Activation State of Muscle Satellite Cells Isolated from Steers Implanted with a Combined Trenbolone Acetate and Estradiol Implant


Animal Growth and Development Laboratory, Department of Animal Science, University of Minnesota, St. Paul 55108

ABSTRACT: Muscle satellite cells were isolated from seven yearling steers implanted for 31 d with a combined implant that contained 120 mg of trenbolone acetate (TBA) and 24 mg of estradiol (E2) and from seven nonimplanted, control steers. Implanted steers had a 28% greater ADG and a 23% greater feed efficiency than did nonimplanted steers. Implanted steers had increased (P < .001) circulating IGF-I concentrations on d 6, 14, and 31 after implantation, and circulating IGF-I concentrations in control steers remained constant or decreased (P < .05) at these times. Maximum fusion percentage was greater (P < .005) in satellite cell cultures isolated from implanted steers (ISC cultures) than in satellite cell cultures isolated from control steers (NSC cultures) (72.8% vs 54.8%, respectively). Satellite cell cultures isolated from implanted steers (ISC cultures) also contained a greater (P < .001) number of myotube nuclei than did NSC cultures (7,998 nuclei/cm² vs 5,150 nuclei/cm², respectively). After 72 h in culture, the number of cells (corrected for plating density) was 43% greater (P < .05) in ISC cultures than in NSC cultures. [3H]Thymidine incorporation rates per 10⁵ cells at 24 and 34 h after plating were greater (P < .05) in ISC cultures than in NSC cultures; however, incorporation rates did not differ at 72 h. These data indicate that TBA + E2 implantation may result in an in vivo activation of muscle satellite cell proliferation that can be detected in cell culture. This activation may play an important role in TBA + E2-enhanced muscle growth.

Key Words: Muscles, Beef, Anabolic Steroids


Introduction

The implantation of feedlot steers with Revalor®-S (Hoechst Roussel Agri-Vet, Warren, NJ), a combined implant containing 120 mg of trenbolone acetate (TBA) and 24 mg of estradiol (E2), results in a 20 to 25% increase in rate of gain and a 15 to 20% increase in feed efficiency (Johnson et al., 1996a). Additionally, serial slaughter studies have shown that carcass protein and longissimus muscle area are increased in implanted steers, compared with nonimplanted steers (Johnson et al., 1996a). Thus, muscle growth seems to be enhanced by Revalor-S. Several studies have established that muscle satellite cells contribute to postnatal muscle growth by providing nuclei to the growing fiber (Powell and Aberle, 1975; Harbison et al., 1976; Swatland, 1977; Trenkle et al., 1978). However, the majority of satellite cells in uninjured adult muscle is in a quiescent (G0) state (Moss and Leblond, 1970; Campion, 1984; Dodson and Allen, 1987; Bischoff, 1990). Furthermore, studies with rats suggest that, as an animal matures, the number of satellite cells decreases and the proportion of the remaining satellite cells that is quiescent increases (Moss and Leblond, 1970; Campion, 1984; Dodson and Allen, 1987; Bischoff, 1990; Johnson and Allen, 1993; Johnson and Allen, 1995). Consequently, the reduced muscle growth rate observed in animals that are approaching market weight may reflect a decrease in the number of active satellite cells as animals mature. Thus, we have hypothesized that Revalor-S-induced enhancement of muscle growth could involve alterations in the proliferation rate and/or activation state of satellite cells. Consequently, we examined these
variables in satellite cell cultures isolated from the semimembranosus muscle (SM) of Revalor-S-implanted and nonimplanted steers. Results of these studies showed that satellite cell cultures isolated from the SM muscle of Revalor-S-implanted steers (ISC) exhibited a shorter lag phase before initiating proliferation than did satellite cell cultures isolated from nonimplanted steers (NSC). This suggests that Revalor-S-induced enhancement of muscle growth involves activation of quiescent satellite cells.

Materials and Methods

Animals. All experimental procedures were approved by the University of Minnesota Animal Care Committee. Crossbred yearling steers \((n = 14)\), matched for age and frame size, were used in this study. The average weight of the steers at the beginning of the experiment was 382 kg. Before implantation, steers were maintained on a high-
roughage backgrounding diet (80% corn silage, 15% cracked corn, and 5% supplement). Beginning 14 d before implantation, steers were fed a step-up diet so that by the day of implantation they were consuming a high-concentrate (85% cracked corn, 10% corn silage, and 5% supplement) diet. This diet (77% DM; 13.2% CP) was provided for ad libitum intake throughout the study. Steers were allocated into four pens (4 steers/pen), and steers in two of the pens were implanted with Revalor-S (120 mg of TBA and 24 mg of E2) on d 0 of the study. Before implantation, steers were maintained on a high-concentrate (85% cracked corn, 10% corn silage, and 5% supplement) diet. This diet (77% DM; 13.2% CP) was provided for ad libitum intake throughout the study. Steers were allocated into four pens (4 steers/pen), and steers in two of the pens were implanted with Revalor-S (120 mg of TBA and 24 mg of E2) on d 0 of the study. Steers were weighed weekly and the feed intake of each pen was determined daily.

Blood samples for IGF-I RIA were taken from the left jugular vein on d 0, 6, 14, 20, 31, and on the day of slaughter. Because facilities allowed preparation of satellite cells from two steers per day, one randomly selected implanted steer and one randomly selected nonimplanted steer were killed on d 32 through 38, and satellite cells were prepared from the SM muscle of each animal. Longissimus muscle area of all carcasses was measured 24 h after slaughter.

Satellite Cell Isolation. Satellite cell isolation was done as described previously (Hathaway et al., 1991, 1994; Frey et al., 1995). Steers were slaughtered by captive bolt stunning followed by exsanguination. Using sterile techniques, approximately 500 g of the semimembranosus muscle was dissected out and transported to the cell culture laboratory. Subsequent procedures were conducted in a sterile field under a tissue culture hood. After removal of connective tissue, the muscle was passed through a sterile meat grinder. The ground muscle was incubated with 1% pronase in Earl’s Balanced Salt Solution (EBSS) for 1 h at 37°C with frequent mixing. Following incubation, the mixture was centrifuged at 1,500 \( \times \) g for 4 min, the pellet was suspended in PBS (140 mM NaCl, 1 mM KH\(_2\)PO\(_4\), 3 mM KCl, and 8 mM Na\(_2\)HPO\(_4\)), and the suspension was centrifuged at 500 \( \times \) g for 10 min. The resulting supernatant was centrifuged at 1,500 \( \times \) g for 10 min to pellet the mononucleated cells. The PBS wash and differential centrifugation were repeated two more times. The resultant mononucleated cell preparation was suspended in cold (4°C) Dulbecco’s Modified Eagle Medium (DMEM) that contained 10% fetal bovine serum (FBS) and 10% (vol/vol) dimethylsulfoxide (DMSO) and frozen. Cells were frozen in liquid nitrogen and stored for use in future studies.

Satellite Cell Culture. Bovine satellite cells from nonimplanted and implanted steers slaughtered on the same day were plated on 2-cm\(^2\) culture plates precoated with reduced growth factor basement membrane Matrigel (Becton Dickinson Labware, Franklin Lakes, NJ), diluted 1:10 (vol/vol) with DMEM. Plating density (satellite cells isolated from .25 g muscle tissue/cm\(^2\)) was empirically established so that all cultures were approximately 40 to 60% confluent after the incubation period. This ensured that cell proliferation rate was not affected by contact inhibition. Cells were plated in DMEM containing 10% FBS and incubated at 37°C, 5% CO\(_2\) in a water-saturated environment. Cultures were fed at 48 h with DMEM containing 10% FBS. Cell number and \[^3\]H\]thymidine incorporation rate were determined at various times after plating.

Cultures that were allowed to fuse were fed with a fusion-promoting medium that consisted of DMEM with 3% horse serum and 1.5 \( \mu \)g BSA-linoleic acid/mL at 96 h, and fusion percentage was determined at 168 h as described elsewhere in Materials and Methods.

\[^3\]H\]Thymidine Incorporation and Satellite Cell Number. \[^3\]H\]Thymidine incorporation into proliferating bovine muscle satellite cells was measured using the method of Yablonka-Reuveni et al. (1990). Briefly, bovine satellite cells that had been isolated from implanted or from nonimplanted steers were plated on 2-cm\(^2\) wells coated with reduced growth factor Matrigel (1:10 dilution) in DMEM containing 10% FBS and were allowed to attach at 37°C, in a 95% air/5% CO\(_2\) humidified atmosphere. \[^3\]H\]Thymidine was added to culture media (1 \( \mu \)Ci/mL final concentration) and allowed to incubate at 37°C for 3 h. Cells were rinsed with cold serum-free DMEM, fixed with 1 mL of cold 5% trichloroacetic acid (TCA), and allowed to incubate overnight at 4°C. Cell number was measured by counting 10 randomly chosen microscope fields per well. Unincorporated \[^3\]H\]thymidine was removed by aspirating the cold 5% TCA and rinsing wells with additional cold 5% TCA. \[^3\]H\]Thymidine incorporation into cellular DNA was measured by dissolving cell material in .5 M NaOH and counting in a scintillation counter. \[^3\]H\]Thymidine incorporation into bovine muscle satellite cells at various times after plating was reported as \[^3\]H\]thymidine incorporated per 10\(^4\) cells. All data points are the average of values obtained from triplicate cultures.
Table 1. Effect of a combined trenbolone acetate (TBA) + estradiol (E₂) implant on performance of feedlot steers

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>TBA + E₂</th>
<th>SEM</th>
<th>% Response</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily gain, kg</td>
<td>1.39</td>
<td>1.79</td>
<td>.09</td>
<td>28.0</td>
<td>.009</td>
</tr>
<tr>
<td>Dry matter intake, kg</td>
<td>8.1</td>
<td>8.49</td>
<td>.14</td>
<td>4.8</td>
<td>.03</td>
</tr>
<tr>
<td>Feed/gain</td>
<td>5.83</td>
<td>4.73</td>
<td>.11</td>
<td>23.0</td>
<td>.03</td>
</tr>
</tbody>
</table>

aSteers were implanted for 32 to 38 d.
bLeast squares means.
c120 mg of TBA and 24 mg of E₂.
dPercentage of control.

Number of Nuclei Incorporated into Myotubes in Satellite Cell Cultures. Photographs of random fields in Giemsa-stained, fused cultures were taken using a 35-mm camera and a Zeiss inverted microscope with a 20× phase-contrast objective. A minimum of 10 fields (containing a total of at least 700 nuclei) per plate and three plates per treatment were photographed. The number of nuclei in myotubes (a myotube was identified by the presence of three or more nuclei within a continuous cell membrane) was determined from these photographs, and fusion percentage was assessed by determining the ratio of myotube nuclei to total nuclei. This method has been used for rat satellite cells (Allen et al., 1984), and we have used it for ovine (Hathaway et al., 1991) and bovine satellite cells (Frey et al., 1995).

IGF-I Radioimmunoassays. The IGF-I RIA was done as described previously (Frey et al., 1994; Johnson et al., 1996b). Sera samples were treated with glycylglycine-HCl (pH 3.7) for 48 h at 37°C to eliminate insulin-like growth factor binding protein (IGFBP) interference. The RIA were performed using a double antibody immunoprecipitation method. The primary antibody was a rabbit anti-IGF-I (UB3-189) obtained from the National Hormone and Pituitary Program. Inter- and intraassay variations were 8.5 and 2.8%, respectively. Recovery of added IGF-I was 90%.

Statistical Analysis. Data were analyzed by analysis of variance using the General Linear Model procedure of PC SAS Release 6.04 (SAS, 1989). For serum IGF-I, the model included treatment, time (day of bleeding), steer, and pen (treatment) as main effects. For proliferation rate, [³H]thymidine incorporation rate, and myotube nuclei, the model included treatment, day of processing, steer, and pen (treatment) as main effects. Day of processing was not significant for any variable tested. When main effects were significant, differences between means for preplanned comparisons were tested using Fisher’s LSD test. Comparisons were considered significantly different if P < .05.

Results

Performance of Nonimplanted and Implanted Steers. Implantation of steers increased average daily gain 28% (P < .05) and feed efficiency 23% (P < .05), compared with nonimplanted steers (Table 1). Dry matter intake was slightly (4.8%) but significantly different between implanted and nonimplanted animals (Table 1). These increases in rate of gain and feed efficiency are similar to those observed in other studies and establish the efficacy of the implant in this study. Even though final weight, hot carcass weight, and longissimus muscle area were numerically greater in implanted than in nonimplanted steers (Table 2), they were not significantly different presumably because of the small number of steers in the study.

Serum IGF-I Concentration in Implanted vs Nonimplanted Steers. Figure 1 shows the average serum IGF-I concentration on d 0, 6, 14, 20, and 31. On d 0, serum IGF-I concentrations of implanted and nonimplanted steers were not different (252 for implanted steers vs 266 ng/mL for nonimplanted steers). By d 6, serum IGF-I concentrations in implanted steers had increased (P < .001) by 32% (Figure 1) relative to concentrations in sera from the same animals on d 0. This TBA + E₂-induced elevation in circulating IGF-I concentration was maintained throughout the 32-d duration of the study. In contrast, serum IGF-I concentrations of nonimplanted steers were not significantly different from d-0 levels except on d 32, when circulating IGF-I concentration was 18% less (P < .05) than the concentration in sera from the same animals on d 0. Direct comparison of the serum IGF-I concentrations in implanted and nonimplanted steers

Table 2. Effect of a combined trenbolone acetate (TBA) + estradiol (E₂) implant on carcass traits of feedlot steers

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>TBA + E₂</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final weight, kg</td>
<td>439.7</td>
<td>466.6</td>
<td>9.7</td>
<td>.07</td>
</tr>
<tr>
<td>Hot carcass weight, kg</td>
<td>263.8</td>
<td>279.5</td>
<td>6.1</td>
<td>.07</td>
</tr>
<tr>
<td>Longissimus muscle area, cm²</td>
<td>73.03</td>
<td>78.97</td>
<td>3.1</td>
<td>.19</td>
</tr>
</tbody>
</table>

aSteers were implanted for 32 to 38 d.
bLeast squares means.
c120 mg of TBA and 24 mg of E₂.
dn = 7.
Figure 1. Serum insulin-like growth factor (IGF-I) concentration of nonimplanted and implanted steers at various times after implantation. Circulating IGF-I concentration in steers implanted with trenbolone acetate + estradiol was significantly greater \((P < .001, n = 7)\) than in nonimplanted steers on all days except on d 0. Pooled SEM is 11.47 ng/mL.

Figure 2. Relative cell number increase in cultures of satellite cells isolated from nonimplanted and implanted steers. Data shown are the fold-increase in cell number relative to the number of cells present at 24 h. Data were obtained from seven nonimplanted and seven implanted steers. The relative cell number after 72 h in culture was 43% greater \((P < .02, n = 7)\) in cultures isolated from implanted steers than in cultures isolated from nonimplanted steers. Pooled SEM is 1.08-fold.

showed that IGF-I concentration in sera from Revalor-S-implanted steers was 20, 29, and 33% greater \((P < .001)\) on d 6, 14, and 31, respectively, than the IGF-I concentration in serum from nonimplanted steers. This Revalor-S-induced increase in circulating IGF-I concentration confirms our previous results and, along with performance data, establishes the effectiveness of the implant in this study.

Relative Proliferation Rate of Cultured Satellite Cells Isolated from Implanted and Nonimplanted Steers. Figure 2 shows the relative increase in cell number at various times after plating in cultures of satellite cells isolated from ISC or NSC steers. At 34 h, the relative increase in cell number in ISC and NSC cultures was not different; however, by 48 h in culture, the relative increase in cell number in ISC cultures was 24% greater \((P < .05)\) than for NSC cultures. At 72 h, relative increase in cell number was 43% greater \((P < .05)\) in ISC than in NSC cultures. These data show that the proliferation rate of ISC cultures was greater than that of NSC cultures.

To further investigate this increased proliferation rate, we examined the \([^{3}H]\)thymidine incorporation/10⁴ cells in ISC and NSC cultures 24, 34, 48, and 72 h after plating (Figure 3). At 34 h after plating, \([^{3}H]\)thymidine incorporation rate/10⁴ cells in ISC cultures was 140% greater \((P < .01)\) than in NSC cultures and, at 48 h, incorporation rate in ISC cultures was 78% greater than in NSC cultures. However, by 72 h after plating, there was no difference in the \([^{3}H]\)thymidine incorporation rate/10⁴ cells in NSC and ISC cultures. These data confirm the cell number results showing that cell proliferation is more rapid in ISC cultures than in NSC cultures.

Additionally, the \([^{3}H]\)thymidine incorporation rates show that the differences in proliferation between ISC and NSC cultures occurred before the 72-h point because \([^{3}H]\)thymidine incorporation rates in NSC and ISC cultures were essentially the same at 72 h.

In order to get a detailed picture of the relationship between cell number and \([^{3}H]\)thymidine incorporation rate in ISC and NSC cultures, cells from a representative implanted and a representative nonimplanted steer slaughtered on the same day were cultured and cell number and \([^{3}H]\)thymidine incorporation rate/10⁴ cells were determined 24, 34, 38, 42, 48, and 72 h after plating (Figure 4). \([^{3}H]\)Thymidine incorporation/10⁴ cells was higher in ISC cultures than in NSC cultures at 24, 34, 38, 42, and 48 h. Cell number in ISC cultures was not detectably greater than in NSC cultures until 48 h (19% more cells in ISC than in NSC cultures) and 72 h (42% more cells in ISC than in NSC cultures). These data indicate that the lag phase before initiation of proliferation was longer in NSC cultures than in ISC cultures. A longer lag phase has generally been interpreted to mean that a higher percentage of the cells are in a quiescent state and must be activated prior to initiation of proliferation. Consequently, our data show that there are more quiescent satellite cells in NSC cultures than in ISC cultures and provide evidence that Revalor-S treatment results in activation of satellite cells.

Comparison of Myotube Nuclei Number and Fusion Percentage in ISC and NSC Cultures. All primary satellite cell cultures are contaminated by cells other
Figure 3. [³H]Thymidine incorporation into cultured satellite cells isolated from implanted and from nonimplanted steers. Data were pooled from measurements made on cultured satellite cells isolated from seven implanted and seven nonimplanted steers. After 24, 34, or 48 h in culture, thymidine incorporation was 140, 78, or 26% greater, respectively, in cultures of satellite cells isolated from implanted steers than in cultures of satellite cells isolated from nonimplanted steers. Bars labeled with different letters are different (P < .01, n = 7). Pooled SEM is 565.

than muscle cells, and the presence of these cells can complicate the interpretation of proliferation data. Consequently, we compared the number of myotube nuclei and fusion percentage in differentiated ISC and NSC cultures. After 96 h in culture, cells were fed a fusion-promoting medium to induce fusion of myogenic cells. Seventy-two hours later (168 h after plating), average fusion percentage in cultures of satellite cells isolated from Revalor-S-implanted steers was greater (P < .005, n = 7) than in cultures isolated from nonimplanted steers (54.8% for NSC and 72.8% for ISC) (Table 3). These data indicate that ISC cultures contained a higher percentage of fusing satellite cells than did NSC cultures. In these same cultures, we also determined the number of myotube nuclei. Table 3 shows that average cell density after 24 h in culture

Figure 4. [³H]Thymidine incorporation and cell number in triplicate satellite cell cultures isolated from a representative implanted and nonimplanted steer. [³H]Thymidine incorporation was 89, 41, and 34% greater at 38, 42, and 48 h, respectively, in satellite cell cultures from the implanted steer (ISC dpm) than in cultures from the nonimplanted steer (NSC dpm). At 72 h, [³H]thymidine incorporation was the same in satellite cells isolated from implanted and nonimplanted steers. Changes in cell number lagged behind [³H]thymidine incorporation, but, by 72 h, cell number in cultures isolated from the implanted steers (ISC number) was 42% greater than in cultures isolated from the nonimplanted steer (NSC number). For points with no standard error bar, the standard error is contained within the point.

Table 3. Fusion percentage and myotube nuclei in satellite cell cultures from cells isolated from the semimembranosus muscle of implanted and nonimplanted steers

<table>
<thead>
<tr>
<th>Item</th>
<th>Cell density a at 24 h. b cells/cm²</th>
<th>Myotube nuclei c at 168 h. b nuclei/cm²</th>
<th>-Fold increase in myotube nuclei relative to cell density d at 24 h.b</th>
<th>Fusion, % e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonimplanted</td>
<td>133 ± 26</td>
<td>5,150 ± 424</td>
<td>41 ± 5</td>
<td>54.8 ± 3.9</td>
</tr>
<tr>
<td>Implanted</td>
<td>117 ± 27</td>
<td>7,798 ± 442</td>
<td>72 ± 10</td>
<td>72.8 ± 2.8</td>
</tr>
<tr>
<td>P-value</td>
<td>NS³</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.005</td>
</tr>
</tbody>
</table>

aAverage cell density from individual cell culture experiments on each of seven nonimplanted and seven implanted steers.

bHours after plating.

cAverage myotube nuclei density from individual cell culture experiments done on each of seven nonimplanted and seven implanted steers.

dAverage-fold increase was calculated by averaging the increases obtained in individual cell culture experiments done on satellite cells from seven nonimplanted and seven implanted steers.

e(Myotube nuclei/total nuclei) × 100 (n = 7).

³NS = not statistically significant.
was not different for NSC and ISC cultures; however, after 168 h in culture, the average number of myotube nuclei was 50% greater (P < .001, n = 7) in ISC cultures than in NSC cultures (5.150 ± 424 for NSC and 7.798 ± 442 for ISC). Dividing the number of myotube nuclei by the cell density at 24 h for each animal yields a relative increase in myotube nuclei that is corrected for initial plating density. The relative increase in myotube nuclei was 1.77 times greater (P < .001, n = 7) for ISC cultures than for NSC cultures (41 ± 5 for NSC and 72 ± 10 for ISC).

Discussion

The number of muscle fibers in meat-producing animals is essentially fixed at birth, so postnatal muscle growth is primarily due to hypertrophy, or increase in length and diameter, of existing multinucleated muscle fibers. This increase in fiber size is accompanied or preceded by an increase in muscle DNA, which is necessary to support the increased size of the fiber (Powell and Aberle, 1975; Harbison et al., 1976; Swatland, 1977; Trenkle et al., 1978). Because the nuclei in muscle fibers are unable to divide, mononucleated cells known as satellite cells are responsible for providing the needed DNA to growing muscle fibers (Mauro, 1961; Campion, 1984). Satellite cells fuse with existing fibers and, in so doing, contribute their nuclei to the fiber (Moss and Leblond, 1970; Moss and Leblond, 1971). From 60 to 90% of the DNA in mature muscle fibers originates from satellite cells (Allen et al., 1979). Thus, proliferation of satellite cells and their fusion with muscle fibers to provide DNA required for fiber growth may be critical rate-limiting steps in muscle growth. It is, thus, significant that the number of satellite cells decreases dramatically as animals become older, and that, in adult animals, the remaining satellite cells normally exist in a quiescent state, in which proliferation does not occur (Moss and Leblond, 1970; Campion, 1984; Dodson and Allen, 1987; Bischoff, 1990). We have observed that satellite cells isolated from sheep that are approaching market weight undergo a 24- to 72-h period of little or no proliferation (lag phase) after being placed in culture (Hathaway et al., 1991). A similar lag phase has been observed in satellite cells isolated from adult rat muscle, and this has been interpreted to indicate that the majority of satellite cells in uninjured adult muscle are in a quiescent (G₀) phase (Dodson and Allen, 1987; Johnson and Allen, 1993). The lag phase in culture is thought to represent the time required for the cells to reenter the G₁ phase and begin to proliferate. Satellite cells isolated from rats of different ages exhibit variable lag periods (Schultz and Lipton, 1982), suggesting that their activation state and(or) their response to growth factors varies with the age of the rat from which they were isolated. Our data show that, between 24 and 72 h in culture, satellite cells isolated from implanted steers proliferate more rapidly than do satellite cells isolated from nonimplanted steers. However, at 72 h the proliferation rates of cells isolated from implanted and nonimplanted steers are the same. These data indicate that satellite cells isolated from implanted steers have a shorter lag phase and, therefore, become activated more quickly in culture than do cells isolated from nonimplanted steers. However, once activated (by 72 h in culture) satellite cells from implanted and nonimplanted steers proliferate at the same rate. Based on these findings, we believe that Revalor-S may enhance muscle growth by directly or indirectly activating quiescent satellite cells. Alternatively, Revalor-S treatment may maintain satellite cells in a proliferative state in vivo.

All primary satellite cell cultures contain nonmuscle cells, and their presence can complicate the interpretation of proliferation data obtained in this study. However, our data show that both fusion percentage and number of myotube nuclei were greater for ISC than for NSC cultures. Assuming that the proportion of satellite cells that fuse in ISC and NSC cultures is the same, these data establish that ISC cultures contain more satellite cells than do NSC cultures. This result is consistent with [³H]thymidine incorporation and cell number data showing that cultured satellite cells from implanted steers begin proliferating sooner than do satellite cells isolated from nonimplanted steers. Viewed together, these results support our hypothesis that the SM muscles from Revalor-S-implanted steers contain a greater number of activated satellite cells than do the SM muscles from nonimplanted steers.

The mechanism by which Revalor-S activates satellite cells and(or) maintains these cells in a proliferative state is not clear from the present data. It has been reported that hepatocyte growth factor (HGF) activates quiescent satellite cells in culture and that quiescent satellite cells possess detectable levels of mRNA for c-met, which is the HGF receptor (Allen et al., 1995). Consequently, it is possible that HGF may be involved in activating satellite cells in Revalor-S-implanted steers. Once satellite cells are activated, proliferation can be stimulated by growth factors such as IGF-I and fibroblast growth factor (FGF). Thus, the increased concentrations of circulating and(or) locally produced IGF-I that we have observed in Revalor-S-implanted steers may play a role in stimulating proliferation of activated satellite cells (Johnson et al., 1996b; Johnson et al., 1998). It is significant that Revalor-S-induced elevation of circulating IGF-I concentration is detectable by 6 d after implantation. Thus circulating IGF-I is elevated during the period between d 0 and 40 after implanta-
tion, when we have shown that muscle growth is most significantly affected by Revalor-S implantation. Additionally, we have recently shown that the level of IGF-I mRNA in the longissimus muscle of steers implanted for 40 d with Revalor-S is 68% greater than in the longissimus muscle of nonimplanted steers (Johnson et al., 1998). These results suggest that muscle tissue from Revalor-S-implanted steers may produce more IGF-I than does muscle tissue from nonimplanted steers. This locally produced IGF-I could function via an autocrine or paracrine mechanism to enhance satellite cell proliferation and muscle growth.

From the current data, it is not possible to determine whether E2, TBA, or a synergistic interaction of the two steroids is responsible for the changes in satellite cell activation, serum IGF-I concentration, and muscle IGF-I mRNA levels that we have observed in our studies. Several studies have shown that E2 treatment increases serum IGF-I level (Breier et al., 1988; Enright et al., 1990), so it is possible that E2 may be primarily responsible for the increased circulating IGF-I observed in Revalor-S-implanted steers. Additionally, satellite cells isolated from trenbolone-treated female rats have been reported to proliferate more rapidly in culture than satellite cells isolated from untreated rats (Thompson et al., 1989). Even though satellite cell activation was not examined, trenbolone-induced satellite cell activation could explain the results obtained by these investigators. Additionally, testosterone treatment has been reported to activate quiescent satellite cells in the rat levator ani muscle (Joubert and Tobin, 1995). Consequently, the closely related compound TBA may be involved in the Revalor-S-induced activation of satellite cells observed in the current study. Even though the possibility that TBA and E2 may act synergistically to stimulate muscle growth via different mechanisms in Revalor-S-implanted steers is intriguing, more evidence is required to establish this hypothesis. Future studies that examine the individual effects of TBA and E2 should provide the data required to establish the role of each of these steroids in the Revalor-S-induced enhancement of muscle growth in feedlot steers.

Implications

Results of this study suggest that activation of muscle satellite cells and( or) maintenance of satellite cells in a proliferative state may play a role in enhanced muscle growth in feedlot steers implanted with a combined implant containing trenbolone acetate and estradiol. These findings contribute to our understanding of the factors that regulate satellite cell activation and the role of satellite cell activation in muscle growth in beef cattle. Ultimately, this information may help to elucidate the mechanism by which anabolic steroids stimulate muscle growth in growing steers.

Literature Cited


