SYMPOSIUM: GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTORS

Role of the Insulin-like Growth Factors and Their Binding Proteins in Lactation

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ABSTRACT

Insulin-like growth factor (IGF)-I is thought to mediate a portion of the effects of bST on lactation in dairy cows. Serum concentrations of IGF-I are increased in lactating cows that were treated with bST, and IGF-I receptors are present in bovine mammary tissue. In addition, close arterial infusion of IGF-I into the mammary gland of goats increases milk yield. Little evidence exists to support a direct galactopoietic effect of IGF-I in ruminants. However, IGF-I is a potent mitogen for mammary epithelial cells and may also influence the inhibition of apoptosis of this cell type.

The IGF are found in association with a family of individual binding proteins. The high affinity of the IGF for these proteins relative to the IGF receptor allows them to modulate IGF-I bioactivity in the mammary gland at the cellular level. Mammary epithelial cells synthesize multiple forms of IGF binding proteins, and one of these, IGF binding protein-3, is specifically regulated by the IGF. Stimulation of DNA synthesis by IGF-I is enhanced in bovine mammary epithelial cells that overexpress the IGF binding protein-3. These data indicate that IGF-I can stimulate the synthesis of an IGF binding protein, which enhances its own mitogenic activity. However, whether this mechanism is operative in the lactating mammary gland in vivo is unknown. Given the complexity of the interactions between the IGF and their binding proteins, more information is needed before the role of these growth factors in regulating growth, differentiation, and apoptosis of mammary epithelial cells is delineated.

(**Key words**: lactation, insulin-like growth factor, binding protein)

Abbreviation key: **IGFBP** = insulin-like growth factor binding protein, **MEC** = mammary epithelial cell, **ST** = somatotropin.

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INTRODUCTION

Both IGF-I and IGF-II are polypeptide growth factors that are present in significant concentrations in the circulation of lactating ruminants (43, 72). The liver is thought to be the primary source of circulating IGF-I, which may act in an endocrine manner and directly affect target tissues, such as the mammary gland. However, IGF-I mRNA is expressed in the bovine mammary gland (25, 65), suggesting that local production of IGF-I also may be important.

The idea that IGF-I is important in lactation originated from studies of somatotropin (**ST**). Although treatment with exogenous ST stimulates milk production, the addition of ST to mammary systems in vitro did not stimulate cell proliferation or synthesis of milk components [reviewed in (5)]. In contrast, IGF-I is a potent mitogen for mammary epithelial cells and stimulated the synthesis of milk components in some studies. In addition, researchers (2, 17) have been unsuccessful in detecting ST binding to ruminant mammary tissue in vitro, although this tissue binds IGF-I. These findings led to the idea that IGF-I mediates the effects of ST on the mammary gland in a manner that is similar to its role in skeletal growth.

Although considerable evidence suggests that the IGF influences lactation, data are limited that define the specific mechanisms of action. Understanding of these mechanisms is complicated by the association of IGF with eight or more IGF binding proteins (**IGFBP**). These IGFBP consist of a family of six individual proteins, IGFBP-1 through IGFBP-6, which exhibit high affinity for the IGF (12, 32). Recently, two proteins that bind the IGF with low affinity have been identified and designated as IGFBP-7 and IGFBP-8 (34, 49, 73). The IGFBP have several functions, including prolonging the half-life of the IGF in the circulation, transporting the IGF from the vasculature to the tissues, and localizing IGF to specific cell types and tissues. At the cellular level, IGFBP have been shown both to potentiate and inhibit the biological activity of IGF (12, 32). In addition, several effects have been described for the IGFBP that are independent of IGF (33, 47). The objective of this paper is to review the current

knowledge of the role of the IGF and IGFBP in the regulation of ruminant lactation.

REGULATION OF CIRCULATING IGF AND IGFBP

Both IGF-I and IGF-II are abundant in the circulation compared with concentrations of other polypeptide growth factors. The well-documented roles of the IGF as autocrine and paracrine regulators of cellular growth and differentiation have led to speculation over the importance of circulating concentrations of IGF. However, the regulation of serum IGF-I by ST and nutritional status (see the following sections), both of which serve as primary controls for normal growth and lactation, suggest that circulating concentrations of IGF-I are somehow involved in supporting the growth and lactation processes. The high concentrations of IGF in the circulation may act as a reserve, allowing IGF to regulate overall growth and lactation in a systematic, endocrine manner (45).

The majority of IGF in the circulation is bound to a large ternary complex of IGF, IGFBP-3, and an acid-labile subunit; the remainder of IGF is associated with the lower molecular mass IGFBP (7). The recent development of radioimmunoassays for all six IGFBP in the human has made it possible to estimate that the total concentration of IGFBP-1, -2, -4, -5, and -6 amounts to approximately 50% of the IGFBP-3 concentration in serum of adults (45). Serum IGFBP concentrations have been quantitated by radioimmunoassay in lactating ruminants for IGFBP-2 only (13, 42, 72).

Stage of Lactation

Concentrations of IGF-I in the serum of dairy cows are lowest during early lactation when serum ST concentrations have increased and milk yield is high (60, 65, 72). Sharma et al. (65) reported that the abundance of hepatic IGF-I mRNA was lower during early lactation than during late lactation, suggesting that the lower serum IGF-I concentrations observed during early lactation are a result of decreased IGF-I synthesis rather than increased clearance. In contrast, the circulating concentrations of IGF-II are not affected by stage of lactation (72). Serum concentrations of IGFBP-3 are unchanged over the course of lactation in dairy cows, but IGFBP-2 concentrations are higher during early lactation (65, 72). Serum IGFBP-2 concentrations are similar in pregnant and lactating ewes. However, expression of IGFBP-2 mRNA was higher in the liver but lower in the mammary gland of lactating ewes versus pregnant ewes (36).

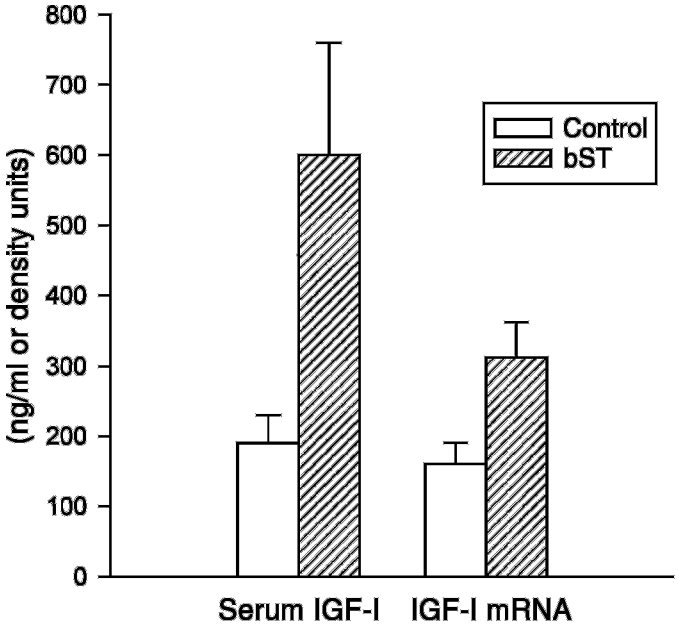

Figure 1. Effect of bST treatment on serum concentrations of IGF-I (nanograms per milliliter) and hepatic abundance of IGF-I mRNA (density units) in late lactation dairy cows. The effect of bST was significant for both serum concentrations of IGF-I (P = 0.03) and IGF-I mRNA (P = 0.01). (Adapted from J. Dairy Sci. 1994 77:2232.)

ST

Somatotropin is a key regulator of circulating concentrations of IGF-I and IGFBP in the bovine, as it is in other species (12). Serum concentrations of IGF-I and IGFBP-3 are increased by ST treatment of lactating cows, but IGFBP-2 concentrations are dramatically reduced (13, 65, 72). The increase in serum IGF-I concentrations corresponds to an increase in the hepatic abundance of IGF-I mRNA (Figure 1), suggesting that an increase in hepatic synthesis may account for the increases observed in circulating IGF-I concentrations during ST treatment (65, 71).

Circulating IGF-II concentrations appear to be less tightly regulated by ST. McGuire et al. (43) reported that bST treatment increased serum IGF-II concentrations, although the response was only 21% compared with 125% for IGF-I concentrations. In contrast, Vicini et al. (72) found no effect of bST treatment on IGF-II concentrations in lactating cows. Similar results have been reported for other species, leading to the belief that ST is not a primary regulator of circulating concentrations of IGF-II.

The decrease in serum concentrations of IGFBP-2 observed during ST treatment in lactating cows is paralleled by a similar decrease of IGFBP-2 concentrations in afferent mammary lymph (13), suggest-

ing that the IGFBP-2 in lymph is transported from the circulation. The IGFBP-2 crosses intact endothelium (3), and [^{125}I]IGFBP-2 injected intravenously into lactating goats is found intact in mammary-derived lymph (56). Infusion of IGFBP-2 into lactating goats increases the plasma clearance of both IGF-I and IGF-II and decreases their secretion into milk, which might suggest that IGFBP-2 decreases the uptake of IGF by mammary epithelium by targeting it to nonmammary tissues (56). Thus, the decrease in circulating concentrations of IGFBP-2 observed during ST treatment may allow for the increased delivery of IGF-I to the mammary gland.

Nutrient Status

Nutrient status is also an important regulator of circulating concentrations of IGF-I and IGFBP (44, 67). Feed deprivation of lactating cows for 2 d decreases the serum IGF-I and increases the IGFBP-2 concentrations (42). Therefore, the lower IGF-I and higher IGFBP-2 concentrations that are observed in early lactation but not in late lactation may be a reflection of the negative energy balance of cows during early lactation. However, a restriction of the energy and protein intakes of midlactation cows to achieve nutrient balances that typically would be observed during early lactation failed to alter circulating basal concentrations of IGF-I or IGFBP-2 (43).

Although IGFBP-3 concentrations in humans and rodents are regulated by energy status, an extended period of feed restriction is required before decreases are observed (67). Similarly, 2 d of feed restriction was insufficient to decrease serum IGFBP-3 concentrations in lactating dairy cows (42). Holstein heifers maintained in negative energy balance for four estrous cycles also showed decreased serum IGF-I and increased IGFBP-2 concentrations but serum concentrations of IGFBP-3 were not affected (70). In addition, hepatic expression of IGF-I mRNA was decreased in these heifers relative to its expression in heifers that were maintained in positive energy balance.

The ST-IGF-I axis is uncoupled during nutrient restriction (42, 72) during which circulating ST concentrations are elevated and IGF-I concentrations are reduced. Uncoupling of the ST-IGF-I axis may allow ST to increase the availability of nutrients to nonmammary tissues via its effects on adipose tissue lipolysis (28, 37) while minimizing nutrient use by the mammary gland (5). Although basal concentrations of IGF-I and IGFBP-2 were not affected in cows that were fed suboptimal concentrations of energy and protein, the ability of exogenous ST to increase IGF-I and to decrease IGFBP-2 is attenuated (43). More severe feed restriction abolishes the ability of bST to increase IGF-I (42). The ability of bST to increase IGF-I and to decrease IGFBP-2 is also lower in early lactation than in late lactation (72).

IN VIVO EFFECTS OF IGF-I

Close arterial infusion of either IGF-I or IGF-II into the mammary gland increases both milk yield and mammary blood flow in lactating goats (52, 54). Because the effect is rapid (within 2 to 4 h) and only observed in the infused gland, a direct effect is suggested. However, these studies do not indicate whether the increase in blood flow is responsible for the increase in milk yield or whether the converse is true. Mammary blood flow is increased more by close arterial infusion of des-IGF-I, a truncated variant of IGF-I with lower affinity for the IGFBP, than by infusion of intact IGF-I (53).

Treatment with bromocriptine (to inhibit prolactin secretion) and an antiserum to ST to inhibit lactation in rats is accompanied by reductions in mRNA concentrations of several enzymes that are required for the synthesis of mammary lipids (4). Concurrent systemic treatment with ST partly prevented these changes; systemic injections of IGF-I or IGF-II had no effect (4). In a subsequent study (21) using the same model, treatment with a complex of IGF-I and IGFBP-3 failed to reinitiate milk production. Treatment with IGF-I analogues, which have reduced affinity for IGFBP but activate the IGF-I receptor, also failed to reinitiate milk production. However, milk production was reinitiated by subsequent treatment with prolactin or ST (21). Those studies suggest that IGF-I does not have a direct galactopoietic effect in rodents. However, the difficultly of achieving a physiological balance between exogenous IGF-I and endogenous IGFBP should be kept in mind when the results of in vivo studies with the IGF are being interpreted.

Studies utilizing transgenic mice have identified an additional mechanism by which the IGF and IGFBP may influence the lactating mammary gland. Mice overexpressing either IGF-I or IGFBP-3 in mammary lobulo-alveolar cells were able to nurse their pups normally, and the only structural difference observed was overall smaller alveoli (46). However, involution was modified in both transgenic lines, and fewer apoptotic cells were present. Results were similar with targeted mammary expression of des-IGF-I, a truncated form of IGF-I that has a low affinity for IGFBP (26).

The ability of IGF-I to inhibit apoptosis has also been demonstrated in several cell lines in vitro (18, 58, 63). However, a role for IGF-I in preventing apoptosis was not supported by a recent study in which

mammary involution was induced in rats by treatment with bromocryptine and ST antiserum. In this study (69), the increased loss of secretory cells associated with mammary gland involution was partially prevented by treatment with ST; treatment with IGF-I and IGF-II complexed to IGFBP-3 had no effect. However, large amounts of an unidentified IGFBP were also produced by the involuting gland. Recently, this IGFBP was identified as IGFBP-5, and IGFBP-5 concentrations in milk and IGFBP-5 mRNA concentrations in mammary tissue were reported (68) to have increased in the involuting rat mammary gland. This effect is inhibited by concurrent prolactin injections but is unaffected by other hormones, including ST or an antiserum to IGF-I. Therefore, it has been proposed (68) that IGFBP-5 inhibits IGF-I-mediated cell survival, which is normally inhibited by prolactin and milk removal.

IN VITRO EFFECTS OF IGF

The ability of IGF-I to stimulate cellular proliferation of ruminant mammary cells is well established [reviewed in (23)]. For both sheep and cattle, IGF-I stimulates the growth of primary mammary epithelial cells (**MEC**) grown on or within collagen (41, 64, 74, 75) and also stimulates the growth of the bovine MEC line MAC-T grown on plastic (79). Synthesis of DNA also was enhanced in multiple cell types by the addition of IGF-I to serum-free cultures of bovine tissue slices (6). Cellular proliferation of ruminant MEC also is stimulated by IGF-II, although with a lower potency, most likely because of its lower affinity for the IGF-I receptor (50, 74). Des-IGF-I stimulates the proliferation of bovine MEC either to a similar (50) or greater (41) extent than does intact IGF-I. In addition, the IGF have been recognized as being potent mitogens for MEC of human breast tumors (38) as well as primary MEC of rodents (16, 31).

Although the effects of IGF-I on cell proliferation appear to be consistent across species, various results have been reported for the effects of IGF-I on the regulation of differentiated secretory functions. Casein gene expression and synthesis, α-LA activity, and glucose transport were all stimulated by IGF-I addition to murine mammary gland explants that were cultured on floating collagen gels in serum-free media containing prolactin and hydrocortisone (55). The addition of IGF-I enhanced prolactin-induced casein synthesis by mammary organ cultures from pregnant rabbits (19). In contrast, IGF-I failed to stimulate α-LA production by primary murine MEC cultured on floating collagen gels, and IGF-I failed to enhance the effect of lactogenic hormones in stimulating production of α-LA (20). Neither fatty acid synthesis nor α-LA secretion was stimulated by IGF-I in cultures of bovine mammary tissue (50, 64), suggesting that IGF-I has no direct galactopoietic effect in the bovine.

MAMMARY SYNTHESIS OF IGF-I

The IGF are synthesized by most tissues of the body in which they act in an autocrine or paracrine manner (12). Although circulating concentrations of IGF-I may exert an endocrine effect on the mammary gland, evidence also exists for a paracrine or autocrine role of IGF-I in the mammary gland. The IGF-I mRNA has been detected in mammary tissue of the pregnant (27) and lactating (25, 65) bovine. Hauser et al. (27) separated mammary tissue from pregnant heifers into fractions enriched in epithelium, stroma, and blood components and found IGF-I mRNA to be localized in the stromal component of the mammary gland. The IGF-I mRNA also has been identified in the stroma of normal and neoplastic human mammary tissue but not in the epithelia (77). Therefore, the stromal components of the mammary gland appear to be responsible for the local synthesis of IGF-I.

Although mammary IGF-I mRNA has been detected in the bovine, the question remains as to whether ST acts directly on mammary gland tissues to increase local synthesis of IGF-I. The inability of preparations of bovine mammary membrane to bind ST led to the widely held view that ST receptors are not present in the bovine mammary gland. However, ST receptor mRNA has been detected in bovine mammary tissue (24, 27), suggesting that a direct effect of ST is possible. Somatotropin receptor mRNA was found by in situ hybridization (24) to be primarily expressed in the alveolar epithelial cells of lactating tissue; ST receptor mRNA was found to be evenly distributed among the epithelium, stroma, and blood components of mammary tissue from pregnant heifers (27). Interestingly, both of those studies identified the ST receptor mRNA in the epithelial component, which does not appear to synthesize IGF-I. Two studies have now shown either no effect (25) or a decrease in mammary abundance of IGF-I mRNA following ST treatment (65), which is in contrast to the results of studies of rodents in which ST stimulated IGF-I mRNA abundance within the mammary gland (35, 61). However, these studies utilized hypophysectomized male rats that had been castrated and were sexually immature.

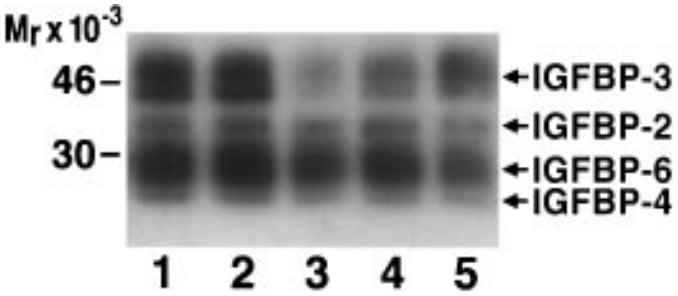

Figure 2. Regulation of concentrations of IGF binding protein (IGFBP) in conditioned media of MAC-T cells by IGF and insulin. Confluent cultures of MAC-T cells were incubated in serum-free media with or without treatments for 24 h. Treatments were 100 ng/ml of IGF-I (lane 1), 100 ng/ml of IGF-II (lane 2), serum-free control (lane 3), 50 ng/ml of insulin (lane 4), and 5 μg/ml of insulin (lane 5). Conditioned media (20 μl) were ligand blotted with [^{125}I]IGF-II. M_r = Relative molecular mass.

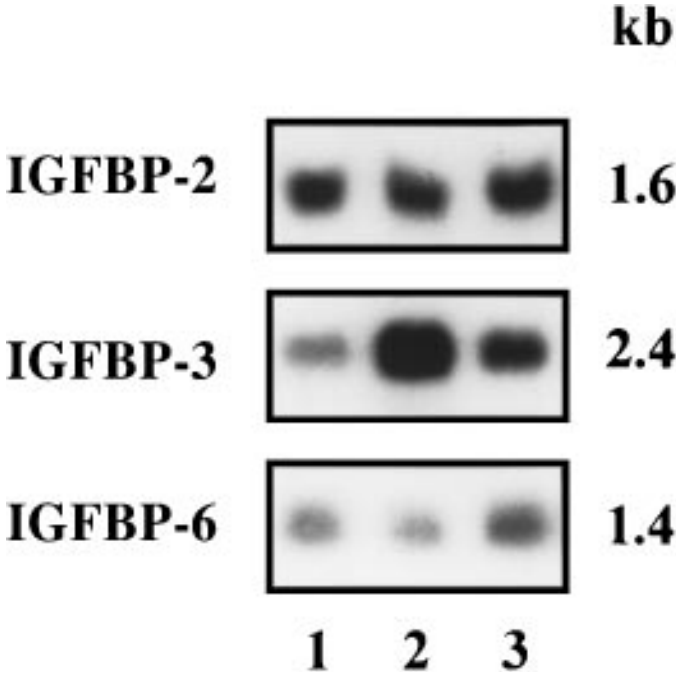

Figure 3. Regulation of IGF binding protein (IGFBP) mRNA concentrations by the IGF. Confluent cultures of MAC-T cells were incubated in serum-free media with or without treatments for 18 h. Treatments were serum-free control (lane 1), 100 ng/ml IGF-I (lane 2), and 100 ng/ml IGF-II (lane 3). Total RNA (15 μg) was sequentially hybridized with cDNA for bovine IGFBP-2, IGFBP-3, and IGFBP-6.

MODULATION OF IGF ACTION BY IGFBP

Insulin-like growth factor-I does not appear to be synthesized by MEC; however, IGFBP synthesized by MEC may localize IGF-I to this cell type. Studies utilizing in situ hybridization or immunocytochemical techniques to determine either synthesis or localization of individual forms of IGFBP to specific cell types in the mammary gland have not been reported in the bovine.

Delineation of the roles of the individual IGFBP at the cellular level is complicated by the finding that most cells in culture synthesize multiple forms of IGFBP. All six forms of the high affinity IGFBP are synthesized and secreted by various human breast tumor cell lines, depending on the status of the estrogen receptors and hormonal stimulation (1, 10, 66). However, information is limited regarding the regulation of IGFBP synthesis by normal MEC.

The MAC-T cell line represents a useful model of the effects of IGFBP on IGF action in the bovine MEC. This cell line was derived from primary mammary secretory alveolar cells and retains several characteristics of differentiated MEC (30, 78). As shown in Figure 2, these cells synthesize IGFBP-2, -3, -4, and -6 (11). The IGFBP-6 is only detected by ligand blot when [^{125}I]IGF-II is used as the radioligand, reflecting the much higher affinity of IGFBP-6 for IGF-II relative to IGF-I (22, 39). Primary MEC that were isolated from pregnant, nonlactating heifers have been shown to secrete IGFBP with approximate molecular masses of 48, 34, 28, and 21 kDa (41). The 48- and 34-kDa forms were identified as IGFBP-3 and IGFBP-2, respectively. Campbell et al. (8) reported that primary bovine MEC that were isolated from pregnant nonlactating and lactating nonpregnant cows secreted IGFBP with molecular masses of 44, 37, 33, and 29 kDa. Although the identities of these forms were not determined, there were no differences in the forms secreted caused by the physiological state of the tissue.

The IGF are potent regulators of IGFBP-3 secretion by MAC-T cells (11, 59). As shown in Figure 2, the basal secretion of IGFBP-3 into serum-free conditioned media is relatively low but is increased up to 10-fold by treatment with 100 ng/ml of IGF-I. This effect is specific for IGFBP-3, as secretion of the other forms of IGFBP is not significantly increased by IGF treatment (Figure 2). McGrath et al. (41) also reported that IGF-I increased the secretion of IGFBP-3 by primary bovine MEC. The increases in IGFBP-3 protein secreted into conditioned media correspond to increases in the abundance of IGFBP-3 mRNA (Figure 3). The IGFBP-3 mRNA concentrations are increased dramatically by treatment with IGF-I in a dose-dependent fashion, and maximal increases are observed between 8 and 12 h of treatment (11). A similar but smaller response is observed with IGF-II treatment (Figure 3). This effect is mediated by the IGF-I receptor because treatment with B-chain IGF-I,

an IGF analog that fails to activate the IGF receptor but has normal affinity for IGFBP, does not elicit the response (11). Exposure of MAC-T cells to IGF-I or -II has no effect on the mRNA abundance of IGFBP-2 or -6 concentrations (Figure 3).

The affinities of the IGFBP for the IGF in solution are actually greater than the affinities of the IGF for the IGF-I receptor (57). Therefore, the IGFBP that are present in the extracellular environment have the potential to modulate IGF bioactivity. There are reports (12, 32) on the ability of the IGFBP to potentiate or inhibit IGF bioactivity in vitro in a multitude of cell types. Each of the six forms of high affinity IGFBP inhibits IGF action when added to culture media in excess relative to IGF. The mechanism for this inhibitory activity appears to be a competition for binding to the IGF receptor. In contrast, potentiation of IGF action by IGFBP has been reported for IGFBP -1, -2, -3, and -5.

Because IGF-I is a potent regulator of DNA synthesis in MEC, and IGF-I stimulates IGFBP-3 synthesis, we examined the ability of IGF-I to stimulate DNA synthesis in MAC-T cells transfected with IGFBP-3. As shown in Figure 4, the ability of IGF-I to stimulate DNA synthesis is enhanced in MAC-T cells transfected with IGFBP-3 relative to mock-transfected controls. Increases in DNA synthesis are similar when mock-transfected cells are exposed either to 5 μg/ml of insulin or to a maximal dose of IGF-I. In contrast, treatment of the IGFBP-3 transfected cells with 50 to 200 ng/ml of IGF-I stimulates DNA synthesis more than insulin does. Because 5 μg/ml of insulin activates the IGF-I receptor but does not bind to IGFBP, these data indicate that IGFBP-3 enhances the action of high concentrations of IGF-I in these cells. However, at lower IGF-I concentrations, IGFBP-3 transfected cells were not more responsive to IGF-I than were mock-transfected control cells. Therefore, the ratio of IGF-I to IGFBP-3 may be important in determining how IGFBP-3 modulates IGF-I action in this cell type. Potentiation of IGF-I action has also been shown with human breast tumor cells (MCF-7) that were transfected with IGFBP-3 (9). The addition of conditioned media collected from MAC-T cells that were transfected with IGF-I, which contains high concentrations of IGFBP-3, enhanced DNA synthesis that was stimulated by IGF-I in wild-type MAC-T cells (59).

The association of IGFBP-3 with the cell surface has been suggested to affect its ability to potentiate IGF action. The association of IGFBP-3 with the cell surface has been demonstrated in MAC-T cells, MCF-7 cells, and fibroblasts (9, 15, 76). The IGFBP-3 that is associated with the cell surface has a lower affinity

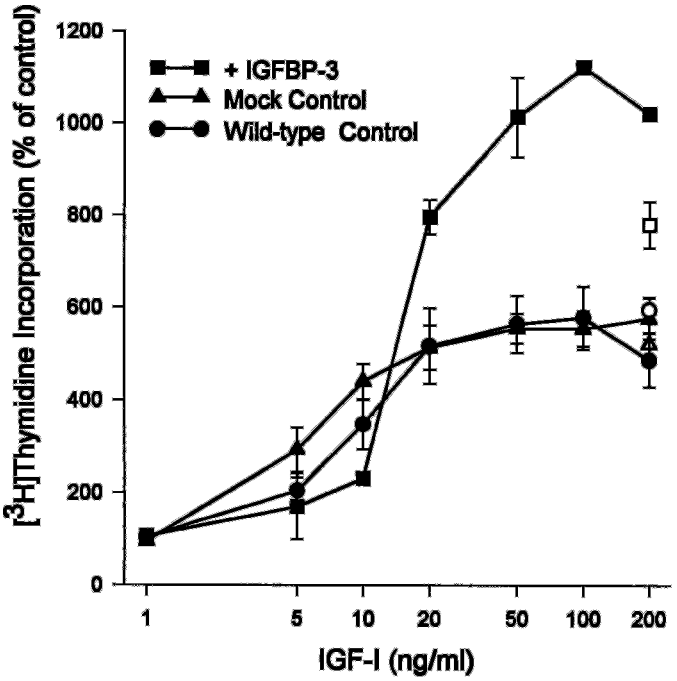

Figure 4. Effects of IGF-I on DNA synthesis in MAC-T cells transfected with the IGF binding protein (IGFBP)-3 (+IGFBP-3), vector alone (mock control), or untransfected (wild-type control) cells. MAC-T cells were grown to confluence, incubated in serum-free media overnight, and then incubated an additional 24 h in serum-free media. Treatments were added with [^{3}H]thymidine, and the assay was harvested after 24 h. Values are expressed as a percentage of serum-free controls. Solid symbols represent the response to IGF-I; open symbols represent the response to 5 μg/ml of insulin.

for IGF-I than does soluble IGFBP-3 (14, 40), which may permit a more favorable balance with the IGF-I receptor, resulting in potentiation of IGF activity. Conover (14) suggested that cell surface association was accompanied by processing of IGFBP-3 to a lower molecular mass form with a lower affinity for IGF-I. Furthermore, the addition of a purified 30-kDa proteolytic fragment of IGFBP-3 with reduced affinity for IGF-I potentiated IGF-I-stimulated DNA synthesis in osteoblasts, but native IGFBP-3 was inhibitory (62). Whether proteolysis must occur on the cell surface or in the media to obtain this effect is unknown.

The data presented suggest a role for IGFBP-3 in potentiating IGF action. However, there is considerable evidence obtained from human breast cancer cells that suggests an inhibitory role for IGFBP-3 in cellular growth. In human breast cancer cells that are negative for estrogen receptors, the addition of IGFBP-3 alone inhibits DNA synthesis in a dose-dependent fashion. The specificity of this response was demonstrated by the lack of a similar effect with the addition of IGFBP-1. This response has been

postulated to be independent of IGF because neither IGF nor IGF analogs with reduced affinity for IGFBP stimulate growth in this cell line (47). The growth inhibitors transforming growth factor-β and antiestrogens stimulate the synthesis of IGFBP-3 by breast cancer cells (29, 48). The ability of IGFBP-3 antisense oligonucleotides to attenuate the inhibition of cell growth by these factors suggests that IGFBP-3 is involved in the growth inhibitory response (29, 48). Furthermore, in cells positive for estrogen receptors, IGFBP-3 addition inhibits both basal and cellular proliferation and cellular proliferation stimulated by estradiol (51). Whether these mechanisms are relevant to the growth regulation of normal MEC is unknown.

CONCLUSIONS

Considerable data exist to support a role for IGF-I in the regulation of lactation in ruminants. However, the precise mechanisms by which this regulation occurs remain undefined. The existence of multiple forms of IGFBP with which the IGF associate makes definition of their mechanism of action in the lactating mammary gland more complicated.

During ST treatment and feed deprivation, circulating concentrations of IGF-I and milk yield change similarly, suggesting that an endocrine mechanism of IGF-I action may be important. Similar directional changes in hepatic concentrations of IGF-I mRNA indicate that alterations in the hepatic synthesis of IGF-I are partially responsible for alterations in circulating concentrations. Although IGF-I mRNA is expressed in the lactating mammary gland, what role, if any, local production of IGF-I has in the regulation of milk synthesis is unclear.

Regardless of its origin, the mechanism by which IGF-I affects the lactating mammary gland presently is unknown. Little evidence exists for a direct galactopoietic effect of IGF-I in the bovine. In light of the increased lactational persistency that is observed with long-term bST treatment, it seems logical that either increased proliferation or decreased apoptosis of mammary secretory epithelial cells must occur in addition to any changes in secretory activity to support the sustained increase in milk yield over time. Clearly, IGF-I has the ability to stimulate the proliferation of MEC; however, the extent to which this occurs in the lactating mammary gland is not well defined. The recent reports of IGF-I inhibition of apoptosis suggest an appealing mechanism by which IGF-I may influence milk yield. Further delineation of such mechanisms will require a much better understanding of the roles of the IGFBP in modulating IGF bioactivity.

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