Bovine growth hormone and its effects on the local production of prostacyclin I_2 and mammary blood flow in dairy cows

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ABSTRACT

Our objective was to test the hypothesis that bovine growth hormone (bGH) can induce the secretion of vasodilators by the bovine mammary gland. Three adult lactating cows were surgically implanted with catheters in the left costoabdominal artery, in the left subcutaneous abdominal vein (milk vein) and in a lymph duct afferent to the left supramammary lymph node. In addition, a transit time flow probe was implanted on the left pudic artery. Cows were injected s.c. with either saline or 25 mg of bGH according to a crossover design. Samples from arterial and venous blood and from lymph fluid were collected from 2 hours before to 16 hours after the injection. Mammary blood flow was recorded at each sampling. Injection of bGH induced a gradual increase in mammary blood flow in lactating cows while it remained constant after the saline injection. Venous concentrations of 6-keto-prostaglandin $F_{1\alpha}$, a stable metabolite of the vasodilator prostacyclin I_2, were not affected by bGH injection. However, lymphatic concentrations of the prostacyclin I_2 metabolite increased when cows were injected with bGH. The time course of the increase in plasma IGF-I concentration was similar to that of mammary blood flow and 6-keto-prostaglandin $F_{1\alpha}$. In conclusion, a local release of prostacyclin I_2 may be responsible for the bGH-induced increase in mammary blood flow. IGF-I might mediate this effect of bGH.

Keywords: Mammary blood flow; prostacyclin I_2; growth hormone; cows; 6-keto prostaglandin $F_{1\alpha}$.

INTRODUCTION

Mammary blood flow is a major determinant for the rate of substrate supply for milk synthesis (Davis and Collier, 1985). Therefore, it is not surprising that there is a close correlation between mammary blood flow and milk production (Linzell, 1974). It is well established that mammary blood flow is affected by several factors such as physiological state (Linzell, 1974), level of milk production (Linzell, 1974), frequency of milk removal (Prosser and Davis, 1992), mammary infection (Dhondt *et al.*, 1977) and fasting (Chaiyabutr *et al.*, 1980).

The nature of the control mechanism of mammary blood flow has not been fully elucidated. Reduction of mammary blood flow occured during fasting even when the gland had been denervated (Chaiyabutr *et al.*, 1980) implying that vasoconstriction is achieved by local or humoral factors. Prostaglandins and related compounds (eicosanoids) may be involved in controlling mammary vascular activity. Indeed, systemic treatment of goats with indomethacin (prostaglandin synthetase inhibitor) produced strong and sustained vasoconstriction of mammary blood vessels (Burvenich and Peeters, 1982). Prostacyclin I_2 (PGI_2) is a potent vasodilator (Moncada and Vane, 1979) and it has been reported that bovine aortic endothelial and smooth muscle cells can produce it *in vitro* (Ingerman-Wojenski *et al.*, 1981). However, it is not known if PGI_2 has any effect on mammary gland vasculature.

It is difficult to study paracrine secretion *in vivo* as it is complicated to harvest interstitial fluid and blood concentrations are not always representative of the interstitial fluid concentrations of locally produced molecules. However, in dairy cows, selection for milk yield has enlarged the mammary gland to the point that it is possible to harvest primary lymph fluid from a catheterized mammary lymph duct. The similarity between the fluid present in primary lymph vessels and the interstitial fluid (Renkin, 1979) allows the study of paracrine secretion *in vivo*.

The purpose of this experiment was to investigate whether PGI_2 (measured as its stable metabolite 6-keto-prostaglandin $F_{1\alpha}$ (6-kPGF$_{1\alpha}$)) is associated with changes in mammary blood flow produced by exogenous bovine growth hormone (bGH) in dairy cows.

MATERIALS AND METHODS

Animals

Three lactating (more than 200 d in lactation) non pregnant, adult dairy cows were used for this project. A primary lymph duct afferent to the supramammary lymph node was surgically implanted with a catheter according to the technique described by Obel *et al.* (1989). Another catheter was implanted in the costoabdominal artery (Haibel *et al.*, 1988). An ultrasonic flow probe (Transonic System Inc., Ithaca, NY, USA) was installed around the left pudic artery close to the

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inguinal ring (Gorewit *et al.*, 1989). At least, one week was allowed for recovery. The day before the first challenge, a third catheter was implanted into one of the subcutaneous mammary veins.

Experimental design

Each animal was injected s.c. in the cervical area with either saline or 25 mg bGH (American Cyanamid Co, Princeton, NJ, USA, Lot AC 6969-53 D/3) according to a crossover design. There was a minimum of 3 days between injections. Samples of arterial and venous blood and lymph were collected at 30 min intervals for the 2 h preceding the injection (1000 h), then at 20 min intervals for the next 14 h and, finally, hourly for the last 2 h. Blood flow was recorded at each sampling.

Assays

Blood samples were split into regular glass tubes (sera) and EDTA coated tubes (plasma). All samples were stored at -20°C until radioimmunoassay. Serum and lymph GH were assayed according to a double antibody RIA procedure described by Petitclerc *et al.* (1987). Plasma and lymph IGF-I were also analyzed by a double antibody RIA as described by Abribat *et al.* (1990). Prostacyclin I_2 is chemically unstable at physiological pH ($t^{1/2} \approx 3$ minutes) and results in the formation of 6-kPGF$_{1\alpha}$ which is stable (Gryglewski *et al.*, 1988). The 6-kPGF$_{1\alpha}$ concentrations were measured using a commercial kit (Amersham International Kit TRK 790). Prior to RIA, plasma and lymph sample extraction was performed with Sep Pak silica cartridge (Waters Associates, Milford, MA). The inter- and intra-assay coefficients of variation were 6.1 and 5.2% for GH, 8.0 and 4.5% for IGF-I and, 12.0 and 4.0% for 6-kPGF$_{1\alpha}$, respectively.

Statistics

Variables were analyzed as a repeated measurement design using the GLM procedure of SAS (1985). In addition, linear coefficients were evaluated on blood flow, 6-kPGF$_{1\alpha}$ and IGF-I profiles and an analysis of variance was performed on these coefficients.

RESULTS

Growth hormone responses to bGH or saline injection are depicted in Fig. 1. Saline injection had no effect on GH concentrations. After bGH injection, blood GH concentration steeply increased ($P < 0.001$) and peaked within 3 hours. There was no significant difference between arterial and venous blood GH concentrations ($P > 0.25$; venous concentration not shown).

Bovine growth hormone injection induced a gradual increase in mammary blood flow ($P < 0.01$; Fig. 2), which was still increasing at the end of the sampling period. Mammary blood flow remained fairly constant after saline injection.

Venous plasma concentrations of 6-kPGF$_{1\alpha}$ were not affected by saline or bGH injections ($P > 0.25$) and averaged 0.83 ± 0.28 ng/ml. However, concentrations of 6-kPGF$_{1\alpha}$ in lymph gradually increased after treatment with bGH ($P < 0.001$; Fig. 3) but not after saline injection ($P > 0.25$).

FIGURE 1: Arterial serum bGH responses to slaine (O), or 25 mg of bGH (•) sc injections in lactating dairy cows. Post-injection concentrations were different ($P<0.001$; see statistic in methods section).

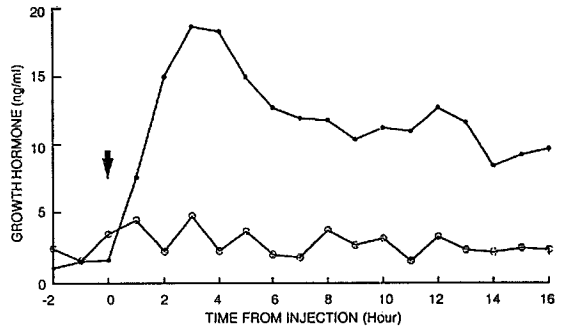


FIGURE 2: Mammary gland blood flow responses to saline (O), or 25 mg of bGH (•) sc injections in lactating dairy cows. The increase in blood flow after bGH was significant ($P<0.01$; see statistic in methods section).

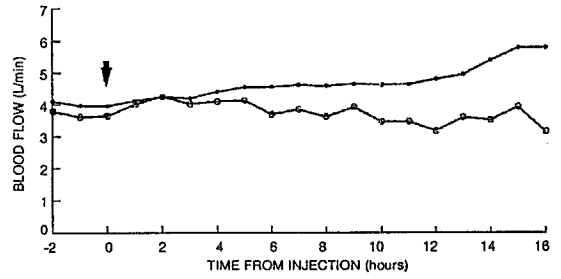


FIGURE 3: Lymphatic 6-kPGF$_{1\alpha}$ responses to saline (O), or 25 mg of bGH (•) sc injections in lactating dairy cows. The increase in 6-kPGF$_{1\alpha}$ after bGH was significant ($P<0.001$; see statistic in methods section).

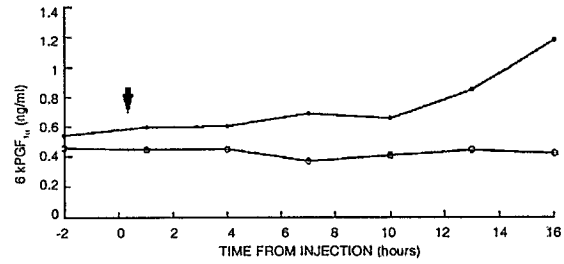
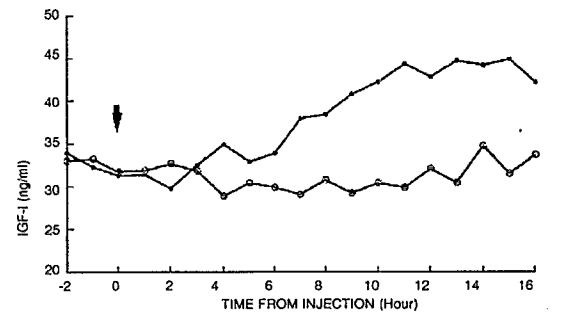


FIGURE 4: Arterial plasma IGF-I responses to saline (O), or 25 mg of bGH (•) sc injections in lactating dairy cows. The increase in IGF-I after bGH was significant ($P<0.01$; see statistic in methods section).



The bGH injection induced a linear increase of arterial plasma IGF-I ($P < 0.01$; Fig. 4); venous concentrations were similar to arterial concentrations ($P > 0.25$).

DISCUSSION

The increase in mammary blood flow following bGH injection in lactating cows agrees with previous results reported in the literature (Mepham *et al.*, 1984; Davis *et al.*, 1988). The comparison of Fig. 2 and Fig. 3 clearly suggests that this effect is mediated by the release of PGI_2. The lack of effect on venous plasma 6-k$PGF_{1\alpha}$ suggests that the endothelial cells of the mammary vasculature released prostacyclin into the interstitial fluid. Christensen *et al.* (1989) reported that milk 6-k$PGF_{1\alpha}$ increased in 2 goats treated with bGH for 7 days. Another vasodilator (endothelium-derived relaxing factor; EDRF) may also have been released in bGH-treated lactating cows. Indeed, most stimulants of PGI_2 release also stimulate EDRF release (Gryglewski *et al.*, 1988). However, EDRF is a labile vasodilator with a half life counted in seconds and is, therefore, very difficult to measure *in vivo*.

The difference between the shape of mammary blood flow, 6-k$PGF_{1\alpha}$ and GH profiles argues against a direct effect of GH on the mammary endothelium. Insulin-like growth factor-I profile in lactating animals coincides with blood flow and 6-k$PGF_{1\alpha}$ profiles. Intra-arterial infusion of IGF-I into the mammary gland of lactating goats also increased mammary blood flow (Prosser *et al.*, 1990; Prosser and Davis, 1992).. It has been established that the receptor-mediated release of PGI_2 and EDRF are coupled with the activation of phospholipase C (De Nucci *et al.*, 1988), which is the key step in the phosphatidyl inositol pathway. There are two types of IGF receptors, but only binding to the type I increases the phosphatidyl inositol turnover (Phillips *et al.*, 1990). Recently, Bornfeldt *et al.* (1993) have observed that IGF-I induces migration of smooth muscle cells in human arteries and binding to the type I receptor was essential for this effect. While these results do not necessarily imply an effect of IGF-I on PGI_2 secretion, they clearly demonstrate that IGF-I can affect vasculature.

It is tempting to hypothesize that the reduction of mammary blood flow observed during fasting (Chaiyabutr *et al.*, 1980) is related to IGF-I concentration. Indeed, severe feed restricton (or negative energy balance) reduces considerably blood IGF-I concentration (Clemmons and Underwood, 1991) with a dynamic compatible to that of blood flow. Control of mammary production of PGI_2 is not exclusive to IGF-I. Acetylcholine and bradykinin, for example, are two mammary gland vasodilators (Dhondt *et al.*, 1973) which lead to the generation of PGI_2 (and EDRF) in several other systems (Gryglewski *et al.*, 1988).

In conclusion, a local release of PGI_2 may be responsible for the increase in mammary blood flow usually observed in bGH-treated dairy cows. However, bGH may not affect the release of PGI_2 directly but indirectly through an increase in blood IGF-I concentration. In light of these results, we believe that lymphatic vessel cannulation is a good model to study the control of blood flow *in vivo*.

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