Recombinant Bovine Somatotropin Decreases Hepatic Amino Acid Catabolism in Female Rats

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ABSTRACT The effect of recombinant bovine somatotropin (rbST) on hepatic amino acid catabolism in female rats was investigated. Daily injections of rbST for 5 d decreased liver homogenate lysine α-ketoglutarate reductase (EC 1.5.1.8) activity (P < 0.05) and liver homogenate lysine oxidation (P < 0.05) ~35%. Liver homogenate methionine and valine oxidation were depressed ~20 (P = 0.13) and 35% (P < 0.05), respectively. These data decrease in hepatic capacity to oxidize amino acids in rats administered rbST. Whether depressed liver amino acid degrading enzyme activity plays a role in amino acid oxidation in vivo remains to be evaluated. J. Nutr. 126: 1657–1661, 1996.

INDEXING KEY WORDS:
- rats
- somatotropin
- lysine
- methionine
- valine

The effect of somatotropin and various repartitioning agents (such as ractopamine, cimaterol and clenbuterol) on whole-animal metabolism has generated much interest (for reviews see Boyd and Bauman 1989, Boyd et al. 1991). This interest has focused on the apparent repartitioning of energy from fat accretion to protein accretion that occurs with the administration of such agents. Little attention has been paid to the effect of these agents on metabolic alterations that affect amino acid catabolism. Many reports suggest that amino acid catabolism is decreased based on suppression of blood/plasma/serum urea nitrogen concentrations (Boisclair et al. 1994, Houseknecht et al. 1992, McGuire et al. 1992, Wray-Cahen et al. 1991). The following studies were conducted to determine the effect of recombinant bovine somatotropin [rbST] on hepatic amino acid catabolism in female rats.

MATERIALS AND METHODS

Chemicals. Lysine-HCl, methionine, valine, and methylcellosolve were purchased from U.S. Biochemical (Cleveland, OH). Monsanto (St. Louis, MO) generously provided the rbST. Other chemicals were obtained as follows: sucrose, Schwarz-Mann (Orangeburg, NY); perchloric acid, MCB Reagents (Cincinnati, OH); MgCl2, Columbus Chemical Industries (Columbus, WI); sodium carbonate, J. T. Baker Chemical (Phillipsburg, NJ); EDTA, potassium phosphate and monothiolamine, Fisher (Fair Lawn, NJ). [1-14C]-lysin (a generous gift from Purina Mills, St. Louis, MO) and [1-14C]-L-Methionine were from American Radiolabeled Chemicals (St. Louis, MO). [1-14C]-Valine was from Dupont NEN Products (Wilmington, DE). The radiolabeled methionine was purified by dilution in 0.01 mol/L HCl, followed by washing over a Dowex 50 (H+ form) column and elution with a buffer containing 57 mmol/L sodium citrate, 34 mmol/L NaOH and 35 mmol/L thiodiglycol, pH 2.88. Radiochemical purity of all three isotopes was evaluated via HPLC using a Beckman Ultrasphere C-18 column (Fullerton, CA) (5-μm packing in a 4.6 mm × 25 cm column) with a 25% methanol (v/v%) 1 mmol/L sodium decysulfonate, pH 3 mobile.

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phase flowing at 0.6 mL/min. Detection was via an inline radioactivity detector (Flo 1/8 detector, Radiomatic Instruments, Boca Raton, FL). No radiochemical impurities were detected. All other reagents were from Sigma Chemical (St. Louis, MO).

**Animals and diets.** Sixteen female Sprague-Dawley rats [Harlan Sprague Dawley, Indianapolis, IN], weighing 180–190 g were individually housed in suspended wire-mesh cages in a room maintained at 24°C. Rats were allowed access to diets containing 20% casein (based on AIN 1977) only during the 12-h dark cycle which began at 2300 h. When rats weighed ~210 g, they were assigned to daily injections at 0800 h of either 12 mg rbST (n = 8) or an equivalent amount of the excipient [control, n = 8] subcutaneously over the midscapular region. The excipient was 25 mmol/L NaOH neutralized with 1 mol/L NaHCO₃ (Azain et al. 1993). Injections were given over 5 d. Animal care and conduct of experiments were approved by the University of Wisconsin-Madison, College of Agricultural and Life Sciences Animal Care committee, and met the National Institutes of Health guidelines (NRC 1985).

**Tissue preparation.** Rats were killed by decapitation and exsanguinated between 1100 and 1300 h. Livers were removed and homogenized as described by Blemings et al. (1994), except that a portion of 330 g/L homogenate was set aside for use in the methionine oxidation assay.

**Measures of amino acid catabolism.** Lysine α-keto glutarate reductase. Liver homogenate lysine α-keto glutarate reductase activity (LKR, EC 1.5.1.8) was assayed at room temperature as previously described (Blemings et al. 1994); the CV for this assay was <5%.

Lysine oxidation. Liver homogenate lysine oxidation was determined by a published procedure (Soliman and Harper 1971) except for the use of a lysine concentration of 1 mmol/L with a specific radioactivity of 4.2 Bq/nmol. The assay was performed at 1 mmol/L lysine because this is our estimate of the average daily portal amino acid concentration; the CV for this assay was <5%.

**Methionine oxidation.** Liver homogenate methionine oxidation was assessed based on a system described by Mitchell and Benevenga (1978), using 25-mL Erlenmeyer flasks covered with a rubber septum from which a well was hung containing a monoethanolamine:methylethanolamine:122 trap (0.5 mL in a 0.5 mL microfuge tube). The incubation was started by the addition of 0.25 mL of 20 mmol/L methionine solution [3.9 Bq/nmol] in 25 mmol/L potassium phosphate pH 6.5 to a flask which contained 2.4 mL of 330 g/L liver homogenate and reagents prewarmed to 37°C. The final concentration of reagents in a 5-mL volume was as follows: methionine, 1 mmol/L; ATP, 1 mmol/L; NAD, 1 mmol/L; MgCl₂, 4 mmol/L; HEPES, 25 mmol/L; EGTA, 1 mmol/L; CoA, 0.5 mmol/L; mannitol, 179 mmol/L; sucrose, 45 mmol/L, and potassium phosphate buffer 10 mmol/L, pH 7.5. The assay was performed using 1 mmol/L methionine because this is our estimate of the average daily portal amino acid concentration. Incubations were terminated by injecting 1 mL of 35% perchloric acid (PCA) via needle through the rubber septum into the bottom of the flask. Samples remained in the shaking water bath for at least 2 h to collect CO₂, the amount of time required to recover all CO₂ from the gas phase in the 25-mL Erlenmeyer flask. Incubations were terminated after 10 or 20 min. Each time point included a heat inactivated blank (10 min in boiling water). The assay was linear for at least 20 min; the CV for this assay was <10%.

**Valine oxidation.** Liver homogenate valine oxidation, based on a system described by Shinnick and Harper (1976), was assayed in the same manner as lysine oxidation except for the final concentrations of reagents in a 0.4-mL final volume which were as follows: valine, 1 mmol/L; NAD, 5 mmol/L; CoA, 1 mmol/L; MgCl₂, 4 mmol/L; EGTA, 0.25 mmol/L; Na₂CO₃, 5 mmol/L; α-ketoglutarate, 10 mmol/L; HEPES, 25 mmol/L; mannitol, 141 mmol/L; sucrose, 35 mmol/L, and potassium phosphate buffer 5 mmol/L, pH 6.8. The assay was performed at 1 mmol/L valine, our estimate of the average daily portal amino acid concentration. The specific radio activity of valine was 5.3 Bq/nmol. Samples were incubated for 10 min and heat inactivated blanks were assayed in parallel. Recovery of 14CO₂ was linear for at least 10 min in this system. Incubations were terminated by injecting 0.25 mL 35% PCA via a needle through the rubber septum into the bottom of the flask; the CV for this assay was <10%.

**Statistics.** Data were analyzed by ANOVA for a one-way design using SAS (version 6.07, Cary, NC). Differences between control and rbST-injected animals were considered significant at P < 0.05. Values in the text are means ± SEM.

**RESULTS**

**Effect of somatotropin on growth and relative liver size.** When rats attained a weight of 210 ± 2 g, injections were initiated. Over the 5 d that rats received injections, rbST-treated rats consumed more diet (13.8 ± 0.4 g/d vs. 12.3 ± 0.4 g/d; P < 0.05) than controls. As expected (Azain et al. 1993), after 5 d of treatment, rbST-treated rats were heavier (251 ± 2 vs. 219 ± 2 g; P < 0.05) than controls. In these studies, rbST injections consistently increased relative liver size (RLS, g liver/100 g body wt) ~27% (4.7 ± 0.1 vs. 3.7 ± 0.1%; P < 0.05). Increased RLS has been reported following administration of recombinant porcine somatotropin to pigs (Caperna et al. 1990, Evock et al. 1988, Hansen et al. 1994), and of rbST to growing heifers (Sandles and Peel 1987).
Effect of recombinant bovine somatotropin (rbST) on hepatic amino acid catabolism in rats.

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<td>24.8</td>
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Values are means and the pooled SEM.

Enzyme activity was determined in a known amount of liver homogenate. The calculated total liver activity was divided by the body weight and multiplied by 100, resulting in the units shown.

LKR, lysine α-ketoglutarate reductase.

**Effect of somatotropin on liver homogenate lysine α-ketoglutarate reductase activity and lysine oxidation.** Both LKR activity and lysine oxidation were decreased about 35% in rats treated with rbST (Table 1). The mechanism by which rbST injections depress homogenate LKR activity and in vitro lysine oxidation is unknown. A dramatic finding was that lysine oxidation was only 10% of LKR activity, supporting the suggestion from our laboratory that mitochondrial lysine uptake may be rate-limiting for lysine oxidation [Blemings 1994]. The mitochondrion is the exclusive site of sarcosine-dependent lysine degradation in liver [Blemings et al. 1994].

The present results raise the question whether somatotropin decreases lysine uptake by rat liver mitochondria. A decreased mitochondrial lysine uptake should result in decreased lysine degradation, making lysine available for protein synthesis.

**Effect of somatotropin on liver homogenate methionine and valine oxidation.** Because treatment with rbST caused a decrease in both liver homogenate LKR activity and lysine oxidation, the question arose whether the effect of rbST is specific for lysine metabolism or if it is a general phenomenon. Thus, liver homogenate methionine and valine oxidation were also studied. Growth hormone decreased liver homogenate valine oxidation about 35% [P < 0.05] and tended to decrease methionine oxidation [20%, P = 0.13] (Table 1). The data indicate that rbST injections result in a general reduction in amino acid oxidation rather than a specific effect on lysine catabolism.

**DISCUSSION**

**Effect of somatotropin on growth.** Recombinant bovine growth hormone has been shown previously to enhance growth in rats [Azain et al. 1993, Gahl et al. 1994]. Presumably, enhanced growth occurred due to a more efficient use of absorbed amino acids that was accompanied by a decrease in urinary nitrogen loss as reported in cattle [Boisclair et al. 1994, Eismann et al. 1986b and 1989, Houseknecht et al. 1992] and pigs [Wray-Cahen et al. 1991], resulting in increased protein accretion. The increased protein accretion has been well characterized and is largely a result of increased protein synthesis while protein degradation remains relatively constant [Boisclair et al. 1994, Eismann et al. 1986a and 1989, Goldberg 1969, Goldberg et al. 1980]. However, the effect of somatotropin on catabolism of individual amino acids has not been studied extensively.

**Effect of somatotropin on amino acid catabolism.** A few studies have evaluated the effects of somatotropin on whole-body flux of a single amino acid. Somatotropin did not affect the irreversible loss of leucine, but did decrease its oxidation by about one third in growing beef heifers [P < 0.01, Eismann et al. 1986b] and steers [P < 0.01, Eismann et al. 1989]. The decreased oxidation at a constant irreversible loss rate is consistent with an increased flux of leucine into protein. Our observation of a decrease in amino acid oxidation is consistent with the depressed loss of urinary nitrogen noted above.

In a hemi-diaphragm system, both somatotropin [Dawson et al. 1963] and prolactin [Beck et al. 1964] increased the flux of leucine into protein. Prolactin-treated, mature female rats had greater weight gain than did controls [Naismith and Walker 1988]. Prolactin treatment resulted in a nonsignificant decrease in both liver alanine aminotransferase and a key urea cycle enzyme arginosuccinate synthase. Somatotropin decreased arginase activity [Beaton et al. 1953, Fraenkel-Conrat et al. 1943] as well as the other enzymes of the urea cycle, particularly arginosuccinate synthase, the enzyme with the lowest activity of the five enzymes in the urea cycle [McLean and Gurney 1963].

The mechanism by which growth hormone decreases amino acid oxidation is unknown, although part of the decrease observed in vivo likely results from increased protein synthesis [Benevenga et al. 1993, Brookes et al. 1972, Kim et al. 1983]. An increased incorporation of amino acids would decrease the degradation of amino acids and result in less oxidation and more efficient use of amino acids for growth.

The results reported here and the work of others (above) suggest that a new steady state in enzyme activity is achieved by administration of somatotropin. The manner in which this new steady state is realized is unknown, but evidence to date suggests that growth hormone is a homeoergic [long-term] regulator of metabolism as opposed to a homeostatic [short-term] regulator [Bell et al. 1987, Boyd and Bauman, 1989]. Growth hormone activates the tyrosine kinase JAK2 [Argetsinger et al. 1993] and presumably acts via activation...
of latent cytosolic transcription factors (see Wilks and Harpur 1994 for a review). The identity of these factors and how they mediate the somatotropin effects either in muscle cells where amino acid uptake is increased (Albertsson-Wikland et al. 1980) or in liver cells where uptake is depressed [Pacitti et al. 1992] are not yet known. The somatotropin-mediated increase in protein synthesis is an active area of investigation. This brief report indicates that there is a somatotropin-mediated depression of amino acid catabolic capacity that warrants further investigation.

**LITERATURE CITED**


Pacitti, A. J., Inoue, Y., Plumer, D. A., Copeland, E. M. & Souba,
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