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Effect of Somatotropin on Adipose Tissue Net Glucose-Stimulated Lipogenesis in Young Goats

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Summary

Net glucose-stimulated lipogenesis (NGSL: the rate of lipogenesis in the presence of glucose minus the rate of lipogenesis in the absence of glucose) in omental adipose tissue explants from young castrated male goats was evaluated in control animals (n = 3; placebo-treated) and in animals treated with the sustained release of recombinant bovine somatotropin (n = 4; bST; 100 mg at 7-day intervals in a 147 days lasting experiment). The rate of fatty acid synthesis was determined in acute incubations in both freshly prepared and chronically cultured explants. Adipose explants remained metabolically active and retained their ability to respond to hormones when maintained in a tissue culture medium. NGSL in explants cultured for 24 h in the presence of insulin alone or bST alone, was non-significantly increased (more in the controls) and decreased (more in bST-treated animals), respectively. However, cortisol alone decreased ($P<0.05$) NGSL in explants from both control and bST-treated animals. In tissues from bST-treated animals, cortisol acted synergistically with insulin to produce a higher rate of NGSL than that observed in cultures with insulin alone. bST inhibited insulin plus cortisol-stimulated lipogenesis significantly ($P<0.05$) in explants from bST-treated animals but non-significantly in control animals. The rates of NGSL were decreased ($P<0.05$) by catecholamines in explants from both control and bST-treated animals. Norepinephrine (NE) and isoprenaline (ISO) were equally effective in the controls, whereas isoprenaline was more effective than norepinephrine in bST-treated animals.

Key words
Goat • Adipose tissue • Lipogenesis • Somatotropin • Catecholamines

Introduction

Somatotropin (ST) exerts a variety of effects on somatic growth, development, lactation and metabolism. The metabolic effects complement the growth-promoting and galactopoietic effects in that ST tends to influence metabolism by enhancing the production of lean body mass or milk. ST directs the flow of metabolic substrates towards the consumption of fat and production of glucose and away from the oxidation of carbohydrates and proteins (Goodman 1993, Bauman and Vernon 1993, Slaba *et al.* 1994). Although it is now clear that ST alters nutrient partitioning in both growing and lactating animals, the respective biological mechanisms are not clear. ST can act in tissues directly on the cells themselves or indirectly through the actions of locally and systemically produced insulin-like growth factor I (IGF-I) and IGF binding proteins (Scott *et al.* 1985). It is

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probable, however, that ST and members of the IGF system act *via* the following mechanisms: (1) through alterations of the cell ability to respond to homeostatic signals such as insulin and catecholamines (Sechen *et al.* 1990), (2) by diminishing the response to antilipolytic agents such as adenosine and prostaglandins and by a decrease in the production of prostaglandin E_2 (Doris *et al.* 1994, 1996), 3) by enhancing the synthesis of inhibitors of lipogenesis such as polyamines and a short-lived mediator protein (Borland *et al.* 1994), (4) by altering the quantity of GTP-binding proteins (Goodman 1993, Houseknecht and Bauman 1997, Krbeček *et al.* 1998). Furthermore, it appears that many cellular mechanisms by which ST exerts its effects on body growth and metabolism are still unknown.

The aim of the present experiments was to study the mechanism of ST action on net glucose-stimulated lipogenesis (NGSL) using ST-treated growing castrated male goats as a model. *In vivo* bST treatment was coupled to *in vitro* cultures of adipose explants in the presence of insulin (I), cortisol (H) and bST alone or in different combinations. In addition, the abilities of these hormones to modulate the lipogenic responses to catecholamines in explants from control and ST-treated animals were compared in acute incubations. The rate of fatty acid synthesis was determined in acute incubations both in freshly prepared and cultured adipose explants.

Methods

Animals and general procedures

The experiments were carried out on seven young castrated male goats (Czech white breed). The animals were castrated at the age of 2-3 weeks and used for experiments between 2-8 months of age. Three animals served as controls (placebo-treated) and four animals were treated (s.c.) with recombinant bST. Animals had free access to grass hay and fresh grass. A concentrate mixture (oats and barley meal) was given two times daily according to the body weight from 0.2 to 0.4 kg . day^{-1}. $animal^{-1}$. Starting between 2-3 months of age, the placebo-treated animals received a subcutaneous injection of olive oil, whereas the bST-treated animals received 100 mg of recombinant methionyl bST (sometribove, Monsanto Agr. Co., St. Louis, MO, USA) in a lipid-based sustained release vehicle at 7-day intervals in a 147-day experiment. After 147 days of treatment all animals were exsanguinated after stunning with a captive bolt. The greater omentum was aseptically removed and placed immediately into sterile phosphate buffered saline at about 38 °C. The visible blood vessels were stripped away and the adipose explants of about 5-10 mg were cut with scissors.

Source of chemicals

The source of chemicals and hormone solubilization were the same as described earlier (Škarda 1998).

Tissue cultures

Adipose tissue explants (6-8) intended for culture were transferred into plastic Petri dishes containing 3 ml of modified Waymouth's medium (38 °C) (Škarda 1998). To study the effect of different hormones on the lipogenic activity during culture, adipose tissue explants were cultured in the presence of insulin (17 nmol . l^{-1}), cortisol (138 nmol . l^{-1}) and bST (4.5 nmol . l^{-1}) alone or in different combinations.

Tissue incubation

Fresh adipose tissue explants or cultured adipose explants (washed in saline at 38 °C) were transferred into 30-ml polyethylene flasks containing 3 ml of a modified Krebs-Henseleit bicarbonate buffer (Krebs and Henseleit 1932, Škarda 1998). Hormones present during culture were not added during acute incubation as their presence had no effect on lipogenesis (data not shown). To measure the net glucose-stimulated lipogenic activity, the amount of (^{14}C)acetate incorporated into fatty acids of the explant in the buffer supplemented with 4 mmol . l^{-1} of sodium acetate was subtracted from the amount of acetate incorporated in the buffer supplemented with both sodium acetate and 3.5 mmol . l^{-1} of glucose. The lipogenic activities were also assessed in the presence and absence of either norepinephrine (NE, 10 μmol . l^{-1}) or isoprenaline (ISO, 10 μmol . l^{-1}) to measure the responsiveness of the explant to catecholamines. The tissue explants were incubated for 2 h at 38 °C in an atmosphere of O_2:CO_2 (95:5 v/v) with reciprocal shaking at 90 strokes per min. All incubations were run under conditions of linear incorporation of acetate and were terminated by cooling in an ice-cold water bath. The explants were then collected, rinsed in cold saline and frozen at −20 °C until analyzed. All tissue incubations were carried out in triplicate.

Adipose tissue fatty acid synthesis

Fatty acid synthesis was assessed by measuring the incorporation of sodium (1-^{14}C)acetate (20 kBq . ml^{-1}) into total lipids of adipose explants over a 2-h period of incubation. In the present experiments, the synthetic

activities of adipose explants were expressed in nanomoles of acetate incorporated per mg protein per hour.

Protein determination

The protein content of adipose tissue explants was determined after lipid extraction according to Lowry *et al.* (1951) with BSA as the standard.

Statistical methods

All results are expressed as means ± S.E.M. Differences among the groups of uncultured and cultured explants were analyzed by repeated measure analysis of variance, factors comprising hormones in culture, catecholamines in the incubation medium and their interaction. The least squares means and their standard errors (one for paired values for comparison within a treated group and the second for unpaired values for comparing mean values from placebo and bST-treated animals) were calculated. All calculations were carried out by the GLM Procedure (SAS 1989). The effects of NE and ISO in uncultured explants and in explants cultured in the presence of different hormones were compared by the paired t-test.

Results

The rate of NGSL in freshly prepared adipose explants in a modified Krebs-Henseleit buffer was established to provide an information on the effect of long-term bST treatment *in vivo* on fatty acid synthesis *in vitro* and to provide a measure for maintenance of lipogenesis in subsequent incubations of explants cultured in the presence of different hormones. The data showed that goat adipose tissue remained metabolically active for at least 24 h in a modified Waymouth's tissue culture medium. Comparison of the lipogenesis of freshly-prepared explants with explants cultured in the absence of hormones for 24 h showed that NGSL in both control and bST-treated animals was the same after 24 h of culture. However, in explants from bST-treated animals a tendency to lower NGSL values after 24 h of culture was observed (NGSL decreased non-significantly from 38.52 to 24.27 nmol . $mg^{-1}.h^{-1}$).

Overall mean values of NGSL in cultured explants incubated in the absence of catecholamines were consistently lower ($P<0.0003$) in bST-treated animals than in the controls (control, 39.99±3.27; bST-treated, 22.65±2.83 nmol . $mg^{-1}.h^{-1}$) (Table 1).

Fatty acid synthesis in explants cultured in the absence of hormones was taken as a basic measure of the lipogenic response to different hormones added to the culture medium. Addition of insulin stimulated the rate of NGSL in tissues of control animals by 24.8 %, but a much lower effect of insulin was observed in bST-treated animals (13.1 %). However, the effects of insulin were not significant. The presence of cortisol alone in the culture medium decreased ($P<0.05$) NGSL in explants from both control and bST-treated animals. Inclusion of bST alone in the culture medium decreased the rates of NGSL after 24 h of culture by 27.3 % in explants of placebo-treated animals and by 52.9 % in bST-treated animals. However, these direct effects of bST were not significant due to variations between animals.

In tissues from bST-treated animals, cortisol acted synergistically with insulin to produce a higher rate of NGSL (by 49.0 %) than that observed in cultures with insulin alone. In control animals, however, no effect of cortisol plus insulin on NGSL was noted when compared with insulin alone (Table 1). In the presence of insulin, bST did not affect the rates of NGSL in tissues from both control and bST treated animals. However, in the presence of insulin plus cortisol, bST decreased ($P<0.05$) NGSL in explants from bST-treated animals, but in explants from control animals NGSL was decreased non-significantly from 53.40 to 32.51 nmol . $mg^{-1}.h^{-1}$.

The *in vitro* rates of NGSL were decreased ($P<0.05$) by catecholamines in freshly prepared and cultured adipose tissue explants of both control and bST-treated animals (Table 1, values within a column). ISO decreased ($P<0.05$) the rate of NGSL (overall mean 3.32±0.63 nmol . $mg^{-1}.h^{-1}$) more than did NE (overall mean 11.20±1.63 nmol . $mg^{-1}.h^{-1}$) in bST-treated animals but not in the controls. These results suggest that NE and ISO were equally effective in placebo-treated goats, whereas ISO was more effective than NE in bST-treated animals.

Discussion

Acute incubation of both fresh and cultured adipose tissue explants in the presence of (1-^{14}C)acetate demonstrated that the loss of lipids from adipose and muscle tissue observed earlier (Škarda 1998) could at least partly be the result of a reduced rate of fatty acid synthesis. The overall mean values of NGSL in explants cultured in the presence of different hormonal combinations were consistently lower in bST-treated

Table 1. Effects of long-term treatment with bST *in vivo* and chronic (24 h) culture in the presence of various hormones on the rate of net glucose-stimulated lipogenesis (NGSL) during acute incubation (2 h) in the presence and absence of catecholamines in adipose explants from young castrated male goats.

Culture variables	Acetate incorporation into fatty acids (nmol . mg^{-1} . h^{-1}) Incubation variables		
	No catecholamines	NE	ISO
PLACEBO-TREATED ANIMALS			
Before culture			
No hormones	45.22^{Aa}	19.68^{Ab}	7.08^{Ab}
After culture			
No hormones	45.89^{Aa}	4.95^{ABb}	4.93^{Ab}
Insulin (I)	57.25^{Aa}	6.13^{ABb}	7.93^{Ab}
bST	33.37^{ABa}	4.88^{ABb}	3.69^{Ab}
Cortisol (H)	8.31^{Ba}	4.07^{Bab}	2.33^{Ab}
I + H	53.40^{Aa}	9.10^{ABb}	6.26^{Ab}
I + bST	43.97^{Aa}	3.90^{Bb}	4.31^{Ab}
I + H + bST	32.51^{ABa}	5.02^{ABb}	2.60^{Ab}
Overall mean	39.99^{a}	7.22^{b}	4.89^{b}
Pooled S.E.M.$_1$	9.26	5.33	2.04
Pooled S.E.M.$_2$	3.27	1.88	0.72
bST-TREATED ANIMALS			
Before culture			
No hormones	38.52^{Aa}	18.70^{Ab}	5.86^{Ab}
After culture			
No hormones	24.27^{ABa}	11.51^{ABb}	4.66^{Ab}
I	27.45^{ABa}	10.74^{ABb}	5.66^{Ab}
bST	11.42^{Ba}	8.90^{ABab}	1.97^{Ab}
H	7.06^{Ba}	11.55^{ABab}	1.09^{Ab}
I + H	40.90^{Aa}	16.69^{ABb}	2.02^{Ab}
I + bST	20.24^{ABa}	3.94^{ABb}	3.89^{Ab}
I + H + bST	11.31^{Ba}	7.54^{ABb}	1.41^{Ab}
Overall mean	22.65^{a}	11.20^{b}	3.32^{c}
Pooled S.E.M.$_1$	8.02	4.61	1.77
Pooled S.E.M.$_2$	2.83	1.63	0.63
Effect of bST treatment	$P < 0.0003$	NS	NS

Omental adipose explants of three control (placebo-treated) and four bST-treated (100 mg of recombinant methionyl bST in a sustained release vehicle at 7-day intervals for 140 days) animals were exposed before and after culture to (1-^{14}C)acetate (20 kBq . ml^{-1}) for a 2-h period of incubation in the presence or absence of norepinephrine (NE; 10 μmol . l^{-1}) or isoprenaline hydrochloride (ISO; 10 μmol . l^{-1}) in Krebs-Henseleit buffer supplemented either with sodium acetate (4 mmol . l^{-1}) or sodium acetate plus glucose (3.5 mmol . l^{-1}). The rate of lipogenesis in the presence of glucose minus rate of lipogenesis in the absence of glucose was taken as NGSL. Cultured explants were maintained in tissue culture for 24 h in a modified Waymouth's medium (containing 3.5 mmol . l^{-1} glucose) in the absence of hormones or in the presence of insulin (17 nmol . l^{-1}), recombinant bST (somidobove; 4.5 nmol . l^{-1}) and cortisol

animals than in the controls. Borland *et al.* (1994) have demonstrated with sheep adipose tissue explants that ovine ST alone had no effect on the rate of lipogenesis after 24 h of culture, but significantly decreased the rate of lipogenesis after 32 h of culture. We assume that the non-significant decrease of NGSL in goat adipose explants cultured in the presence of bST alone for 24 h was due to both variation of the effect of bST between animals and due to insufficient time for a significant inhibitory effect of bST on lipogenesis to appear. In addition, we observed that bST tended to antagonize the insulin action on lipogenesis and this antagonism was markedly enhanced by cortisol in tissues of bST-treated goats. The exact mechanism by which ST decreases lipogenesis is not clear. In both non-ruminants and ruminants, ST decreases the uptake, oxidation and utilization of glucose, in part due to decreased synthesis of glucose transporters (Goodman 1993, Vernon *et al.* 1995, Zhao *et al.* 1996). ST can also affect the activity of key lipogenic enzymes such as acetyl-CoA carboxylase and their insulin activation. Adamafio and Ng (1984) found that ST-deficient mice have much higher acetyl CoA carboxylase activity in adipose tissue than normal mice.

The addition of cortisol alone to the culture medium decreased NGSL in both placebo-treated and bST-treated goats. Glucocorticoids act by inhibiting both the glucose transport *per se*, perhaps by affecting glucose transporter GLUT4 subcellular trafficking, and glucose metabolism (Weinstein *et al.* 1995). The inhibitory effect of cortisol on NGSL was eliminated by simultaneous addition of insulin. The mechanism by which insulin eliminates the inhibitory effect of cortisol alone, could be related to the stimulatory action of hormonal combination of insulin plus cortisol on insulin receptor synthesis (Salhanick *et al.* 1983), on the synthesis of enzymes involved in glucose utilization and lipid synthesis (Chung *et al.* 1983, Stumps and Kletzein 1984), on the level of an intracellular mediator of insulin action (Walton *et al.* 1986) and on the responses of glucose-6-phosphate dehydrogenase and acetyl CoA carboxylase to insulin (Hillgartner *et al.* 1995).

The inhibition of NGSL in the presence of ISO was higher in explants from bST-treated animals than in explants from the controls. These results are consistent with the increase of isoprenaline-stimulated lipolysis in bovine adipose tissue cultured in the presence of bST (Lanna *et al.* 1995). The higher effect of ISO in bST-treated animals could also be due to ST-induced increase of the number of α_2-adrenergic receptors (Vernon *et al.* 1995), which could limit the effects of NE but not of ISO.

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(138 nmol . l^{-1}) alone or in different combinations. All values from placebo-treated animals are means of three experiments and all values from bST-treated animals are means of four experiments, each performed in triplicate, and expressed per mg proteins. For each column of results, pooled S.E.M.$_1$ (Standard Error LS Mean for paired values) is for comparison of mean values from animals in the same treatment (placebo or bST-treated); pooled S.E.M.$_2$ (Standard Error LS Mean for unpaired values) is for comparing mean values of placebo-treated with those of bST-treated animals. Values within a column which do not have the same upper case (A,B) differ significantly ($P<0.05$). Values within a row (analyzed by paired t-test) which do not have the same superscript (a,b,c) differ significantly ($P<0.05$).

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