The Professional Animal Scientist 22 (2006):283–291

Optimization of Culture Conditions for Determining Protein Degradation in Myoblasts Using Extracts of Adult Bovine Muscle Treated with Muscle Enhancing Compounds[1]

J. L. MONTGOMERY,*[2] W. M. HARPER, Jr.,* M. F. MILLER,* K. J. MORROW, Jr.,[†3] K. W. BRADEN,*[4] and J. R. BLANTON, Jr.*[5]

*Department of Animal Science, Texas Tech University, Lubbock, TX †Department of Cell Biology and Biochemistry, Texas Tech Health Sciences Center, Lubbock, TX

Abstract

The objective of this study was to optimize a methodology in which muscle extracts from animals treated with growth agents are added to growth media and then applied to muscle cell cultures to determine effects of growth agents on indirect cellular protein degradation. Experiments were designed as a 2 × 2 × 2 × 2 factorial arrangement of cell type (C_2C_{12} myoblasts or primary bovine muscle cell cultures), growth medium [(Dulbecco's Modified Eagle's Medium (DMEM)or skeletal muscle cell basal medium (SKBM)], muscle extract medium [potassium phosphate buffer (KPB) or prerigor extraction buffer], and β-adrenergic agonist treatments of the bovine (control or treated) to determine differences in cellular protein degradation. There was a treatment media × β-adrenergic agonist treatment × cell type interaction ($P < 0.0001$). C_2C_{12} myoblasts with the β-adrenergic agonist in DMEM media had less protein degradation than controls in DMEM ($P < 0.05$). Primary bovine muscle cell cultures treated with the β-adrenergic agonist treatment in DMEM media had greater protein degradation than did controls in DMEM ($P < 0.05$). However, primary bovine muscle cell cultures treated with the β-adrenergic agonist treatment in SKBM media had reduced protein degradation than did controls in SKBM ($P < 0.05$). There was a treatment media × β-adrenergic agonist treatment × muscle cell extraction buffer interaction ($P = 0.04$). The β-agonist treatment decreased protein degradation when muscle samples were extracted with KPB or prerigor extraction buffer and made with SKBM culture media ($P < 0.05$). The results indicated SKBM and C_2C_{12} myoblasts had the most consistent differences for showing relative β-adrenergic agonist treatment effects on indirect protein degradation.

Key words: skeletal muscle, cell culture, protein degradation

Introduction

Muscle protein turnover is a ratio dependent on both protein synthesis and protein degradation. Muscle protein degradation has been measured by constant infusion with radiolabeled tracers (Mulvaney et al., 1985; Zhang et al., 1996), flood administering or injection of radioactively-labeled compounds to live animals (Lorenzen et al., 2000), and perfusion of tissue sections with radiolabeled tracers (Ward and Buttery, 1979). Meth-

[1]This research was partially supported by Texas Tech University and the Department of Animal and Food Sciences. This journal paper is no. T-5-447 from the Texas Tech University Agricultural Research Program.
[2]Current address: Intervet Inc., 29160 Intervet Lane, PO Box 318, Millsboro, DE.
[3]Current address: Meridian Biosciences, Inc., 3471 River Hills, Dr., Cincinnati, OH.
[4]Current address: Auburn University, 142 Upchurch Hall, Auburn, AL.
[5]To whom correspondence should be addressed: john.blanton@ttu.edu

ods employed to measure protein turnover in livestock are typically expensive and time consuming due to enormous isotope and animal costs (Skjaerlund et al., 1988). Also, a number of methods used to measure protein turnover are only able to measure either protein synthesis or degradation rates in a single study. Thus, a rapid method that allows researchers to determine treatment effects on relative indirect protein synthesis and degradation rates simultaneously in bovine would be highly beneficial. Another report from this laboratory discusses the measurement of protein synthesis using muscle extracts from bovine treated with pharmaceutical agents (Montgomery et al., 2006). Herein a similar method to measure protein degradation is discussed. Because both methods utilize the same muscle extracts, cell culture system, and supplies, experimental outcomes of treated animals can be determined for either protein synthesis, protein degradation or both simultaneously. Thus, an in vitro incubation system is employed using muscle extracts from treated animals that are added to growth media and applied to cell cultures to screen for relative changes that occur in vivo in response to hormonal, pharmaceutical, or physiological treatments. The results listed indicate how cell culture conditions can be optimized for determining relative treatment differences in protein degradation. However, the methods discussed demonstrated only indirect effects on protein degradation, and further studies will be needed to determine exactly how each individual compound being tested directly affects muscle protein degradation.

Materials and Methods

Chemicals. Amino acid mixture L-[^{14}C(U)] (NEC-445E) was obtained from Perkin Elmer Life and Analytical Sciences, Inc. (Wellesley, MA). Dulbecco's Modified Eagle's Medium (**DMEM**) and gentamycin sulfate were purchased from Invitrogen Corp. (Carlsbad, CA). Fetal bovine serum (**FBS**) was purchased from Atlanta Biologicals (Nocross, GA). Skeletal muscle cell basal medium (**SKBM**) was purchased from BioWhittaker (Walkersville, MD). RPMI-1640 cell culture media and antibiotic and antimycotic (penicillin, 100 U/mL; streptomycin, 100 μg/mL) were purchased from Sigma Chemical Corp. (Saint Louis, MO). Protein concentration determination was made using a protein assay kit from Bio-Rad Laboratories (Hercules, CA). All other chemicals and reagents were of analytical grade and were readily available from commercial sources.

Animals and Muscle Sample Preparations. Steers from a control group of cattle (n = 10) and from a treated group (n = 10) fed a diet with a commercially available β-adrenergic agonist compound for 30 d were harvested at a commercial processing plant after a 5-d withdrawal; muscle samples were collected and prepared as described elsewhere (Montgomery et al., 2006). From each animal, two 10-g muscle samples were collected: 1 sample was extracted in 30 mL of ice-cold 0.01 *M* potassium phosphate buffer (**KPB**, pH 7.4), and the other sample was extracted in 30 mL of ice-cold prerigor extraction buffer (50 mM Tris, 10 mM EDTA, pH 8.3). Tissue samples were homogenized for 45 sec and centrifuged for 30 min at 40,000 $\times$ *g*, prepared as elsewhere described (Montgomery et al., 2006), and the protein concentration determined according to Layne (1957).

Treatment Media. Two different cell culture media preparations were made for each of the 20 animals and extraction buffers. In a 15-mL conical vial, muscle extract was added at a level of 400 μg protein/mL into treatment media consisting of SKBM with 15% FBS, 1% penicillin and streptomycin, and 0.1% gentamycin (vol/vol) The muscle extract averaged approximately 10% of the final level in media. In a second 15-mL conical vial, muscle extract was added at a level of 400 μg protein/mL into treatment media consisting of DMEM with 15% FBS, 1% penicillin and streptomycin, and 0.1% gentamycin (vol/vol) The treatment media were then filter sterilized through a 0.22 μM filter and stored at 4°C until application to cell cultures.

Isolation and Culture of Primary Bovine Muscle Cell Cultures. Primary bovine muscle cell cultures were isolated and cultured as outlined in detail by Kerth (1999) and Pollard et al. (2001). Primary bovine muscle cell cultures were prepared following procedures outlined by Hembree et al. (1991) as adapted by Montgomery et al. (2002).

To test for fibroblast contamination, primary bovine muscle cultures were grown in DMEM in 6-cm petri dishes at a density of 15,000 cells/cm^2. At approximately 80% confluency the DMEM media was replaced with fusion media, which consisted of the DMEM and antibiotics as described above and 2% (vol/vol) horse serum. Fibroblast contamination was presumed low because 60 to 80% of the nuclei fused to form myotubes when incubated in fusion media for 8 d.

Cell Cultures. C_2C_{12} myoblasts (CRL-1772) were purchased from American Type Culture Collection (ATCC; Manassas, VA). C_2C_{12} myoblasts and primary bovine muscle cell cultures were thawed from liquid nitrogen storage and transferred to DMEM growth media (media containing 15% FBS, 1% penicillin and streptomycin, and 0.1% gentamycin; vol/vol) and placed into 75-cm^2 canted neck tissue cell culture flasks and incubated in a culture incubator (37°C, humidified environment, 5% CO_2, 95% air). Cell cultures were grown to approximately 80% confluence in the cell culture flasks and subcultured. Cells were added to 24-well culture plates at a density of 2,500 cells/cm^2 in 1 mL of growth media and incubated in a culture incubator for approximately 48 h until they reached 60 to 70% confluence.

Protein Degradation Assay. The procedure for determining the rate of protein degradation as measured by a pulse-chase uptake of labeled amino