are at least two classes of receptors that bind these hormones. Somatotropic receptors are those that bind ruminant GH more potently than prolactin and are assumed to mediate the somatogenic effects of these hormones. Lactogenic receptors bind prolactin more potently than ruminant GH and are assumed to mediate those actions characteristic of prolactin. Misleading conclusions can be obtained where binding studies using nonhomologous systems have been performed. In particular, studies with human GH as the ligand can be misleading. In subprimate species, hGH appears to bind to a distinct receptor class (108, 109). Recently evidence for a distinct PL receptor has been reported for ruminants (69), and this may be the basis for anomalous GH binding in heterologous systems.

Hepatic somatogenic receptors appear to be under active endocrine and metabolic control, although direct evidence for such control is restricted to the rodent (136, 137). Starvation and malnutrition in the young rat reduce the binding of GH to hepatic membranes. This is associated with a fall in circulating IGF-1 concentrations. Refeeding restores both measures to normal (137). However recent evidence suggests that there may be nutritionally affected postreceptor changes as well as a reduction in GH receptor affinity or number (138).

Hypophysectomy of rabbits and lambs has markedly reduced hepatic GH receptors (178). Because these receptors are restored by GH treatment it is proposed that GH plays a major role in maintaining its own hepatic receptors (17). No data are available on the effects of GH therapy on GH receptor numbers in intact animals.

There are marked ontogenic changes in somatogenic binding. In the fetal and neonatal lamb, no hepatic GH binding is observed. About 4 to 6 d after birth, specific somatogenic binding is first observed (84). This lack of somatogenic receptors is suggested as the basis for the lack of an effect of GH on prenatal growth. The appearance of the GH receptor postnatally is associated with a rise in circulating IGF-1 concentrations and the appearance of GH-dependent growth. The basis for the appearance of the GH receptor in the neonatal lamb liver is not known. It is probably not dependent on parturition as in other species such as man and rats. Growth hormone-dependent growth is first observed well after birth (80, 81).

Somatogenic receptors are also in adipose tissue (76) and have been studied most extensively in rodents. Growth hormone has biphasic effects on adipose tissue to stimulate lipogenesis and lipolysis (89). Goodman (91) has attempted to relate the different functions to different degrees of receptor saturation; however, such studies are limited by use of heterologous hormones. Studies in ruminants are lacking. Recently both pituitary ovine GH and recombinant bovine GH have been shown to stimulate lipogenesis in ovine adipose tissue in vitro (Lewis and Bass, personal communication).

Actions of Growth Hormone

It is now generally accepted that GH either directly or indirectly stimulates anabolic processes such as cell division, skeletal growth, and protein synthesis (growth-promoting and galactopoietic activity). In addition, important metabolic effects are observed, including increased oxidation of fat (lipolytic activity) and inhibition of transport of glucose into body tissues (diabetogenic activity).

Anabolic Effects of Growth Hormone

Effects of GH on cell division and skeletal
Actions of Growth Hormone in Adipose Tissue

Studies of GH on adipose tissue are based on either clinical observations in man or in vitro studies in rats. Thus, extrapolation to the ruminant may be misleading. The initial response to GH in adipose tissue is insulin-like (89); it consists of increased transport and oxidation of glucose and accelerated glucose metabolism to fatty acids and CO₂. This response also includes increased oxidation of leucine and antagonism of the lipolytic actions of epinephrine.

The initial insulin-like effect of GH is transient and cannot be elicited by GH until several hours have elapsed (89, 91). There is evidence that GH does not produce its insulin-like effect by interacting with the insulin receptors and that refractoriness to this effect of GH occurs at a postreceptor site (94). The insulin-like effect of GH, once triggered, appears independent of the continued presence of the hormone. If specific antiserum is added more than 10 min after incubation of GH, the insulin-like response persists (89).

A second response to GH in adipose tissue, the promotion of lipolysis, can be seen 2 h after exposure to GH. This lipolytic response is not subject to refractoriness (94). Induction of lipolysis requires the continuous presence of the hormone for some hours, suggesting a requirement for constant stimulation of surface receptors to obtain a sustained response (89, 94). Goodman (90) used recombinant human GH (hGH) in rat adipose tissue and found support for the conclusion that both the initial insulin-like and the delayed lipolytic actions of GH are intrinsic properties of the molecule and cannot be attributed to contaminants present in the pituitary extract.

Because insulin resistance and carbohydrate intolerance have been reported in acromegaly, it was postulated that GH has diabetogenic properties, producing hyperglycemia and glucose intolerance and causing insulin resistance and hyperinsulinemia (75). This diabetogenic effect of GH requires chronic exposure of the organism or tissue culture to high doses of the hormone (131) and presumably represents interference with a postreceptor step in insulin action (188). Cameron et al. (29) compared different vertebrate GH in rat adipose tissue and showed that the diabetogenic activities are intrinsic properties of GH. This remains a confusing area, and there is conflicting evidence with reports relating the differential actions of GH to different fragments of the GH molecule, to pituitary contaminants, or possibly to different posttranslational products of the GH gene. For example, Hart et al. (102, 103) attributed the multiple metabolic activities of GH to different molecular forms, which were separated from pituitary bovine growth hormone (bGH) by chromatography and consequently tested for their physiological activities.

Studies of the effects of ruminant GH on adipose tissue are limited. Acetate incorporation into lipid was increased by bGH in adipose tissue removed from cows in mid to late lactation (127). However, a direct effect of bGH on lipolysis in ruminant adipose tissue remains to be demonstrated. Certainly in cows in negative energy balance increased mobilization of FFA in response to bGH treatment has been shown (164, 165), but this may simply be due to the increased energy deficit evoked by GH treatment.

Lipolytic and diabetogenic activities of bGH have been studied recently (102). In contrast to pituitary-extracted bGH, recombinant-derived bGH was not lipolytic in studies using rat adipose tissue in vitro. However, both natural and recombinant bGH were of similar potency in increasing concentrations of plasma FFA in nonlactating sheep (103). Similarly, both forms of bGH significantly impaired the ability of insulin to lower the concentration of plasma glucose in goats (102). It was suggested that diabetogenic and lipolytic activity were intrinsic to bGH with the proviso that lipolytic activity may become apparent only after molecular modifications in vivo or activation of a lipolytic intermediate. The insulin resistance
and increased lipolysis evoked by exogenous bGH presumably conserve glucose and fatty acids for utilization by the mammary gland. There is little or no change in plasma concentration of these milk precursors in lactating animals treated with bGH (13), probably due to the increased nutrient drain at the udder. In non-lactating sheep and low producing beef cows, increases in plasma FFA and glucose have been apparent after treatment with bGH (103, 105) but not usually in lactating dairy cows (14).

Actions of Growth Hormone in Mammary Tissue

There are only few conclusive studies on the role of pituitary hormones in mammary function. The important role of prolactin (PRL) in lactogenesis in bovine mammary tissue has been investigated in vitro (78), and the endocrine control of lactogenesis has been reviewed recently by Akers (4). Although participation of bGH in the endocrine control of galactopoiesis in goat mammary tissue has been discussed (205), GH receptors have not been identified in bovine or any of the ruminant mammary tissues. Neither ovine nor bGH significantly increased casein or fat synthesis above controls in bovine mammary gland explants (78). These studies indicate that the in vivo stimulation of milk secretion by exogenous administration of bGH does not result from direct actions on mammary tissue.

In contrast, lactogenic properties of hGH are well-established (130). It was shown by Gertler et al. (77) in explants from bovine mammary glands, that hGH binds specifically to prolactin receptors in the mammary gland of the lactating cow.

Mechanism of Action of Growth Hormone on Ruminant Lactation

Recombinantly derived bGH stimulates lactation in dairy cows with a similar efficacy to material extracted from pituitary glands (11, 12). Previously many trials with pituitary-extracted bGH had elicited milk production responses varying from 10 to 60%, corresponding to an increase in yield, on average, of 4.0 kg/d (40). Some of the variability in response of dairy cows to bGH was attributable to variable doses of bGH. In Holstein cows, maximum yield response of 8.3 kg/d was achieved with a daily injection of 77 mg bGH (68).

Galactopoietic action of GH is associated with increasing partitioning of nutrients for milk synthesis (12, 166) and, after a lag phase of approximately 5 wk, increased feed intake (13). The increased gross efficiency of feed conversion to milk noted in many short-term trials in response to bGH (14) may be simply attributed to body tissue mobilization or increased partitioning by which FFA are preferentially taken up by the mammary gland at the expenses of other tissues. In longer trials, where feed intake was increased, improvement in gross efficiency of feed conversion to milk was still apparent (13), arising by dilution of the "fixed costs" of maintenance at the higher feed intake. There is no need to postulate any change in the partial efficiency of utilization of metabolizable energy for milk synthesis to account for the observed increase in feed efficiency of bGH-treated cows, and indeed, no increase was measured (12).

There are three major questions regarding the mode of action of GH in maintaining or enhancing lactation in ruminants. First, is the galactopoietic action of GH mediated through other hormones or growth factors? Second, does the milk production increase represent a compliant response of mammary tissue to an increase in nutrient supply through diabetogenic and lipolytic actions of GH? Third, is the galactopoietic effect of exogenous GH via actions on the productivity of existing mammary secretory cells, via an increase in the number of secretory cells, or via a reduction in the rate of secretory cell regression and loss?

There are several lines of evidence that indicate that exogenous bGH does not act directly on mammary tissue. No direct effect of GH on milk synthesis has been demonstrated in lactating mammary tissue in culture (15, 77). Efforts to measure specific binding of bGH to mammary membranes from lactating ewes or cows have not been successful (4). Arterial infusion of GH into the udder of lactating ewes did not increase milk production of the infused udder half (144).

It is unlikely that the galactopoietic activity of bGH is achieved simply through increasing blood concentrations of essential (and rate limiting) precursors for milk synthesis. With cows in positive energy balance, milk yield has
never been increased by infusion extra nutrients via either intravascular or abomasal infusion to the same extent as by subcutaneous GH injection (58). Abomasal infusion of a glucose-sodium caseinate mixture in GH-treated cows did not enhance the production response to GH (165).

Similarly, although blood flow (and hence, nutrient supply) to the udder is increased by bGH treatment of cows (56) and goats (147), it is likely that the blood flow response is caused through hormonal stimulation of mammary metabolism and is a response to, rather than a cause of, the increase in milk yield. Bovine GH therapy was associated with an increase in the proportion of cardiac output perfusing the udder from 14.4 to 18.7% in Jersey cows at peak lactation. Only half the increase in cardiac output could be accounted for by the increase in udder blood flow, implying increased perfusion (and a stimulation of metabolism) in nonmammary tissues as a result of GH treatment (56). The increase in udder blood flow may occur through a decrease in mammary vascular resistance through the production of local vasodilator substances. Such vasodilation is likely linked to the mammary metabolism, because similar increases in udder blood flow and milk yield were obtained in thyroxine-treated cows (56).

The evidence cited indicates it is unlikely that bGH has a direct effect on mammary metabolism or an indirect effect through increasing mammary nutrient supply. Therefore, there is a question as to whether a secondary mediator is responsible for the galactopoietic effects of bGH. To answer this, we measured plasma concentrations of IGF-1 and IGF-2 in dairy cows treated with bGH (55). Plasma IGF-1 concentrations increased two- to threefold while milk yield increased by 17%. Concentrations of IGF-2 were unchanged. Evidence is now required to demonstrate the presence of IGF-1 receptors in lactating mammary tissue and to demonstrate the actions of IGF-1 receptors in lactating mammary tissue and to demonstrate the actions of IGF-1 on milk synthesis in vitro and in vivo. However, it seems reasonable to postulate that at least part of the galactopoietic effect of GH is mediated via IGF-1.

The rapidity of the rise and fall of milk yield in response to bGH injections (or their cessation) implies that galactopoiesis is achieved through increased productivity of existing secretory cells rather than an increase in cell numbers. Early studies reported an increase in udder size in response to bGH treatment of dairy cattle (28), but such an observation was almost certainly due to increased milk accumulation in the udder. However, there is evidence of mammogenesis in response to GH injection of heifers and sheep around puberty (124). In one experiment (34), but not another (27), injections of GH during pregnancy resulted in increased milk yield in the subsequent lactation.

If the effect of bGH on milk synthesis is through enhanced productivity of existing cells, the milk production response may be limited by udder capacity. The maximum milking interval tolerated by dairy cattle is 18 to 20 h. An increase in milk yield of 20 to 40% is likely to reduce functional udder capacity by 3 to 6 h. Such a restriction may account, in part, for the curvilinear shape of the dose response curve (68). A 32% increase in milk yield at high doses of GH administration must utilize almost all the functional udder capacity even at 12-h milking intervals. It would seem likely that a synergistic response between thrice daily milking and bGH treatment can be obtained, particularly at high dose rates.

Udder involution is rapid in dairy cattle. Measurements of udder volume in Jersey cows indicate a 40 to 50% decline in udder size during the first 10 to 15 wk of lactation (57). From this stage udder volume is relatively constant, but further involution is indicated by the continuing decline in milk yield and udder capacity (216). Recent results from long-term treatment of Holstein cows with bGH are of interest, because treatment with recombinant-derived methionyl bGH resulted in greater lactational persistency, implying some reduction in the rate of udder regression (13).

**INSULIN-LIKE GROWTH FACTORS**

**Introduction**

The IGF are a family of polypeptides, related structurally and evolutionarily to proinsulin, which have important growth-promoting effects both in vitro and in vivo, including mediation of GH effects on somatic growth. In addition, at least in vitro, they have insulin-like effects.
A historical review of the IGF is complex, because terminology varies among the different approaches from which the biochemistry of the IGF has been elucidated. In their classic experiments, Salmon and Daughaday (191) reported that the actions of GH on skeletal growth were mediated by a GH-dependent serum factor, which they termed "sulfation factor". Subsequently, with the realization that the activity represented by this factor or factors had further anabolic actions, they were renamed "somatomedins" (47). In apparently unrelated studies, Froesch et al. (70) reported the presence of insulin-like biological activity in serum that was not due to immunoreactive insulin. This activity was termed "nonsuppressible insulin-like activity" (NSILA). These factors had no storage pool, and to purify them from blood plasma was a complex problem. Rinderknecht and Humbel (183, 184) purified two polypeptides from NSILA, and upon sequencing, a structural and functional relationship to insulin was apparent. These two peptides were named IGF.

Insulin-like growth factor-1 is a basic peptide that consists of 70 amino acid residues and IGF-2 is a neutral peptide with 67 amino acids. The molecular weights calculated from the amino acid sequences are 7649 for IGF-1 and 7471 for IGF-2 (185, 186). Almost 50% of the amino acids in the A and B domains of the molecules of IGF-1, IGF-2, and human proinsulin are in identical positions. The C-peptide regions of IGF-1 and IGF-2 are analogous in location to the C-peptide region of human proinsulin but show no amino acid sequence identities. In addition, the IGF-1 and IGF-2 molecules have a carboxy-terminal extension not seen in the proinsulin molecule, which has been termed D region ([235] for review]. The three-dimensional structures of IGF-1, IGF-2 and insulin are thought to be similar (23), which is important in the interpretation of the biological responses of these hormones.

Structural studies have shown that somatomedin C (129) and somatomedin A (67) have structures identical to IGF-1. Multiplication stimulating activity (MSA), which is purified from rat liver conditioned media, is the rat homologue of IGF-2 (139). Recently the structure of bovine IGF-1 and 2 have been reported. Bovine IGF-1 has the same sequence as human IGF-1 and IGF-2 is largely homologous except for three substitutions in the C-peptide region (Humbel, personal communication). The investigation of the biological activity of IGF has been restrained by the scarcity of purified material derived from serum. Recent synthesis of IGF-1 by solid phase procedure (135) and of an analog by recombinant DNA technology (195) offer great promise for future studies. The synthetic peptide and the recombinant analog of IGF-1 behave similarly in radioligand systems (195). Furthermore, the synthetic IGF-1 peptide is equivalent to the preparation purified from serum in all important biological respects (218).

Binding Proteins

Measurement of the IGF and assessment of their biological role are complicated by the observation that the IGF circulate noncovalently bound to large molecular weight binding proteins (38, 111, 112, 219). Multiple species of binding protein are present in the circulation. In man, two major forms are observed on column chromatography of serum at neutral pH — a major fraction of approximately 150,000 daltons and a lesser fraction of approximately 50,000 daltons. No unbound IGF is seen in the circulation. Both IGF-1 and IGF-2 distribute in a similar fashion, although there may be multiple forms of binding protein with differing affinities for IGF-1 and IGF-2 (21). The large molecular weight complex dissociates in acid to an acid-labile component and an acid-stable component of about 50,000 daltons (72, 111) and appears to be a GH-dependent complex (158). However, an alternative interpretation has recently been suggested by D’Ercole (61), who postulates that this large molecular weight binding complex is a hexamer of smaller binding units. This would explain why only smaller molecular weight binding proteins can be demonstrated in culture systems.

In species other than human, analogous binding proteins are observed (230). In the
adult sheep IGF-1 circulates primarily in the 150,000 dalton form and a lesser amount in the 50,000 dalton form, but IGF-2 primarily circulates in the 50,000 dalton form (Butler and Gluckman, unpublished observation).

The affinity of the serum binding proteins for IGF interferes with the detection of IGF in whole serum or plasma by radioligand techniques. It is essential that assay systems use an initial separation technique to remove the binding protein. Gel chromatography under acidic conditions or acid-ethanol extraction (48, 50) are the generally used methods.

The presence of IGF serum-binding proteins is unique for a peptide hormone. This could explain why total serum IGF concentrations, which are in the 100 to 1000 mg/ml range in ruminants, are far in excess of the concentrations required for the stimulation of biological activity in most in vitro systems (usually between 1 to 50 ng/ml). Because free IGF is not detectable in native plasma and is not stored in any organ or tissue, the circulating complexes represent the storage forms of these peptides. The binding protein prevents acute insulin-like actions typical of the free IGF (234, 235), it restricts their permeability through capillaries, and it inhibits their access to membrane receptors (46). There are indications that endogenous serum proteases might be involved in dissociation of IGF-binding-complex (32), which makes bioactive IGF available to tissues. The different binding complex forms may play a role in tissue selectivity and delivery of active IGF peptide to the intracellular space (111).

Presence of binding proteins makes assessment of the half-life of the IGF difficult, because exogenously administered labeled IGF-1 does not bind to the serum proteins in a distribution equivalent to that of the endogenous peptide. It is probable that the different species of IGF-binding protein complexes have different half-lives. The plasma half-life of IGF has been variably estimated to be 4 to 18 h in contrast to the much shorter half-life of other peptide hormones (239).

Source of Insulin-Like Growth Factors

It is now recognized that many, if not all, tissues have the capacity to synthesize IGF (60). This has led to the concept that IGF can act locally close to their site of synthesis as paracrine or autocrine factors.

Liver is the major source of circulating IGF (60). A number of studies have utilized hepatic perfusion or hepatocytes in culture to study the regulation of IGF release (21, 51, 194). Hepatic tissue in rats contains substantial IGF-1 and its concentration is GH-dependent (222, 223, 224). In the dog, a hepatic-portal vein difference in plasma IGF concentrations has been reported (196, 197, 198).

Circulating Concentrations of the Insulin-Like Growth Factors

Relatively few data have been published for the ruminant on IGF concentrations measured by specific radioligand assays. The IGF concentrations in the peripheral circulation of humans are relatively stable with no obvious diurnal rhythm due to the long biological half-life (97). Similarly, in cattle, IGF-1 concentrations do not fluctuate during 24-h periods even when animals are undernourished and GH secretion is extremely pulsatile (Breier et al., unpublished data).

In sheep, IGF-1 concentrations are relatively low in the fetus and rise soon after birth (82). This postnatal rise is consistent with the appearance of somatogenic receptors in the liver and probably represents the onset of GH-dependent IGF-1 release (84). Approximately 40 d after birth, IGF-1 in the lamb decreases to adult concentrations (82). The basis of this postnatal decrease remains to be elucidated. It may represent the effect of nutritional changes associated with weaning and the initiation of ruminant function. This ontogenic pattern differs from that seen in man, where IGF-1 concentrations are low at birth (86), remain low through the first year of life, and then rise gradually through childhood with a marked rise at puberty and a decrease postpuberty (97, 123, 232, 239). Ontogenic studies in the bovine are lacking.

In the sheep, IGF-2 activity measured by radioreceptor assay is high in the fetus and decreases at birth to adult values (82). Similarly in the rat, IGF-2 (MSA) activity is high during fetal life (52). However, in humans IGF-2 activity as measured by immunoassay is low, in umbilical cord plasma, although higher activity is measured by radioreceptor assay (97, 193). This discrepancy has led to the suggestion that
there may be a fetal form of IGF, at least in humans. This area remains controversial with
definitive data lacking (83). High IGF-2-like activity in fetal sera have led to the suggestion
that IGF-2 or the related fetal IGF has a particular role in fetal development (81, 83).

Breed differences in IGF-1 concentrations have been reported. These have been studied
most extensively in the dog (66) where breeds of large body size have higher IGF-1 than those
of small body size. This is evidence for genetic determinants of IGF-1 release and suggests a
relationship between IGF-1 release and body size. To date, selection experiments based on
IGF-1 concentrations have not been reported.

Growth Hormone and the
Insulin-Like Growth Factors

Growth hormone is the dominant endocrine
influence on plasma concentrations of the IGF. Insulin-like Growth Factor-1 is clearly GH-
dependent; concentrations are lower in hypopituitary states and elevated in conditions of
GH excess. Insulin-like Growth Factor-2 is often considered less GH-dependent, because
although lower concentrations are seen in hypopituitary states, IGF-2 is not elevated in
conditions of GH excess (38, 39). Alternatively, this could be interpreted as reflecting a greater
sensitivity to GH with maximal secretion of IGF-2 at lower concentrations of GH than are
necessary to stimulate maximally IGF-1 release.

Following the administration of GH to
normal human subjects, there is a threefold rise
in plasma IGF-1 concentration, which begins
within 6 h with peak values between 16 and 18
h (43). These findings and studies on perfused
rat liver (203) suggest that the GH effect on
IGF-1 production is through stimulation of de
novo synthesis rather than through release from
a storage pool. Similarly in the ewe (Bass and
Gluckman, unpublished data) and dairy cow
(55), exogenous GH stimulates IGF-1 concen-
trations, but only after several hours following
its administration. There is limited evidence
that IGF-2 concentrations also rise after GH
administration in the dairy cow (55).

During pregnancy, PL seems likely to
stimulate IGF secretion (74). Following hypop-
hysectomy of pregnant rats, IGF-1 does not
fall until after parturition (52). Insulin-like
growth factors tend to rise during pregnancy
(114). From in vitro experiments, PL also has
been suggested to play a role in regulation of
fetal IGF release (2, 3), because GH does not
(83), presumably because of the deficiency of
GH receptors in fetal tissues. However, no
direct evidence for such a role is yet available
(81).

Insulin and Insulin-Like
Growth Factor Release

There are complex interactions between
insulin, IGF, and their receptors. In addition,
insulin may stimulate the release of IGF (204).
This is likely to be particularly important in the
perinatal period where growth is independent
of GH and dependent on substrate availability
(83). In the pancreatectomized sheep fetus,
IGF-1 is reduced (81). Insulin infusion increases
IGF-1 in the pig fetus (206). It seems likely
that in the postnatal ruminant, where substrate
availability remains an important determinant
of growth, substrate and possibly insulin remain
important determinants of IGF release. In the
adult rat, insulin stimulates IGF-1 release (171,
172). Whether this effect is direct or indirect
via insulin’s actions on the somatogenic receptor
(16) has not been elucidated. The answer to
this question may offer important insights into
methods of manipulating the somatogenic axis.

Influence of Nutritional Status
on Concentrations of Circulating
Insulin-Like Growth Factors.

Evidence from clinical observations and
studies on laboratory animals indicate that IGF
concentrations are decreased severely by
nutritional deficiency (35, 172). It is now clear
that, in the rat and human, protein as well as
calories are important to maintain normal IGF
(36, 39, 116, 117, 170, 171).

Early bioassay studies suggested that the
total bioactive IGF content of plasma is reduced
during fasting in rats and that refeeding restores
IGF concentrations to normal (170, 173). These
findings were confirmed by a number of
experiments using IGF radioassay techniques in
rats (136, 137), humans (35), and dogs (65).
Merimee et al. (150, 151) also observed that
IGF-1 was unresponsive to hGH administration
after a 3-d fast, indicating relative GH resistance
during acute fasting. As has been discussed,
decreased IGF-1 plasma concentrations and
partial GH resistance during fasting are causally
related to reduced GH receptors in the liver.

Because IGF measured by immunoassay rise more slowly during refeeding than IGF activity measured by bioassay, IGF inhibitors were suggested to play a role in the biological regulation of these peptide hormones (171). Insulin-like growth factor inhibitory factors have been described in malnutrition (169). Their nature is poorly understood, but they appear to be proteins (190) with a molecular weight of about 25,000 daltons (7). The IGF inhibitors have broad antianabolic effects; they are metabolically regulated and originate in the liver (171, 222, 224). These inhibitors may provide a potential mechanism for the liver to modulate production and utilization of metabolites.

There are no published data about nutritional regulation of IGF in the ruminant. In the growing lamb (ewe, ram or wether), transfer from pasture to pelleted diet is associated with a doubling of IGF-1 but no change in IGF-2 concentrations (Bass and Gluckman, unpublished data). Fasting in sheep is associated with a reduction in IGF-1 and IGF-2 concentrations (8). These observations suggest that in the pasture-fed sheep, nutritional factors are playing an active role in the regulation of IGF release.

We have investigated the effect of different nutritional status on IGF-1 plasma concentration in young angus steers. Concentrations of IGF-1 in plasma were decreased by 50% in steers fed below maintenance in comparison with steers on high feed intakes. The significance of these changes in relation to changes in GH secretion has been discussed previously. Indeed, it seems probably that nutritional factors will play a greater role in the regulation of IGF release in the ruminant than in monogastric species in view of the greater impact of feed availability on growth in pasture-fed ruminants.

Influence of Sex Steroids on Circulating Insulin-Like Growth Factor

The relationship between gonadal steroids and IGF concentration is still uncertain. In primates, IGF-1 and gonadal steroids rise considerably during puberty, whereas IGF-2 concentrations do not alter (41, 42, 132). Harris et al. (98) provided direct evidence that the pubertal rise in IGF-1 is related to an increase in sex steroid production, because suppression of gonadal sex steroids decreases IGF-1. In contrast, administration of pharmacological doses of estradiol to normal or acromegalic patients results in a depression of both bioassayable and radioimmunoassayable IGF-1 (37, 227, 228).

Recent studies on rhesus monkeys (231) and baboons (42) suggest a positive relationship between physiological doses of estradiol administration and IGF-1 plasma concentrations. This estradiol-stimulated increase in IGF-1 is thought to be mediated at least in part through an increase in plasma GH concentrations.

We have evidence, from preliminary studies in young steers chronically treated with low physiological doses of estradiol, that estrogens not only markedly increase live weight gains but also significantly increase IGF-1 plasma concentrations (Breier et al., unpublished data). Similarly, testosterone increases IGF-1 concentrations in hypogonadal children (122).

Receptors for the Insulin-Like Growth Factors

The IGF exert their biological effects, like other peptide hormones, by reacting with cell surface receptors. Early evidence that IGF bind to membrane sites was obtained indirectly from studies in which IGF-1 competed with [125I]insulin for receptors on isolated adipocytes, liver membranes, and isolated chondrocytes (110, 140, 236). Megyesi et al. (145, 146) demonstrated that IGF bind to specific high affinity, low capacity receptors on cell membranes. Competitive binding studies and experiments that examined IGF receptor structure have described at least two types of IGF receptors.

Massague and Czech (143) characterized these two IGF receptor structures using affinity and crosslinking techniques. The type 1 receptor binds IGF-1 more potently than IGF-2, and insulin shows a weak but significant crossreactivity. The type 2 receptor prefers IGF-2 over IGF-1 but does not recognize insulin (46, 49). In addition, both IGF-1 and IGF-2 have weak affinity to the insulin receptor. Massague and Czech (143) characterized these two IGF receptor structures using affinity and crosslinking techniques. The type 1 receptor shows close structural homology to the insulin receptor (142) with a similar subunit structure and some immunological determinants in common. The type 2 receptors consist of one single chain molecule that is not disulfide-linked to any other membrane component and
The original hypothesis that the IGF receptor mediates the growth-promoting action of both IGF and insulin (160) while the acute metabolic effects of insulin and IGF are mediated by the insulin receptor (128, 219, 220) is viewed as an oversimplification. There are now clear examples of insulin stimulating growth by acting through the insulin receptor and, conversely, instances of IGF stimulating glucose transport by acting through IGF receptors [(159) for review].

Most attention has focused on the type 1 receptor as the mediator of the effects of both IGF-1 and IGF-2. It had been suggested that the type 2 receptor was not a transducer of IGF action but had properties similar to the glucose transporter system (44). However, evidence has been reported recently for IGF actions in some tissues being mediated by type 2 receptors (192).

It seems likely that the mode of action of IGF and insulin in any tissue depends on the distribution of insulin, type 1, and type 2 receptors in that tissue. Insulin-like growth factor receptors have been identified in all tissues. Most binding studies have been carried out on adipocytes, cartilage, heart, and skeletal muscle, and liver and kidney tissue [for reviews see (38, 159, 232)].

With regard to the endocrine involvement of the GH-IGF axis in mammmogenesis and lactation, it is important to note that although all attempts to demonstrate GH receptors in mammary tissue have been unsuccessful, Furlanetto and DiCarlo (73) recently have identified IGF type 1 receptors in human breast cells.

The regulation of IGF receptors has been subject to limited investigation. Acute up regulation of IGF binding to the type 2 receptor by insulin has been reported from several different cell types. This effect is mediated by an action of insulin on the insulin receptor. Schoenel et al. (200) observed that insulin caused a 250% increase in $^{[125]I}$IGF binding to rat adipocytes. There is strong evidence that the rat adipocyte has a typical type 2 receptor, whereas type 1 IGF receptor is absent (143). In contrast, studies in myocytes show that preincubation with IGF or with high concentrations of insulin selectively down regulates the type 1 receptor without affecting type 2 IGF receptors (62).

**Actions of the Insulin-Like Growth Factors**

The functions of the IGF that formed the basis of their identification are first, their ability to stimulate mitosis in cell culture and to promote somatic growth in the hypophysectomised animal and, second, their insulin-like actions. Most actions of the IGF have been studied only in vitro; sufficient materials only now are becoming available for in vivo studies. Conclusions on their biological role in vivo are based, therefore, on extrapolation from in vitro data and correlative studies between IGF concentrations and physiological states.

The different biological functions of IGF can be distinguished on a temporal basis (232, 235); first the acute metabolic effects onset of which occurs within seconds to minutes, and second, long-term effects, onset of which occurs within hours.

**Metabolic Effects of Insulin-Like Growth Factors**

The acute nonsuppressible insulin-like function of IGF have most commonly been studied in the classic insulin target tissues: adipose tissue, striated muscle, and heart muscle. In adipose tissue or isolated fat cells, IGF-1 and IGF-2 stimulate glucose transport, glucose oxidation, and lipid synthesis from glucose; they also inhibit lipolysis (234, 236). Compared with insulin, IGF-1 and IGF-2 are only 1:50 to 1:100 as potent in enhancing glucose metabolism in fat cells. The small but significant potency difference between IGF-1 and IGF-2 in these cells is consistent with their potency difference in competing for the insulin receptor of the adipocyte (233). Zapf et al. (232) have postulated that IGF exert their acute metabolic insulin-like function in adipose tissue through the insulin receptor.

In the perfused rat heart, IGF stimulate glucose transport, glucose uptake, and lactate production. The biological potency ratio between IGF and insulin is 1:2 to 1:5; thus, potency of IGF in the perfused rat heart is 20 to 40 times higher than in adipose tissue (152, 153, 154). IGF-1 shows also insulin-like
effects on striated muscle (175). Its potency ratio to insulin is 1:10 and 1:20, which is five times higher than in adipose tissue. Insulin-like growth factors act on muscle tissue through IGF receptors rather than through the insulin receptor (232).

When IGF are injected intravenously into rats, blood glucose falls significantly (235). Insulin-like growth factor-factor-1 is more effective in this respect than IGF-2. When $[^{14}C]$glucose is injected simultaneously, incorporation of $[^{14}C]$glucose into diaphragm glycogen is increased 15-fold for IGF-1 and 8-fold for IGF-2 compared with control rats.

The blood glucose lowering effect of IGF in vivo is accompanied by a preferential utilization of glucose in striated muscle. In contrast to insulin, the stimulatory effect of IGF on lipid synthesis from glucose in adipose tissue in vivo is poor (235). These results are consistent with in vitro findings, where the potency difference between insulin and IGF is much greater in adipose tissue than in heart or skeletal muscle.

However, such experiments with acute IGF administration do not mimic a physiological situation, as they lead to a marked increase of free IGF in the circulation. As unbound IGF normally are not in circulation, it is not known whether IGF normally have these metabolic effects in vivo. It is possible that, as for GH itself, IGF may have differing and opposing actions on adipose tissue over time.

**Anabolic Effects of Insulin-Like Growth Factors**

Mitogenic properties of IGF are well-established. Their actions to promote mitosis have been demonstrated in many cell lines in culture (189, 202). They have been shown to act late in the G1 phase of the cell replication cycle as "progression factors" prior to the phase of DNA synthesis [for review see (189)]. Insulin-like growth factors stimulate ribonucleic acid (RNA), DNA, and protein synthesis in organ cultures of rat calvaria (30), chick embryo fibroblasts (232), and mouse soleus muscle (155). The onset of the stimulatory effect on RNA synthesis is rapid and IGF-1 is 5 to 7 times more potent than IGF-2 and 50 times more potent than insulin. The pronounced potency difference between IGF and insulin in stimulating RNA synthesis suggests these effects are mediated through specific IGF receptors (235).

Physiological concentrations of IGF-1 stimulate DNA synthesis in human breast cell lines through type 1 IGF receptors (73). In a number of cell types, the mitogenic effect of insulin is the result of the binding of insulin to the type 1 IGF receptor (128). An important criterion by which IGF are biologically characterised is their effect of stimulating sulphate incorporation into cartilage. On a molar basis, potency of IGF-1 is 3 times that of IGF-2 and 25 times that of insulin in chick embryo cartilage (71).

The somatomedin hypothesis of Daughaday et al. (47) postulates that GH stimulates growth indirectly through IGF. Although the weight of indirect evidence supports this hypothesis, there are few direct data addressing this important question. Pure IGF-1 and IGF-2 were assessed for their growth-stimulating effects on hypophysectomized rats by Schoenle et al. (201, 202). Insulin-like growth factor-factor-1 was considerably more effective than IGF-2 at the same dose. Insulin-like growth factor increased tibial epiphysial width in a dose-dependent manner. Weight gain in the IGF-1-infused groups of hypophysectomized rats was comparable with that in the groups infused with GH. Insulin-like growth factor-factor-2 had no significant effect on body weight at the dose used in this experiment, but only a single dose was tested (201). It was shown clearly in this experiment that a rise of circulating IGF-1, whether produced indirectly through GH administration or by IGF-1 infusion in the absence of GH, is accompanied by a stimulation of growth. No studies of IGF administration to intact animals have been reported and in intact animals, the evidence for a central role of IGF in growth regulation is inferential only. For example, there is evidence that the growth rate in steers due to varying feed intakes correlates well with IGF-1 concentrations (Breier et al., unpublished data).

**CONCLUSIONS**

Knowledge is accumulating rapidly regarding the physiology of the somatotropic axis, although relatively few definitive studies have been performed in the ruminant. Growth hormone clearly causes an increase in lactation and this effect appears commercially viable.
This effect is probably indirect and may be mediated by the IGF. The somatotropic axis may be manipulated in other ways; however, the importance of such manipulations to production indices in ruminants other than lactation remains to be elucidated.

ACKNOWLEDGMENTS

Our own studies in this review were supported by the Medical Research Council of New Zealand, The Combined Beef Breeders Association of New Zealand, and by the Ministry of Agriculture and Fisheries.

REFERENCES


80 Gluckman, P. D. 1984. Functional maturation of


107 Heretlydy, F., and D. N. Kipnis. 1973. Studies on growth hormone secretion: II Influence of
plasma free fatty acid levels. Endocrinology 92:402.


136 Maes, M., L. E. Underwood, G. Gerard, and J. M. Ketelslegers. 1984. Relationship between plasma somatomedin-C and liver somatogenic binding site in neonatal rats during malnutrition and after...


