Effects of Recombinant Equine Somatotropin on Wound Healing, Carbohydrate and Lipid Metabolism, and Endogenous Somatotropin Responses to Secretagogues in Geldings¹

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ABSTRACT: The primary purpose of this experiment was to assess the possible beneficial effects of recombinant equine somatotropin (reST) administration on wound healing in adult geldings. The effects of the 21-d reST treatment on carbohydrate and lipid metabolism and on endogenous ST characteristics were monitored as well. Single, full-thickness skin incisions (7.62 x 7.62 cm) were made in the pectoral region of all geldings on d 0. Treated geldings received reST at 20 µg/kg BW i.m., and control geldings received vehicle (10 mM sodium borate) at equivalent volumes daily from d 0 (immediately after surgery) through d 20. Tracings of the wounds were made with acetate transparencies, and wound areas were calculated via a digital analyzer. In addition to once-daily blood samples collected at specified days throughout the treatment period, an i.v. glucose tolerance test was performed on d 16, and three assessments of endogenous ST secretion were performed in the 2 d immediately following the end of treatment: epinephrine administration during the morning of d 21, an exercise test during the afternoon of d 21, and i.v. aspartic acid infusion on d 22. There was no effect (P > .1) of reST treatment on wound healing as assessed by changes in wound areas. Daily plasma ST, IGF-I, glucose, and insulin concentrations were higher (P < .05) and urea-nitrogen concentrations were lower (P < .001) in geldings receiving reST relative to controls. Glucose, NEFA, and insulin concentrations were all higher (P < .01) in reST-treated geldings before glucose infusion on d 16, and the responses to glucose were greater (P < .05) as well. Epinephrine administration increased (P < .02) ST concentrations in control geldings on d 21 but not in reST-treated geldings; a similar suppressive effect of reST treatment was observed for the ST response to exercise (P < .001). After aspartic acid infusion on d 22, reST-treated geldings had a much smaller (P < .001) ST response than did control geldings. Conclusion: reST administered to geldings at 20 µg/kg BW i.m. caused hyperglycemia, hyperinsulinemia, insulin insensitivity, mobilization of fatty acids, and an apparent negative feedback on the pituitary’s ST response to various stimuli known to induce ST secretion. However, there was no beneficial effect of reST treatment with the wound model used in this experiment.

Key Words: Somatotropin, Healing, Insulin-like Growth Factor, Metabolites, Horses

Introduction

Endogenous ST is generally known to be involved in protein, lipid, and carbohydrate metabolism in adult mammals (Guyton, 1996). Given that tissue repair and remodeling after injury involves active protein synthesis, a dependency on ST and its associated growth factor, IGF-I, is possible. In rats, ST has been shown to stimulate granulation tissue (Steenfos and Jansson, 1992), and GHRH administration increases wound strength (Garrel et al., 1991). Moreover, recombinant human ST has been shown to accelerate wound healing in children with large cutaneous burns (Gilpin et al., 1994).
Buonomo et al. (1996) first reported that bovine and porcine ST were biologically active in horses. Malinowski et al. (1997) treated aged mares with either 6.25 or 12.5 mg of recombinant equine ST (reST) daily for 43 d and reported no alteration in feed intake, BW, or body condition scores; however, a subjective score for muscle definition was increased by the higher dose of reST. Other hormonal and metabolic effects of reST in horses have been briefly reported (Christensen et al., 1996a,b; Ralston et al., 1996; Julen Day et al., 1998).

The primary purpose of the present experiment was to assess the possible beneficial effects of reST administration on wound healing in adult horses. The effects of the 21-d reST treatment on carbohydrate and lipid metabolism and on endogenous ST characteristics were monitored as well.

Materials and Methods

**Animals and Treatments.** Twelve light-horse geldings (mean BW 540 kg; mean age 8 yr; mean body condition score 6) were used. The geldings were randomly assigned (six/treatment) to either a treated group receiving reST (EquiGen, BresaGen Ltd., Adelaide, SA, Australia) at 20 µg/kg of BW i.m. daily (dose recommended by the company) or to a control group receiving an equivalent volume of vehicle (10 mM sodium borate). Geldings were weighed weekly during the experiment to ensure that a proper dosage was administered throughout the project. The geldings were treated for 21 d beginning immediately after lesioning on d 0 of the experiment; this duration of treatment was designed to provide elevated ST concentrations throughout the major portion of the healing process. Geldings were housed in covered stalls throughout the experiment. They were brought into the stalls from the pasture 1 wk before the beginning of the experiment (d -7) to allow for adaptation to the new environment and diet. They were fed a commercial pelleted feed (12% CP on DM basis) twice daily at .5% of their BW per feeding, and bermudagrass hay was available for ad libitum consumption. Except when noted otherwise, the routine schedule of events involved blood sampling first, followed by treatment injections, and feeding last.

**Wounding, Wound Care, and Wound Evaluation.** All surgical procedures were performed by a licensed veterinarian (D. D. French) and were approved by the LSU Agricultural Center Institutional Animal Care and Use Committee. On d 0, full-thickness skin incisions were made in the pectoral region. A 7.62- × 7.62-cm template centered over the midportion of the right pectoral was used to mark the incision site (Fretz and Li, 1992). The area was clipped and prepared using standard surgical procedures. The horses were sedated with an i.v. injection of xylazine (1.0 mg/kg of BW; Rompun, Bayer, Shawnee Mission, KS) and butorphanol tartrate (.02 mg/kg of BW; Torbugsic, Fort Dodge, Overland Park, KS). A line of local anesthetic solution (Carbocaine, 2% mepivacaine hydrochloride, Pharmacia & Upjohn, Kalamazoo, MI) was infiltrated over the dorsum of the template in a semicircular manner to block local pain receptors. Following the removal of the skin flap, the wound was left to heal by second intention (Lindsay, 1988). No bandage was required for the area and the location of the wound did not interfere with the normal daily activities of the horses.

The wound region was cleaned daily with hydrotherapy and mild washing to remove all scabs and debris. The wounds were evaluated clinically on a daily basis by personnel familiar with the treatment assignment of each gelding. Tracings of the wounds were made with acetate transparencies and permanent markers on d 1, 2, 4, and 7; then at 3-d intervals until d 22; and then every 9 d until d 40. The wound areas were calculated from the manual tracings using a digital analyzer (Snowden, 1981). Photographs of the wounds were also taken on d 1, 2, 4, and 19 for later evaluation.

**Blood Sampling.** Samples of jugular blood were drawn twice daily (before treatment injections and approximately 12 h later) from d -1 to d 3, once daily (before the daily treatment injections) from d 4 to 7, and then every 3 d through d 33. Samples were taken by venipuncture into 7-mL evacuated tubes containing sodium fluoride and potassium oxalate. Immediately after withdrawal, blood samples were refrigerated at 5°C and were subsequently (within 1 h) centrifuged for 15 min at 1,600 × g at 5°C. Plasma was stored at -15°C. These plasma samples were used for the measurement of ST, IGF-I, glucose, insulin, NEFA, and urea-N concentrations (described later).

**Metabolic Tests.** Several metabolic tests were performed near the end of treatment to characterize the long-term effects of reST treatment on glucose and lipid metabolism and endogenous ST production and storage. An i.v. glucose tolerance test (IVGTT) was administered on d 16. All feed and hay was removed at 1900 the night before (water was available ad libitum). Horses were fitted with 14-gauge indwelling jugular catheters at 0700 and allowed to rest for approximately 1 h. Blood samples (5 mL) were drawn at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min relative to infusion of glucose at 200 mg/kg BW (as a 50% solution in sterile saline). Blood samples were placed in 12- × 75-mm glass tubes containing heparin and sodium fluoride, refrigerated at 5°C, and subsequently centrifuged for 15 min at 1,600 × g at 5°C. The plasma was then transferred into labeled polypropylene tubes and stored at -15°C. These samples were used for the measurement of glucose, insulin, and NEFA concentrations.

On the 1st d after the end of treatment (d 21), two assessments of endogenous ST status were performed on all geldings: 1) an epinephrine challenge in the morning (0800) and 2) an exercise bout in the
Results

Body Weights, Injection Sites, and Antibody Production. Geldings in both groups gained (P < .05) an average of 4 kg over the 1st 2 wk of treatment and another 3 kg by the 3rd wk of treatment. However, there was no effect of reST treatment on BW nor any interaction with week (P > .1).

Over the 21 d of injections, two of the six reST-treated geldings displayed swelling on their necks for 1 to 2 d, after which the swelling subsided. Neither gelding displayed subsequent swelling in response to the injections. There was no difference (P > .1) between groups in the amount of radiolabeled equine ST that was precipitated with the IgG fraction of plasma samples from d 21 and 42, indicating that no antibody formation occurred in these horses during this period of time.

Wound Healing. The average wound areas determined from the acetate tracings are presented in Figure 1. Average wound area decreased (P < .001) in all geldings over time; however, daily administration of reST at 20 μg/kg did not alter (P > .1) wound healing, based on wound areas, in treated geldings relative to controls. Subjective evaluation of the wounds on site and later from the photographs did not indicate any noticeable differences between those in reST-treated and control geldings.

Daily Plasma Hormone and Metabolite Concentrations. Plasma ST concentrations (Figure 2) averaged over the 33-d sampling period were higher (P < .05) in reST-treated geldings than in control geldings. Concentrations of IGF-I (Figure 2) were also higher (P < .001) in reST-treated geldings than in controls. Administration of reST also increased concentrations of glucose (P < .001; Figure 3) and insulin (P < .05; Figure 3) in plasma. Plasma urea-N concentrations (Figure 3) were lower (P < .001) in reST-treated geldings than in controls. There was no difference (P >
Figure 2. Daily ST and IGF-I concentrations in geldings treated with vehicle (control) or recombinant equine somatotropin (reST) daily for 21 d beginning immediately after lesioning on d 0. There was a treatment × time interaction for ST ($P < .05$) and for IGF-I ($P < .001$) concentrations. The pooled SE from the analysis of variance was .23 ng/mL for ST and 39.6 ng/mL for IGF-I.

.1) in daily NEFA concentrations between the two groups over the treatment period (data not shown).

Intravenous Glucose Tolerance Test. Data from the IVGTT are presented in Figure 4. Glucose concentrations were higher ($P < .001$) in reST-treated geldings than in controls immediately before glucose infusion and stayed higher ($P < .001$) in reST-treated geldings throughout the 3-h sampling period.

Insulin concentrations were higher ($P < .002$) in reST-treated geldings relative to controls immediately before glucose infusion, and the glucose-induced insulin response in reST-treated geldings was approximately twice ($P = .02$) that of control geldings. Plasma NEFA concentrations were higher ($P < .003$) in reST-treated geldings before glucose infusion and remained higher ($P < .05$) in that group throughout the IVGTT.

Epinephrine Challenge. In response to i.v. injection of epinephrine on d 21, ST concentrations increased ($P < .02$) in the control geldings but not in reST-treated geldings (Figure 5). Glucose and NEFA concentrations both increased ($P < .001$) after epinephrine injection, but the increases were similar ($P > .1$) for both groups (data not shown). There was also an increase ($P < .001$) in plasma cortisol concentrations following epinephrine administration; however, cortisol concentrations did not differ ($P > .1$) between the reST-treated and control geldings (data not shown).

Exercise Challenge. Similar to the epinephrine challenge, 5 min of exercise induced an immediate increase ($P < .001$) in plasma ST concentrations in control geldings, whereas there was no increase in the reST-treated geldings (Figure 6). Prolactin concentrations were lower ($P < .03$) in reST-treated geldings relative to controls before exercise; after exercise, the rise in prolactin concentrations (Figure 6) also tended to be lower ($P < .08$). There was no change ($P > .05$) in cortisol concentrations after exercise in either group of geldings (data not shown).
Aspartic Acid Challenge. Similar to the two previous tests, the plasma ST response following i.v. infusion of aspartic acid on d 22 was greater ($P < .001$) in control geldings than in reST-treated geldings (Figure 7). In fact, only one of the six reST-treated geldings displayed an increase in plasma ST concentrations, accounting for the small rise seen for that group in Figure 7.

Discussion

This mode of lesioning used in the present experiment was chosen based on accessibility of the wound, reliability of measurements of wound area, and similarity in degree to lesions commonly encountered on the farm. Daily administration of reST at 20 $\mu$g/kg of BW did not affect healing of this skin lesion model.

Administration of ST has been shown to improve mechanical strength of wounds after skin grafts in rats (Jorgensen et al., 1995). Also, Christensen and Oxlund (1992) reported that ST increased collagen deposition and breaking strength after colon surgery in rats. Even though the dosage of reST in this study was lower on a BW basis than those used in other wound healing studies, it was high enough to increase ST and IGF-I concentrations in the blood and to alter various metabolic systems. Thus, it is unlikely that higher doses of reST would have any different effects on this wound healing model than those observed herein. It is possible that other types of lesions, such as in areas less vascularized than the pectoral region (e.g., on the lower leg), or more serious lesions (e.g., resection of the gut in colic surgeries), would be better tests of the possible beneficial effects of reST on wound healing. Gilpin et al. (1994) reported that ST administration accelerated healing of large cutaneous burns in children. Similarly, ST administration improved mortality rates of patients suffering from body surface area burns (mean burn area 58% of body; Knox et al., 1995). The inflammatory response to severe injury, such as thermal injury, causes extreme catabolism, which impairs wound healing and decreases lean body mass (Nuytich et al., 1988). The protein anabolic effects of ST likely help combat these deleterious effects of inflammation.

The effects of ST on carbohydrate and lipid metabolism in this experiment agree well with previous reports. Buonomo et al. (1996) reported that administration of bovine or porcine ST to horses resulted in hyperglycemia and hyperinsulinemia as assessed by daily blood samples. In contrast, Christensen et al. (1996a,b) reported no elevation in daily
Figure 6. Somatotropin and prolactin concentrations after exercise on d 21 in geldings treated with vehicle (control) or recombinant equine somatotropin (reST) daily for 21 d beginning immediately after lesioning on d 0. There was a treatment × time interaction for ST (P < .001) and for prolactin (P < .08) concentrations. The pooled SE from the analysis of variance was .99 ng/mL for ST and .79 ng/mL for prolactin.

Figure 7. Somatotropin concentrations after aspartic acid infusion on d 22 in geldings treated with vehicle (control) or recombinant equine somatotropin (reST) daily for 21 d beginning immediately after lesioning on d 0. There was a treatment × time interaction (P < .001) for ST concentrations. The pooled SE from the analysis of variance was 1.13 ng/mL.

glucose or NEFA concentrations but did find an elevation of daily insulin concentrations in aged mares treated with 12.5 mg of reST daily. Christensen et al. (1996a,b) also reported that reST treatment of those aged mares increased the normal postprandial rises in plasma glucose and insulin. In the present experiment, plasma glucose and insulin concentrations were elevated in reST-treated geldings within 2 d and remained elevated for most of the 21-d treatment period. In addition, treatment with reST decreased the rate of clearance of glucose after the IVGTT in the face of greatly enhanced insulin concentrations evoked by the infused glucose. This indicates that reST treatment not only results in hyperglycemia and hyperinsulinemia, but also causes the geldings to be less glucose tolerant as well as insulin insensitive, characteristic of the classic diabetogenic effect of long-term ST treatment (Guyton, 1996).

Daily urea-N levels were decreased in reST-treated geldings relative to controls, which is similar to results in mares treated with reST (Christensen et al., 1996a). The effect on plasma urea-N concentrations was rapid, with concentrations being lower within 12 h after the first reST injection. Plasma NEFA concentrations, although not affected overall in daily samples, were higher before and after glucose infusion on d 16 in the reST-treated geldings relative to the controls. It is likely that the difference in NEFA concentrations between groups on d 16 (vs no difference in daily samples) was detectable because all geldings were deprived of feed overnight in preparation of the IVGTT, whereas they were not deprived of feed prior to the drawing of routine daily blood samples. Overnight feed deprivation typically elevates plasma NEFA concentrations from a "fed" average of 100 to 200 µEq/L to at least 600 µEq/L or greater (DePew et al., 1994; Nadal et al., 1997).

From previous reports on other species (Nakamoto et al., 1986; Guyton, 1996), we expected reST to alter the normal production and secretion of endogenous ST due to the feedback effects of IGF-I and perhaps insulin (Harvey, 1995b). From these data, we concluded that treatment with reST did in fact result in feedback that reduced the endogenous ST secretion in response to known secretagogues. We have shown previously that exercise (Thompson et al., 1992, 1994; Sticker et al., 1995a) and epinephrine administration (Thompson et al., 1992; Sticker et al., 1995b) consistently produce an immediate increase in plasma ST concentrations in horses. Moreover, we have recently found (our unpublished data) that aspartic acid infusion also consistently induced an ST response when administered i.v. at 380 mg/kg of BW. The responses in the control geldings in the present experiment confirmed these observations, and the lack of responses in the reST-treated geldings to exercise and epinephrine 1 d after the last reST treatment indicated that the normal stimulus-response axis in these geldings was absent or suppressed. Within 2 d after the last reST injection, only one of the six reST-
treated geldings responded to aspartic acid infusion, whereas all control geldings responded. From what is known for other species (Harvey, 1995a), failure to respond to these stimuli may be due to 1) reduced ST stores in the pituitary, 2) enhanced somatostatin production and secretion and, therefore, maximum suppression of somatotropes, or 3) diminished GHRH stores and secretion from hypothalamic neurons.

The administration of reST at the dosage used herein did not seem to have any visible negative side-effects in any of the geldings other than the two isolated injection site swellings in two of the six reST-treated geldings. The lack of persistent site-of-injection swelling and soreness is consistent with the lack of specific anti-reST antibody formation in these geldings. That is, in a previous experiment in which pony mares were administered 4 mg of recombinant porcine prolactin s.c daily, antibodies were detected by the methods used herein within 14 d after the first injection, and were fourfold higher by 28 d (Thompson et al., 1997); those pony mares also exhibited swelling at the injection sites on several occasions.

In conclusion, administration of reST to adult geldings at 20 μg/kg of BW daily for 21 d resulted in many of the perturbations in glucose, fat, and protein metabolism that one would expect from previous reports on other species, thereby confirming the biological activity of the reST. However, treatment with reST did not result in any beneficial effect with the wound model used in this experiment. Whether similar treatment might be beneficial in other forms of lesions or traumas in horses needs to be tested in future studies.

Implications

Treatment of adult geldings with recombinant equine somatotropin daily for 21 d resulted in hyperglycemia, hyperinsulinemia, reduced plasma urea nitrogen concentrations, and exaggerated glucose and insulin responses to an intravenous glucose tolerance test. There was no beneficial effect of treatment on the healing of a skin lesion in the pectoral region; however, it is not possible to know whether other types of lesions or traumas in horses might benefit from similar treatment. Treatment with exogenous somatotropin reduced the geldings’ ability to respond to stimuli that normally increase endogenous somatotropin concentrations in the blood, and this fact should be considered when devising future treatment protocols that involve exogenous somatotropin.

Literature Cited


