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Plasma Profiles of Somatotropin and IGF-I in Dairy Cows Following Application of Two Preparations of Recombinant Bovine Somatotropin in a Sustained Release Vehicle

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Summary

Milk production, plasma bovine somatotropin (bST) and insulin-like growth factor I (IGF-I) were measured in dairy cows following a single subcutaneous injection of a slowly released preparation of either recombinant enterokinase linker bST (somidobove: 640 mg) or recombinant methionyl bST (sometribove: 500 mg). There was a 3–7-fold increase in plasma bST concentrations during the first three postinjection hours in cows treated with both sometribove (from 3.4±0.8 to 11.2±3.0 ng ml^{-1}) or somidobove (from 2.3±0.3 to 17.5±2.6 ng ml^{-1}). In the next 8 days the bST concentration in the bST-treated cows varied, but was still significantly increased above the controls. In the following days, the concentrations of bST did not differ from the controls. Plasma concentrations of IGF-I increased nearly 2-fold as early as 24 h following recombinant bST administration and then continued to rise so that by 48 h postinjection they were nearly four times higher (control 16.2, bST-treated 61.7 ng ml^{-1}). From 48 h after sometribove injection, IGF-I concentrations remained at a plateau (varying between 60.4 and 85.7 ng ml^{-1}) till day 11. Then it decreased slowly, but still remained higher on day 14 than those in placebo-treated cows (44.4±17.8 ng ml^{-1} in bST-treated animals; 12.2±7.5 ng ml^{-1} in the controls). Although IGF-I level was increasing in all bST-treated animals, the absolute IGF-I increase was not related to the increase in milk production.

Key words

Somatotropin – IGF-I – Plasma – Milk production – Dairy cow

Introduction

The productivity of domestic animals is a function of their genetic potential and their interaction with the environment. Environmental factors are often changing independently of the farmer's activity. On the other hand, the genetic potential is a much more stable property. The genetic potential for milk production has been successfully improved by selective breeding. However, the progress in genetic gain is slow, averaging at best, about 1–3 % per year (Ward and Nancarrow 1991). Advances in basic science, particularly in molecular biology, have created new possibilities for supplementing traditional methods of improving of lactational performance of dairy cows. A recombinant DNA technology has opened the way for the production of large quantities of the recombinant bovine somatotropin the stimulating effect of which on milk production (an increase between 3.8 to 40 %) has repeatedly been demonstrated in lactating cows (Bauman *et al.* 1985, 1989, Schams *et al.* 1991a,b, Škarda *et al.* 1992a,b). Recently, unpractical daily injections of bST have been replaced by economically feasible preparations containing recombinantly derived bST in sustained release vehicles. However, the concentrations of bST in the blood plasma of dairy

cows treated with slowly released recombinant bST were usually not measured. There are only few published data from three laboratories related to bST profiles after injection of sustained release bST formulation (Cisse *et al.* 1991, Schams *et al.* 1991a,b, Škarda *et al.* 1992a).

Using intensive blood sampling the present trials investigated bST plasma profiles after injections of recombinant methionyl bovine somatotropin and recombinant enterokinase linker bovine somatotropin in sustained release vehicles in multiparous dairy cows. The effect of recombinant bST on plasma IGF-I level and milk production responses were also studied.

Materials and Methods

Trials were conducted in three commercial herds (34 cows). Cows were crossbred Bohemian spotted Friesian-Ayrshire which had never been treated with exogenous bST before experiment. Cows were housed in tie stalls and offered corn silage, haylage and alfalfa or clover hay and concentrate mixtures to meet their nutrient requirements for maintenance and milk production (NRC, 1978).

Two forms of recombinantly derived bST were used. The first, somidobove, consists of the 190 amino acid form of pituitary bST with a short additional moiety of nine amino acids at the aminoterminus (enterokinase linker bovine somatotropin). Somidobove (640 mg, 28.08 μmol) was supplied as the formulated product in cartridges (OPTIFLEX^R) in a prolonged release vehicle (lipid based vehicle) by Eli Lilly/Elanco (Indianapolis, IN, U.S.A.). The second, sometribove, consists of the 190 amino acid form of pituitary bST with additional methionine at the aminoterminus (methionyl bovine somatotropin). Sometribove (500 mg, 22.86 μmol) was supplied as the formulated product (SOMATECH^R) in syringes in a prolonged release vehicle (lipid based vehicle) by Monsanto Agricultural Co. (St. Louis, MO, U.S.A.).

The cows were arranged in randomized two-member blocks (somidobove-treated and placebo-treated; sometribove-treated and placebo-treated) for treatment assignment based on calving date (cows did not differ by more than 20 days of lactation duration since calving) and milk production (cows did not differ by more than 3 kg of average daily milk yield) during pretreatment. Their milk yield was recorded daily or every 2 days.

Beginning at 90–150 days postpartum, cows of two herds received one subcutaneous injection of somidobove (640 mg) or placebo (olive oil) and hormone concentrations and milk production were determined for 28 days, while the cows of the third herd received one subcutaneous injection of sometribove (500 mg) or placebo and the hormone concentrations and milk production were determined for 14 days.

Blood samples for analysis were taken from control and bST-treated cows by jugular venipuncture at 3 and 6 h postinjection and then daily (heparin was used as an anticoagulant) or every other day in the morning between 0800 to 0900 h at intervals following bST administration and the plasma was stored at −20 °C until analysed. Concentration of bST in the plasma of cows was determined following the first recombinant bST administration by radioimmunoassay (Škarda *et al.* 1992a) with pituitary-derived bST purified by ionex chromatography (potency: 1.4 IU mg^{-1}) for iodination and reference standards. The first antibody was produced in rabbits against pituitary-derived bST NIH-GH-B-1003A (a gift from the National Institutes of Health, Bethesda, MD, U.S.A.) conjugated with human serum albumin by means of tolylene-2,4-diisocyanate according to Likhite and Sehon (1967). The second antibody against bovine gamma-globulin was produced in pigs (SwAR/G-RIA, SEVAC, Institute of Sera and Vaccines, Prague, Czech Republic). Iodination of bST was performed by the lactoperoxidase method (Thorell and Johansson, 1971) with lactoperoxidase (B grade from Calbiochem, Los Angeles, CA, U.S.A.) using ^{125}I ($Na^{125}I$, carrier free) from Amersham International (Amersham, England). Intra- and inter-assay coefficients of variation averaged 5.9 and 10.2 %.

Plasma IGF-I levels were measured following the first recombinant bST administration by radioimmunoassay according to Furlanetto *et al.* (1977). All plasma samples were pretreated with an equal volume of 0.2 mol l^{-1} glycine-glycine HCl buffer for 1 h at 37 °C to separate IGF-I from its binding protein(s). The samples were then neutralized with 2 mol l^{-1} NaOH. 20 μl of these extracts were incubated for 3 days with antiserum (UBK 487) directed against human IGF-I before the tracer is added. The antiserum was kindly provided by Dr. L. E. Underwood, the National Institute of Diabetes, Digestive and Kidney Diseases (Bethesda, MD, U.S.A.) and NHPP University of Maryland School of Medicine. This antiserum had 0.5 % cross-reactivity with IGF-II and a minimal cross-reactivity with insulin at 10^6 mol l^{-1} (Huybrechts *et al.* 1989). Intra- and inter-assay variabilities of acid-treated samples were 6.5 % and 15 % respectively.

Statistical differences between results were estimated by Student's t-test.

Results

In the control groups, mean basal plasma concentrations of bST were low and varied throughout the experimental period between 1.3±0.6 to 4.2±1.5 ng ml^{-1} (Fig. 1, 2). Endogenous bST secretion was shown to be pulsatile. The episodic spikes of high plasma bST concentrations ranging from 6.8 to 20.0 ng ml^{-1} were observed in only four out of 267 analysed plasma samples.

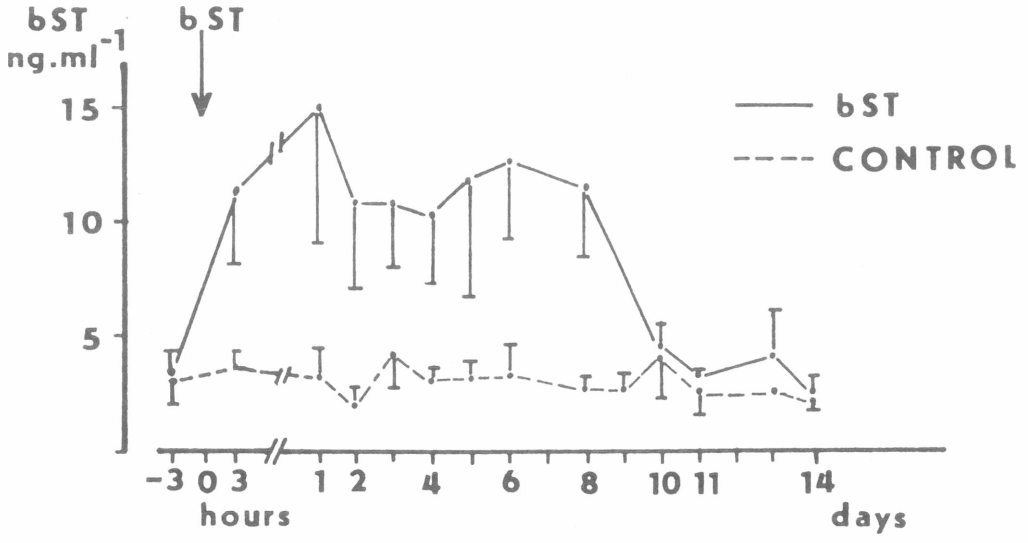

Fig. 1
Effect of sometribove administration in a sustained release vehicle (SOMATECH^{R}) on bovine somatotropin (bST) plasma profiles in lactating dairy cows. Crossbred Bohemian spotted Friesian-Ayrshire cows were subcutaneously injected with 500 mg of sometribove. The values are the mean concentrations of bST ± S.E.M. obtained from twelve animals.

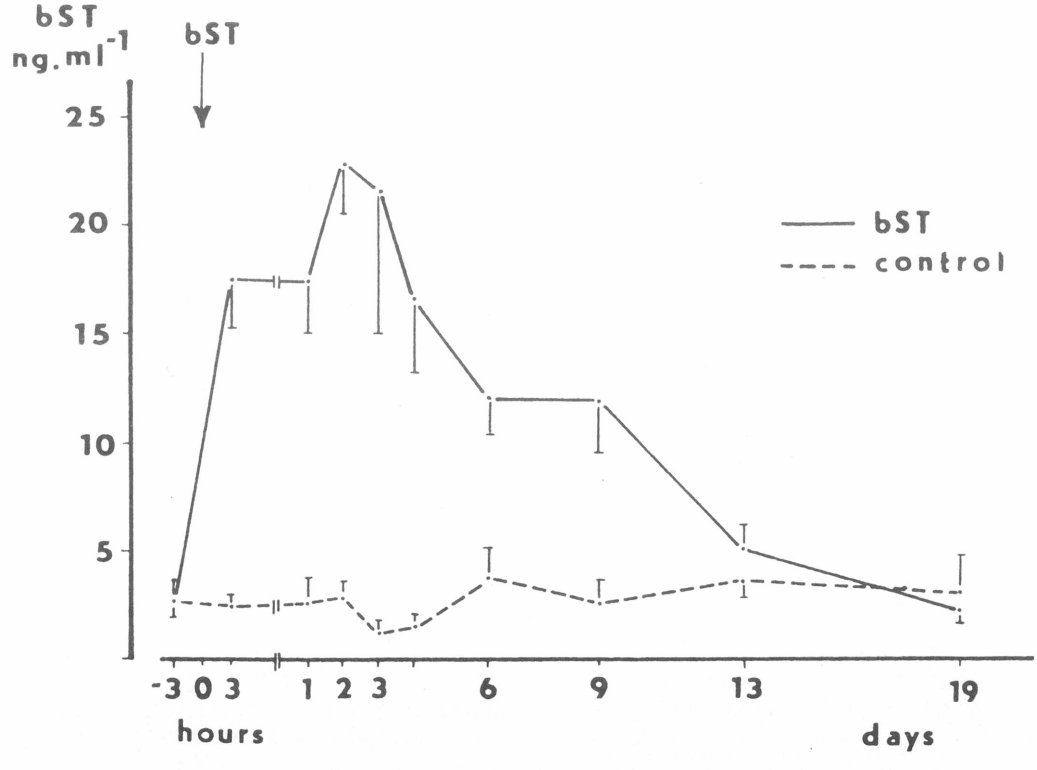

Fig. 2
Effect of somidobove administration in a sustained release vehicle (OPTIFLEX^{R}) on bST plasma profiles in lactating dairy cows. Crossbred Bohemian spotted Friesian-Ayrshire cows received a subcutaneous injection of 640 mg of somidobove. The values are the mean concentrations of bST ± S.E.M. obtained from five animals.

In the treated groups, plasma bST concentrations increased more than 3-fold during the first 3 h postinjection in cows treated with 500 mg of sometribove (from an average pretreatment concentration of 3.4±0.8 to 11.2±3.0 ng ml^{-1}) and more than 7-fold in cows treated with 640 mg of somidobove (from 2.3±0.3 to 17.5±2.6 ng ml^{-1}). In the following days, bST concentrations remained elevated for 8–9 days and then decreased rapidly and returned to preinjection or control levels within 10 and 12 days for sometribove and somidobove treated cows, respectively (Figs 1 and 2). The bars designating S.E.M. in Figs 1 and 2 show high individual variations in the level of plasma bST following recombinant bST administration. For example, a range of individual plasma bST concentrations from 10.8 to 23.5 ng ml^{-1} at 24 h following OPTIFLEX administration and even a range from 3.0 to 58.4 ng ml^{-1} at 24 h were found following SOMATECH administration.

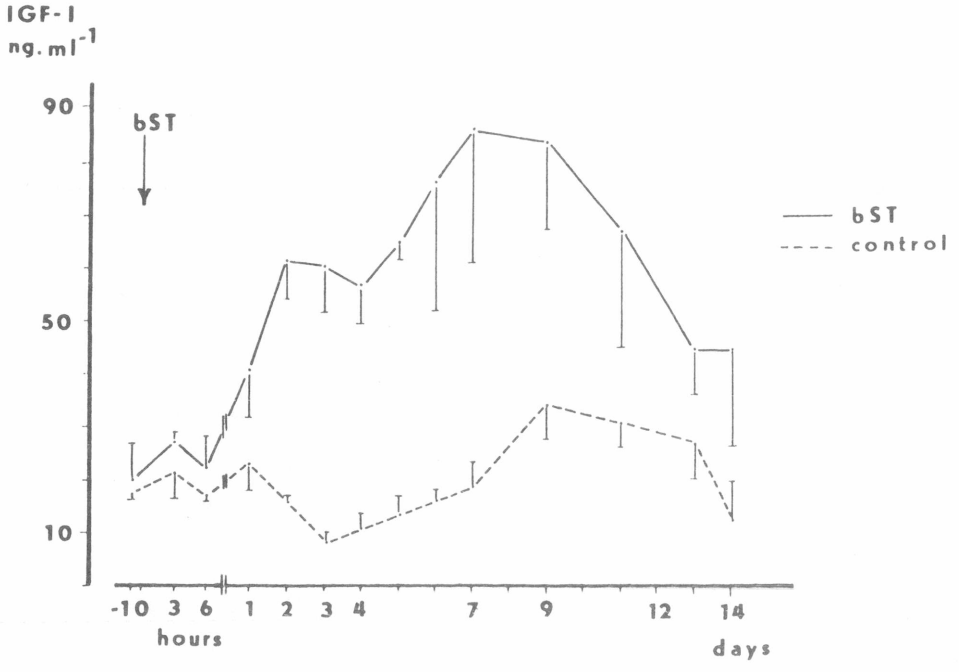

Fig. 3
Effect of SOMATECH^R administration on IGF-I plasma profiles in lactating dairy cows. Crossbred cows were received a subcutaneous injection of 500 mg of sometribove in a sustained release vehicle. The values are the mean concentrations of IGF-I±S.E.M. obtained from three animals.

Table 1
Effect of administration of OPTIFLEX^R on IGF-I plasma concentration (ng ml^{-1}) in lactating dairy cows

Hours	Control	bST
−1	18.3±2.1^a	14.4±2.1^a
3	18.0±2.1^a	16.4±2.1^a
24	17.0±1.8^a	34.3±6.5^b
48	19.6±2.0^a	42.1±5.0^b
72	19.1±0.9^a	43.3±7.8^b
96	18.2±1.9^a	43.1±8.9^b

The values are the mean concentrations of IGF-I ± S.E.M. obtained from five animals. ^{a,b}Means with different superscripts differ significantly ($P < 0.05$).

Plasma IGF-I levels in control animals were quite stable at an average 19.3±3.7 ng ml^{-1} throughout experimental period (Fig. 3, Table 1).

Following sometribove (500 mg) administration, the concentrations of IGF-I increased so that by 24 h postinjection the levels were elevated to nearly 2-fold and by 48 h to nearly 4-fold values (control: 16.2±1.2 ng ml^{-1}; bST-treated 61.7±7.2 ng ml^{-1}). In the next few days, the mean IGF-I concentration varied between 56.7 to 85.7 ng ml^{-1}. Then it decreased but still remained higher on day 14 (before next sometribove administration) than those in placebo-treated cows (44.4±17.8 ng ml^{-1}). Table 1 shows the concentrations of plasma IGF-I following somidobove (640 mg) administration. In five treated animals, plasma IGF-I concentrations significantly increased ($P<0.05$) from an average pretreatment level of 14.4±2.1 ng ml^{-1} at 1 h before bST injection to 34.3±6.5 ng ml^{-1} at 24 h postinjection. The 96 h IGF-I

level increased to 43.1±8.9 ng ml^{-1} while levels in control animals remained low and relatively stable (18.2±1.9 ng ml^{-1}). The variability of IGF-I values in bST-treated animals (Fig. 3 and Table 1) shows a significant individual animal effect.

Table 2
Effect of a single injection of recombinant bovine somatotropin in a sustained release vehicle on plasma bST and IGF-I concentrations and on milk production in lactating dairy cows

	Control	bST
Cows, no.	7	7
Plasma bST (ng ml^{-1})		
before treatment^{+}	2.9±0.6^{a}	3.1±0.6^{a}
1–96 h post treatment	3.2±0.5^{a}	18.7±4.4^{b}
difference	0.3	15.6
% increase	37	588
Plasma IGF-I (ng ml^{-1})		
before treatment^{++}	18.2±1.3^{a}	16.4±3.2^{a}
24–96 h post treatment	17.1±1.1^{a}	45.9±5.6^{b}
difference	−1.1	29.4
% increase	−4	232
Milk production (FCM: kg d^{-1})		
before treatment^{+++}	15.6±1.4^{a}	16.4±1.9^{a}
5–8 d post treatment	15.3±1.0^{a}	19.3±1.5^{b}
difference	−0.3	2.9
% increase	−0.2	20.9

Multiparous crossbred cows received a subcutaneous injection of either 500 mg of sometribove (3 cows) or 640 mg somidobove (4 cows); both preparations were in a sustained release vehicle. Seven cows were controls after olive oil injection. All values are the daily means ± S.E.M. ^{a,b} Means with different superscripts differ significantly ($P<0.05$). FCM = 4 % fat corrected milk. $^{+}$ Mean plasma bST concentration of 3 and 1 h before treatment; $^{++}$ mean plasma IGF-I concentration of 1 h before treatment; $^{+++}$ mean production 2 days before treatment.

In general, the first 96 h postinjection are characterized by a maximal level of plasma bST and an increasing level of plasma IGF-I, while maximal milk production responses were achieved later between day 5–8 following both somidobove and sometribove administration. The increases in all three parameters (determined only in 7 bST-treated and 7 placebo-treated animals) were quite similar and due to the large interindividual variation not significantly different after administration of both preparations of recombinant bST in the sustained release vehicles. The obtained results were thus pooled for further evaluation (Table 2). Plasma bST and IGF-I in the treated cows rose by 588 % and 232 % respectively and the milk production increased by 20.9 % only. Milk production responses of individual cows did not correlate positively with both the IGF-I and bST plasma concentrations.

Discussion

The mean plasma bST concentration in both OPTIFLEX- or SOMATECH-treated cows increased rapidly during the first 24 h following hormone administration and returned to pretreatment levels by day 10 postinjection. This is not in agreement with the results obtained by Schams *et al.* (1991a) who reported a gradual increase of plasma bST concentration until day 3 after the injection of OPTIFLEX (640 mg of somidobove) and with the results reported by Cisse *et al.* (1991) who found higher plasma bST concentrations at day 10 and at day 3 after the administration of SOMATECH (500 mg of sometribove). The latter finding is very surprising because both the present results and those of Schams *et al.* (1991a) have shown that plasma bST concentration returned on day 10 following injection of recombinant bST in a sustained release vehicle to preinjection or control levels. The maximal plasma bST concentration in treated cows was higher in the experiments of Schams *et al.* (1991a,b) and Cisse *et al.* (1991) than in the present report. The difference in plasma bST concentrations and profiles in the treated cows could be related to the different breeds of cows investigated, parity, stage of lactation, protein and energy intake on the one hand and differences in bST antibodies, plasma processing and procedures used to measure and adjust for non-specific binding during radioimmunological bST assay on the other hand. Further experiments on plasma bST profiles are needed to understand better the clearance rates of injected recombinant bST in sustained release vehicles.

Plasma bST concentrations in cows treated with OPTIFLEX were nonsignificantly lower than those in cows treated with SOMATECH. This was mainly due to the lower amount (cca 5 μmol) of recombinant bST in the SOMATECH dose than in OPTIFLEX rather than due to differences in the rate of bST absorption at the injection site caused by the different quality of vehiculum. This interpretation is supported by our previous finding obtained in cows which had received an injection of different amounts of somidobove. The plasma bST concentrations were again non-significantly lower in cows treated with 640 mg than in those treated with 960 mg of somidobove in a sustained release vehicle (Škarda *et al.* 1992a).

The results of the present investigation indicate that a considerable variation exists between animals in the plasma bST levels and in the magnitude of IGF-I and milk production responses to the administration of a formulated product of recombinant bovine somatotropin in a sustained release vehicle.

Numerous factors may contribute to these variations, e.g. differences in body weight of individual animals, differences in the rate of bST absorption from the vehiculum at the injection site, differences in the rate of enzymatic breakdown of the bST molecule in the tissues at the site of injection and in the blood plasma, differences in the concentration of bST binding proteins in blood plasma of individual animals, differences in the number of specific binding sites on the cell membranes of the liver and other tissues, differences in nutritional factors, feed intake and in the partitioning of nutrients among body tissues, etc. (Bauman *et al.* 1985b; Breier *et al.* 1986, 1988; McGuire *et al.* 1992). Animal-related variations in the level of plasma bST presented in this paper were not higher than those in cows treated with soluble pituitary derived bST though their doses were accurately adjusted per kg of body weight (Kerr *et al.* 1991). Thus plasma variations of bST concentration are more affected by factors other than molecular modifications in the recombinant bST such as addition of one (sometribove) or nine (somidobove) amino acid residues to the amino terminal of the peptide chain.

The direct effect of bST on mammary epithelial cells has not been demonstrated. The increase in circulating concentration of IGF-I after bST administration therefore suggests a direct role of IGF-I in the bST-stimulated increase in milk production. The exact mechanism is not known, but McGuire *et al.* (1992) indicated that both increased rate of synthesis and an increased number of mammary cells are likely to occur. IGF-I stimulate both DNA synthesis (an increase in cell number) (Winder *et al.* 1989) and lactose synthesis (Baumrucker 1986) in mammary epithelial cells *in vitro*. The rapidity for the rise and fall of milk yield in response to bST injections and their cessation implies that stimulation of milk production is achieved through increased productivity of existing secretory cells rather than through an increase in their number. The selective increase in alpha-lactalbumin synthesis which is a subunit of lactose synthetase in bST-treated cows is consistent with a change in secretory activity of existing cells induced by IGF-I. Increased lactose synthesis is a prerequisite of increased milk yield because lactose is the primary osmotic constituent of milk (Eppard *et al.* 1985b). Milk production of bST-treated cows was increased but was not significantly correlated with both IGF-I and bST plasma concentrations due to a significant interindividual variation. High milk yield responses were found in cows with both relatively high and relatively low plasma levels of bST and IGF-I. This is in agreement with earlier findings showing that even under the relatively uniform conditions in a single herd, the obtained results vary in both lactational response and bST plasma level. The pattern of diminishing marginal response of milk yield to increasing bST doses was also demonstrated. Significant increases in milk production were not evident between groups of cows receiving daily either 13.5, 27.0 or 40.5 mg of solubilized sometribove or 320, 640 or 960 mg of somidobove in a sustained release vehicle (Eppard *et al.* 1985a, Bauman *et al.* 1985a, Škarda and Mader 1991, Škarda *et al.* 1992a). In contrast, Kerr *et al.* (1991) have demonstrated that plasma IGF-I and milk production responses to bST administration were dose-dependent as significant increases in milk yield and IGF-I plasma levels were found between groups of cows receiving injections of 66 and 165 $\mu g\ kg^{-1}$ of body weight of solubilized pituitary-derived bST. To obtain this effect, the authors corrected the considerable variation in serum bST and IGF-I levels by calculating areas under the serum bST and IGF-I concentration profiles and related them to the percentage of milk production responses. However, in spite of the fact that circulating IGF-I levels were increased in response to bST administration, the more significant autocrine or paracrine action of locally produced IGF-I cannot be excluded, as was demonstrated in sheep mammary tissue (Wheatley *et al.* 1989). It seems that the variation in plasma IGF-I cannot explain the differences in the milk production response following bST administration alone due to the vast complexity of processes controlling the magnitude of milk production at the level of regulation of both extramammary and mammary metabolism.

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