Effect of Bovine Somatotropin on the Distribution of Immunoreactive Insulin-Like Growth Factor-I in Lactating Bovine Mammary Tissue

ABSTRACT

The distribution pattern of immunoreactive insulin-like growth factor-I in normal lactating bovine mammary tissue and in tissue obtained after bovine somatotropin treatment was determined by indirect immunofluorescence. In normal tissue, insulin-like growth factor-I immunoreactivity was observed almost exclusively associated with stromal elements. Intralobular stromal cells, small blood vessels, and capillaries all expressed moderate to high immunoreactivity. In contrast, mammary epithelial cells displayed only sparse cytoplasmic immunoreactivity. Immunoreactive material was also present in the periductular connective tissue area, possibly associated with the basal plasma membrane of epithelial cells. Somatotropin treatment of animals resulted in elevated serum insulin-like growth factor-I concentrations and altered the distribution of insulin-like growth factor-I-stainable material in mammary tissue. After somatotropin treatment, immunoreactivity was still detected in mammary stroma; however, prominent staining was also observed in the cytoplasm of mammary epithelial cells. Given the possible role of insulin-like growth factor-I in the regulation of bovine mammary epithelial cell growth and function, our findings raise the possibility that somatotropin may induce insulin-like growth factor-I production in mammary tissue, or other tissues, to influence indirectly the growth or function of the epithelial cells. This offers a possible mechanism for bovine somatotropin stimulation of lactation.

INTRODUCTION

Much recent attention has been paid to the ability of injected bovine somatotropin (bST) to stimulate milk production markedly in lactating dairy cattle (4). This phenomenon has considerable biological interest as well as economic significance for the dairy industry. However, the biochemical details of bST action in the mammary gland are not well understood. Enhanced substrate processing by the mammary epithelial cells or increased numbers of epithelial cells are necessary for milk production to increase. However, bST cannot interact directly with the epithelial cells, because they apparently do not possess specific bST receptors (2, 15, 22). To stimulate milk production, bST must therefore act directly on other mammary cell types of nonmammary cells to influence indirectly the growth and activity of mammary epithelial cells.

A number of recent studies indicate that the stimulatory effect of somatotropin on tibial cartilage growth in the epiphyseal growth plate of hypophysectomized rats is the result of local somatotropin-induced insulin-like growth factor-I (IGF-I) production (20, 26). That somatotropin may exert a similar indirect action on other target tissues is suggested by the observations that IGF-I has mitogenic action on several types of cultured cells, such as human fibroblasts and porcine smooth muscle cells, that also produce IGF-I in response to somatotropin (1, 7). Furthermore, multiple rat tissues (e.g., kidney, lung, heart, liver, testes) produce IGF-I in response to somatotropin (12). The abundance of IGF-I mRNA in a variety of rat tissues has also been shown to be regulated by somatotropin (19). Thus, it is possible that somatotropin acts indirectly on a number of tissues by stimulating local production of IGF-I.

The presence of specific IGF-I receptors on bovine mammary epithelial cells (6) and the recent finding that IGF-I exerts mitogenic action on these cells in culture (5) point to a
role for IGF-I in the regulation of mammary epithelial cell growth in dairy cattle. In addition, bST administration to lactating dairy cattle results in an increase in serum IGF-I concentration (11). The objectives of this study were to characterize the cell-specific localization of immunoreactive IGF-I in mammary tissue of lactating dairy cattle and to determine the effect of bST injections on the distribution of IGF-I in mammary tissue.

MATERIALS AND METHODS

Animals and Experimental Design

Experiment 1. An experiment was undertaken to determine the time course response in serum IGF-I concentration to a single bST injection. Four lactating Holstein cows (second lactation) between 40 and 53 d postpartum were used. Animals were fed a complete mixed diet containing 60% concentrate and 40% forage. All animals received a single saline injection (control) followed by periodic blood sampling for 24 h, and then a single bST injection followed by periodic blood sampling for 72 h. The bST used in all experiments was a preparation produced by recombinant DNA technology (Lot No. PR6776C-169A, American Cyanamid Company, Princeton, NJ). Physiological saline (2.0 ml, .9% NaCl) and bST (20.6 mg in 2.0 ml physiological saline) were administered as single subcutaneous injections in the shoulder area. The bST was dissolved in physiological saline just prior to administration.

A catheter for blood withdrawal was inserted into the jugular vein of each animal the day before the experiment started. Blood samples (10 ml) were taken just prior to administering the saline injection and at 4-h intervals following injection. After bST injection, blood samples were taken at 2 or 4-h intervals. Blood samples were allowed to clot at room temperature for 4 h and centrifuged at 1000 × g for 15 min. Serum was stored at -20°C until processed for use in the IGF-I radioimmunoassay (RIA).

Experiment 2. Three Holstein cows in second or later lactation were used. Two animals were in late lactation (240 and 276 d postpartum) and one was in early lactation (97 d postpartum). Animals were fed a complete mixed diet containing 50% concentrate and 50% forage. All animals received daily saline injections for 3 consecutive d (control) and then bST (20.6 mg/d) injections for another 3 consecutive d. Injection volumes and administration route were the same as in Experiment 1. Mammary tissue was sampled by percutaneous biopsy 10 h after both the last saline and bST injections. Animals were milked 3 to 4 h prior to taking mammary biopsies. Blood samples (10 ml) were withdrawn by puncture of the coccygeal vein or artery 4 h before the first saline injection and at 12-h intervals over the entire experimental period. Blood samples were collected into siliconized Vacutainers (Beckton Dickinson and Company, Rutherford, NJ) and serum obtained and stored as in Experiment 1.

Analysis of Serum IGF-I Concentration

The IGF-I in serum samples was separated from its serum binding proteins by acid gel filtration using a previously published procedure (30) and suggested modifications in the procedure (9). The IGF-I was measured by RIA using antiserum (UB 286) provided by J. J. Van Wyk and L. E. Underwood, University of North Carolina, and distributed by the National Hormone and Pituitary Program of the National Institutes of Arthritis, Diabetes, Digestive, and Kidney Diseases. This antiserum is highly specific for IGF-I and has only .5% cross-reactivity with IGF-II.

The RIA for IGF-I was carried out in 12 x 75-mm polystyrene tubes (Fisher Scientific, Edmonton, AB) using a nonequilibrium technique (14). The assay buffer contained .05% Tween 20 (Fisher Scientific) as a substitute for bovine serum albumin (9). An appropriate volume of the gel filtrate of serum was dried under nitrogen gas in an assay tube and re-suspended in 100 µl of assay buffer to obtain 1:200 dilution. Standards (.125 to 5.0 ng/ml IGF-I in 100 µl assay buffer) or unknowns were preincubated with the antiserum (1:8000 final dilution) for 3 d at 4°C before addition of [125I]IGF-I. The standard used was a preparation of IGF-I synthesized by solid phase methodology and purified by high performance liquid chromatography (Lot No. 588C, Bachem Fine Chemicals, Torrance, CA). This preparation was also used for iodination (125I, ICN.
Radiochemicals, Irvine, CA) by the chloramine-T method (30). Approximately 10,000 cpm $^{[125]}$ IIGF-I in 100 μl of assay buffer were added and the incubation carried on for another 24 h at 4°C. The final volume of the reaction mixture was .5 ml.

Separation of antibody-bound and free $^{[125]}$ IIGF-I was accomplished by adding 100 μl goat antirabbit gamma globulin (diluted 1:25 in assay buffer, Gibco Canada Inc., Burlington, ON) and 100 μl of normal rabbit serum (diluted 1:150 in assay buffer). After 24 h at 4°C the tubes were centrifuged (3000 × g, 15 min, 4°C), the supernatant aspirated, and the precipitate counted in a gamma counter. The gel filtrate of each serum sample was tested in duplicate. Displacement of $^{[125]}$ IIGF-I by gel filtrates of bovine serum was parallel to IGF-I standards. All samples were assayed in a single assay. The within assay coefficient of variation was 3.5%. Recovery of synthetic IGF-I added to bovine serum, incubated for 1 h at room temperature, and chromatographed was 97%. Recovery of $^{[125]}$ IIGF-I from bovine serum was 93% of the added counts using this method. The reported concentration values are not corrected for the apparent loss.

Mammary Biopsy Technique

Parenchymal tissue was aseptically excised from the left rear quarter of the mammary gland of each animal using a Tru-Cut biopsy needle (Travenol Laboratories, Deerfield, IL). The second biopsy was taken approximately 10 cm distant (to the right) from the first biopsy site. Tissue samples (approximately 15 mm long × 1 mm o.d.) were taken from an area about 15 cm dorsal to the base of the teat at a depth of approximately 8 cm. Sampling from this location yields tissue relatively free of large ducts (22). Prior to each tissue excision, animals received a local anesthetic (2.0 ml of 2% lidocaine) that was injected under the skin surrounding the site of incision and biopsy needle insertion. An incision (approximately .5 cm) was required to facilitate biopsy needle insertion. Tissue excision caused no external bleeding. In a preliminary experiment, post-mortem mammary tissue was examined the day after performing a single percutaneous biopsy. Evidence of mild tissue trauma (i.e., slight reddening) was confined to the immediate area around the excision site (maximum 1 cm radius). Puncture track tissue damage was not visible.

Morphological Techniques

Tissue Fixation and Cryoprotection. Excised tissue was immediately placed in a fixative solution of 4% paraformaldehyde in 100 mM phosphate buffer, pH 7.2, and immersion-fixed for 90 min at 4°C. The fixative solution was prepared fresh daily using powdered paraformaldehyde (Fisher Scientific Company). Following fixation, tissue was washed with three changes of phosphate-buffered saline (PBS, pH 7.4), 30 min each, at 4°C. Tissue was then cryoprotected by immersion in 30% sucrose (wt/vol) in PBS for 3 h at 4°C.

Embedding and Sectioning. Tissue was mounted in OTC compound (Ames Company, Elkhart, IN) by rapidly freezing in isopentane (–150°C) cooled by liquid nitrogen. Mounted tissue was stored in air tight polypropylene bags at –70°C until cryosectioning. Tissue sections for immunocytochemistry were cut at 8 μm in a cryostat (Ames Company) at –20°C. Serial sections were periodically taken and stained with hematoxylin and eosin for examination of tissue morphology and preservation by light microscopy.

Immunocytochemistry. After cryosectioning, frozen tissue sections were picked up onto rubber cement (Lepage's Limited, Bramalea, ON) coated coverslips and allowed to dry for 30 min at room temperature. Immediately after drying, sections were washed in PBS for 30 min at room temperature. To minimize nonspecific binding of the secondary antibody, sections were incubated with normal goat serum (30% in PBS) for 30 min at 4°C.

After incubation with primary antibody, sections were washed three times with PBS, 15 min each at room temperature. Secondary
antibody (fluorescein isothiocyanate (FITC)-labeled goat antibody to rabbit immunoglobulin G, Sigma Chemical Company, St. Louis, MO) at 1:30 dilution in PBS was incubated on sections for 60 min at room temperature. The incubation with secondary antibody, as well as all subsequent procedures in the immunocytochemical protocol, was carried out in the dark. After incubation with the secondary antibody, sections were washed three times with PBS, 15 min each at room temperature. A final wash comprising three brief immersions in distilled water was performed before mounting sections. Sections were mounted in glycerol-containing paraphenylenediamine to reduce immunofluorescent fading (21).

Immunocytochemical Controls. Controls to check the specificity of the immunocytochemical reactions included: omission of the primary or secondary antibodies, substitution of the primary antibody with serum from an unimmunized rabbit, and incubation with antiserum (K1792) depleted of anti-IGF-I antibodies by solid phase immunoabsorption (29). The solid phase immunoabsorbent was prepared by coupling 20 μg of synthetic IGF-I in PBS (Bachem Fine Chemicals) to cyanogen bromide-activated sepharose 4B (Sigma Chemical Company) using a previously described protocol (29). A control sepharose gel was prepared by the same protocol, using PBS containing no IGF-I. Liquid phase absorption was also performed by incubating K1792 antiserum (1:1000) with .1, .25, .5, 1.0, 1.5, 2.0, and 5.0 μg/ml IGF-I for either 30 min or 3 h at 37°C prior to applying antiserum to the tissue sections. Liquid phase absorption was also performed by incubating K1792 antiserum with IGF-I for 30 min at 37°C and then for 18 h at 4°C. Following the 18-h incubation, the antiserum containing IGF-I was centrifuged at 8000 × g for 5 min (4°C), and the supernatant was removed and applied to tissue sections.

The antiserum used has been previously well characterized and shown to be highly specific for IGF-I (17). Specificity of the antiserum has been demonstrated by RIA, double-immunodiffusion, Western and dot blotting (17). Relaxin, IGF-II, and proinsulin were shown to have no significant crossreactivity with the antiserum.

Fluorescence Microscopy and Photography. Fluorescent microscopic images were recorded on Kodak Ektachrome 200 film using a Leitz Dialux photomicroscope equipped with epillumination for fluorescence microscopy. A standard exciter/barrier filter combination for fluorescein was used. All fluorescent photomicroscopy was performed immediately after sections were mounted. Preparations were observed with 25X and 40X PL, and 100X NPL oil immersion objectives. Typical exposures were about 10 s. Images of control sections were recorded using the same exposure times.

RESULTS

Effect of Bovine Somatotropin on Concentration of Insulin-Like Growth Factor-I in Serum

The effect of a single bST injection on serum IGF-I concentration is shown in Figure 1. The basal concentration of IGF-I averaged throughout the period before bST injection was 261.7 ± 12.3 (SEM) ng/ml; an increase was first detected 8 h after the bST injection, and the maximum value (509.0 ± 26.4 ng/ml) was reached at 18 h. The IGF-I concentration at 72 h after injection (333.8 ± 33.8 ng/ml) was still slightly higher than the basal value.

One of the cows (late lactation) in Experiment 2 failed to show a significant rise of serum IGF-I above the basal value after any of the bST injections (data not shown). This cow exhibited nervousness during blood sampling, injection administration, and the biopsy procedure. At initiation of bST treatment, this

![Figure 1. Effect of a single injection of bovine somatotropin on serum insulin-like growth factor-I concentration. 20.6 mg bST were administered subcutaneously at time 0 (arrow). Data points are means for four cows.](image-url)

cow's feed intake had decreased to less than half of the intake at the start of the experiment. Because fasting and protein-caloric deficiency are known to inhibit markedly the somatotropin-stimulated rise of serum IGF-I (25), lack of an IGF-I response in this animal was assumed to be related to her nutrient-deprived state.

The effect of three consecutive daily bST injections on serum IGF-I concentration is shown in Figure 2. The basal concentration of IGF-I averaged throughout the control period (saline treatment) was 372.9 ± 12.2 ng/ml. By 8 h after the first bST injection, an increase in the serum IGF-I concentration was detected. A further elevation in IGF-I concentration was observed 20 h after the first injection and again within 8 h after the second bST injection. The magnitude of the response to the second injection was similar to that observed for the initial injection. An additional injection of bST did not seemingly result in a further increase in the serum IGF-I concentration; however, the peak response was maintained and still present at 8 h after the last bST injection. At peak response, serum IGF-I concentration was raised about twofold above the basal value. Based on the information obtained concerning the time course of the IGF-I response to bST, serum IGF-I concentrations were at or near peak response values when the second mammary biopsies were taken.

Morphology of Lactating Mammary Tissue

Light microscopic examination of cryostat sections of mammary biopsies stained with hematoxylin and eosin revealed satisfactory morphological preservation (Figure 3). Tissue was characterized by distended alveoli that were lined by a single layer of epithelial cells. Varying amounts of stainable secretory material and some cell debris were observed within the lumens of alveoli. Epithelial cells were frequently seen to contain large supranuclear fat globules abutting into the alveolar lumen. Myoepithelial cells, that lie between the bases of the epithelial cells, were difficult to identify because in the distended alveoli they are mostly flattened beyond recognition. When observed, however, they were seen to contain small nuclei surrounded by clear cytoplasm.

Alveoli were surrounded by compressed connective tissue of the intralobular stroma. Numerous small blood vessels and dilated capillaries were present in the intralobular stroma. Fibroblast-like cells as well as other cell types, presumably lymphocytes, blast cells, and plasmacytes, were also observed in the stromal matrix.

Distribution of Immunoreactive Insulin-Like Growth Factor-I in Control Tissue

In mammary tissue from saline-injected animals the distribution of IGF-I immunoreactive material was qualitatively similar for all animals. The IGF-I immunoreactivity was primarily seen associated with stromal elements (Figure 4). The most intense staining was consistently present as a cytoplasmic accumulation of aggregate granules in cells located in the intralobular stroma. Some intralobular cells also showed circumferential staining patterns. This immunofluorescent pattern may represent IGF-I binding to cell surface receptors. In contrast, the attenuated fibroblasts that lie adjacent to the connective tissue surrounding each individual alveolus were seemingly negative.

Small blood vessels showed moderate to high IGF-I immunoreactivity. Staining of the media in vessel walls may represent IGF-I associated with smooth muscle cells or extracellular IGF-I.

Figure 2. Effect of three consecutive daily bovine somatotropin (bST) injections on serum insulin-like growth factor-I concentration. The bST (20.6 mg/d) was administered subcutaneously (arrows). Mammary biopsies (arrowheads) were taken before and after the bST treatment period. Data points are means for two cows.
in the connective tissue or both. Occasionally IGF-I immunoreactive material was seen in the tunica intima, presumably associated with the endothelial cells. Capillaries in the intralobular stroma also stained. This staining presumably represents IGF-I associated with endothelial cells of capillaries, or with the modified smooth muscle cells (i.e., pericytes) that surround capillaries.

Myoepithelial cells, which surround the glandular alveolus, were seemingly negative. Occasional fine elastic fibers located in the intralobular stroma showed slight autofluorescence (yellowish color) in control sections. Such autofluorescence was very rarely detected in sections stained with antiserum because of faint staining of other elements in the intralobular stroma. It is possible that some intralobular stromal staining represents IGF-I immunoreactivity associated with plasma cells (e.g., lymphocytes), fibroblastic processes, or simply free IGF-I in this extracellular matrix.

Although immunoreactive IGF-I was not detected in the cytoplasm of most epithelial cells, some displayed a few discrete cytoplasmic fluorescent granules (Figure 4B). In contrast, the circumferential distribution of intense IGF-I immunoreactivity around each individual alveolus suggests that this growth factor is mainly associated with the basal plasma membrane of epithelial cells. Alternatively, this fluorescent pattern may represent IGF-I immunoreactive material in periductular connective tissue.

**Distribution of Immunoreactive Insulin-Like Growth Factor-I in Bovine Somatotropin-Treated Tissue**

The distribution of IGF-I detected by immunofluorescence in tissue obtained after bST treatment was qualitatively similar for all animals. The distribution was also similar in many respects to that seen in control tissue.
Figure 4. Representative immunofluorescent micrographs of insulin-like growth factor-I (IGF-I) localization in mammary tissue from saline treated (control) lactating Holstein cows. Cryostat sections 8-μm thick were stained with 1:1000 dilution of anti-IGF-I antiserum (K1792). A) Tissue from control animal illustrates IGF-I immunoreactivity primarily associated with stromal elements. ×470. B) At higher magnification, intensely IGF-I immunoreactive blood vessels (arrow) and unidentified stromal cells (arrowhead) are observed. Note that the alveolar epithelium in control tissue contains only sparse IGF-I-stainable material. ×740.
Cells and tissue elements in the intralobular stroma that were IGF-I immunoreactive in control tissue were also stained in tissue obtained after bST treatment (Figure 5). The epithelial cells, however, more frequently displayed cytoplasmic IGF-I immunofluorescence when compared with epithelial cells in control tissue (compare Figure 4 and 5). Most epithelial cells contained a diffuse distribution of IGF-I immunofluorescent granules. Sparing of nuclei and probable lipid globules or secretory vesicles could be visualized in these cells. Although convolutions in the basal plasma membrane were not visible at the resolution used, the rounded bases of bulging epithelial cells were occasionally discernable, presumably because of immunofluorescent IGF-I molecules bound to basal plasma membrane receptor sites. Occasionally, cells occupying locations typical of myoepithelial cells in lactating mammary tissue were observed to contain a moderate amount of diffuse IGF-I-stainable material. Although the method used here is not strictly quantitative, there also appeared to be an increase in overall staining intensity after bST treatment (compare Figure 4 and 5).

Immunocytochemical Controls

Control sections showed no IGF-I immunoreactivity when the primary antibody K1792 or the secondary antibody was omitted (Figures 6A, B). Incubation with serum from an immunized rabbit resulted in only a low level of nonspecific background staining (Figure 6C). Liquid phase absorption of K1792 with various concentrations of IGF-I and incubation conditions failed to abolish staining (not shown). Staining was sometimes enhanced by this treatment. After solid phase absorption of K1792, specific staining was abolished, and only background staining was observed (Figure 6D). Preincubation of K1792 with the control sepharose gel did not change either the intensity or pattern of staining (not shown). The failure of liquid phase absorption to abolish staining and, in some instances the increase of staining after liquid phase absorption have been reported by other researchers using antibodies against different antigens (27, 28). The added antigen used for liquid phase absorption likely explains enhanced staining. Failure of liquid phase absorption to abolish staining is thought to be caused by the presence of high antigen density in the tissue section, resulting in higher functional affinity of specific antibodies for antigen in the tissue than for antigen in the solution (27).

DISCUSSION

Indirect immunofluorescence was used to determine the localization pattern of immunoreactive IGF-I in mammary tissue obtained from both normal and bST-treated lactating dairy cattle. The immunocytochemical results show that under normal conditions IGF-I immunoreactive material is present in lactating bovine mammary tissue, and that it is primarily found associated with stromal elements in this tissue. Injection of cows with bST altered the distribution of IGF-I immunoreactivity in this tissue. After bST treatment, IGF-I immunoreactivity was still detected in mammary stroma; however, there was also prominent staining in the alveolar epithelium. Most IGF-I immunoreactivity in the alveolar epithelium was present as diffuse cytoplasmic granules in epithelial cells, but occasionally myoepithelial cells also displayed immuno-stained material. The observed changes in the distribution of IGF-I in mammary tissue correspond temporally with increased circulating IGF-I and milk production responses, which are consistently observed by 3 d after starting daily bST injections at dosages similar to that used in the present study (4).

The subcellular localization of IGF-I immunoreactivity present in the cytoplasm of epithelial cells after bST treatment could not be determined with the method used in the present study. Regardless of its subcellular location, this staining pattern suggests either local synthesis or internalization of IGF-I-stainable material. Local synthesis cannot be excluded, but bST-induced local synthesis in the epithelial cells is considered to be unlikely because these cells apparently do not possess specific bST receptors (2, 15, 22). Internalization of receptor bound IGF-I is more likely because these cells possess specific IGF-I receptors (6). Furthermore, because biological action of IGF-I on target cells presumably requires ligand-receptor binding and internalization, the finding that IGF-I has mitogenic action on cultured bovine mammary epithelial...
Figure 5. Representative immunofluorescent micrographs of insulin-like growth factor-I (IGF-I) localization in mammary tissue from bovine somatotropin (bST)-treated lactating Holstein cows. Cryostat sections 8-μm thick were stained with 1:1000 dilution of anti-IGF-I antiserum (K1792). A) Tissue from bST-treated animal shows staining of stromal elements (as in control tissue), however there is also staining in the alveolar epithelium. ×470. B) At higher magnification, prominent staining is observed in the cytoplasm of epithelial cells (arrows). After bST treatment, probable myoepithelial cells (arrowhead) are also seen to contain IGF-I-stainable material. ×740.
Figure 6. Immunofluorescent micrographs of control series demonstrating the specificity of the immunocytochemical reaction. No specific staining is observed in any of the control tests. X740. A) Omission of the primary antibody (K1792). B) Omission of the secondary antibody. C) Substitution of the primary antibody with serum from an unimmunized rabbit. D) Incubation with serum depleted of anti-insulin-like growth factor-I antibodies by solid phase immunoabsorption.

cells also suggests that the staining observed in these cells is the result of internalization of receptor bound IGF-I (5).

The source of the IGF-I that was observed in mammary epithelial cells after bST treatment is unknown. The twofold increase in serum IGF-I concentration in bST treated animals suggests that the IGF-I found in epithelial cells was synthesized in other tissues and delivered to the mammary gland by the blood. But it is also possible that this IGF-I is both bloodborne and locally synthesized IGF-I that has been internalized by the epithelial cells. Consistent with the hypothesis of local IGF-I synthesis is our recent finding that IGF-I mRNA is synthesized in lactating bovine mammary tissue (unpublished observation). Regardless of the source, the presence of more IGF-I in mammary epithelial cells after bST treatment seems to have considerable relevance to the biological action of bST on mammary tissue. The results of this study suggest that IGF-I mediates the stimulatory effect of bST on mammary tissue. Such a mechanism would likely involve bST-induced IGF-I interacting directly with the epithelial cells to stimulate mitogenesis, or alter specific metabolic processes.

Some of the IGF-I immunoreactivity detected in mammary stroma in the present study may represent IGF-I associated with intralobular fibroblasts. Consistent with this possibility is evidence that fibroblasts in other species have specific IGF-I receptors (24) and that cultured fibroblasts synthesize IGF-I (1). Insulin-like growth factor-I has a wide range of biological effects on fibroblasts in culture (8). Thus, these observations not only suggest that mammary fibroblasts would stain for IGF-I, but may also explain both the circumferential staining pattern and cytoplasmic staining seen in unidentified stromal cells in this study. That fibroblasts in culture synthesize IGF-I in response of somatotropin further suggests that these cells in mammary tissue may respond to bST in a similar fashion. Currently, we are undertaking in situ hybridization studies to examine which cell types synthesize IGF-I mRNA in bovine mammary tissue utilizing a cloned IGF-I gene probe.

Both before and after bST treatment, IGF-I immunoreactivity was also visualized in small blood vessels and capillaries located in the intralobular and interlobular mammary stroma. This observation agrees with the recent finding that several elements in rat blood vessel walls display immunofluorescence when stained by indirect immunofluorescence with specific anti-IGF-I antibodies (16). Mammary vascular staining after bST injection, a treatment known to increase mammary blood flow in lactating dairy cattle (10), suggests a role for IGF-I in adaptation to increased blood supply. This idea is also supported by the finding that IGF-I immunoreactivity is increased in rat blood vessels in response to a vascular load (16). An IGF-I role in other mammary vascular processes is suggested by the observations that IGF-I has mitogenic action, and alters various metabolic processes in cultured capillary endothelial cells isolated from bovine retinas (23) and adipose tissue (3). These findings and the results of the present study suggest that IGF-I may be involved in regulating various vascular processes during both normal and bST-stimulated lactation in dairy cattle.

An alternative hypothesis to explain the vascular staining, both before and after bST treatment, is that it represents bloodborne IGF-I being stored or processed by the endothelial cells. This is supported by the recent finding that cultured endothelial cells from bovine capillaries and blood vessels have specific surface receptors for IGF-I as well as the ability to store intact IGF-I for extended periods (3). Although a role for the capillary endothelium in modulating the delivery of bloodborne IGF-I to different tissues has not been established, the possibility exists that it may be part of a complex regulatory system to control differentially the amount of IGF-I available for binding to receptors on specific target tissues. A similar function has already been proposed for some forms of the IGF-I serum binding proteins (13). If this type of regulatory system operates, it would likely be very important during bST-stimulated lactation because of the elevated serum IGF-I concentrations. Furthermore, control of mammary specific stimulation of growth by bloodborne IGF-I during bST treatment could be explained by this type of regulatory system. Alternatively, specific cells could modulate their responsiveness to increased IGF-I in the extracellular environment by simply altering the concentration of IGF-I receptors on their cell surface.
A model whereby bST stimulates IGF-I production in only nonmammary tissues to influence indirectly the growth or function of mammary epithelial cells appears overly simplistic. The adaptations required for increased milk production in response to bST probably involve actions of bST and IGF-I as well as other unidentified hormones and growth factors on both mammary and nonmammary tissues. The data presented in this paper provide evidence suggesting that IGF-I mediates the action of bST on at least mammary epithelial cells. This finding offers a possible mechanism for bST stimulation of lactation. Our results also suggest that IGF-I may be involved in regulating the growth or metabolism of other mammary cell types important in mammary gland function. Whether local IGF-I synthesis occurs in mammary tissue and is stimulated by bST could not be determined from the results of the present study. Studies are currently underway to investigate this possibility.

ACKNOWLEDGMENTS

The authors greatly acknowledge the kind gifts of antiserum (K1792) for immunocytochemistry from KabiVitrum and bST from Cyanamid. We also thank Gerry de Boer, Brian Turner, and David Kerr for valuable advice; Ingrid Moffat and Gary VanDoesburg for excellent technical assistance; and Janice McNeil for careful preparation of the manuscript. This work was supported by The National Science and Engineering Research Council of Canada and the Farming for the Future Program of the Government of Alberta.

REFERENCES

5 Baumrucker, C. R. 1986. Insulin like growth factor 1 (IGF-1) and insulin stimulates lactating bovine mammary tissue DNA synthesis and milk production in vitro. J. Dairy Sci. 69(Suppl. 1):120. (Abstr.)