β-agonist Regulate Skeletal Actin Gene Expression Post-transcriptionally

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Summary and Implications.

Previously reported studies have provided evidence that ractopamine regulates the expression of skeletal α-actin, but the molecular mechanisms underlying these effects are poorly understood. This was the first study to look specifically at the effects of ractopamine on the transcriptional regulation of a contractile protein in myotube cultures. Our model was sufficient to evaluate the anabolic effects of ractopamine in porcine skeletal muscle. However, we were unable to identify a cis-acting DNA element within the cloned porcine skeletal α-actin promoter that conferred transcriptional responsiveness to ractopamine in porcine skeletal muscle. Thus, it appears that ractopamine promotes the accumulation of skeletal α-actin mRNA in porcine skeletal muscle via a post-transcriptional mechanism. Elucidation of the mechanism whereby ractopamine increase skeletal muscle growth will aid in the development of new strategies, both genetic and pharmacological, to improve lean tissue deposition in livestock species.

Introduction

Ractopamine, as well as other β-adrenergic agonists, stimulate muscle hypertrophy *in vivo*. In porcine skeletal muscle, ractopamine positively augments net protein accretion, as fractional protein synthesis increases with no apparent change in proteolysis. Similarly, Skeletal α-actin mRNA abundance in pigs and steers increases in response to ractopamine, which indicates that the actin promoter may be used as a reporter to understand the mechanism whereby ractopamine regulates skeletal muscle growth.

Materials and Methods.

Porcine satellite cells were isolated from three-week-old gilts. Briefly, hindlimb muscles (180 to 200 g) were mechanically dissociated and digestion with bacterial protease, followed by differential centrifugation. Satellite cells were then plated in growth media on culture dishes coated with Matrigel and cultured standard cell culture conditions.

At 85 to 90% confluence, satellite cells were stimulated to differentiate into myotubes for 36 hours by the addition of differentiation media. This regime consistently produced cultures in which myotube nuclei, defined as more than three nuclei per cell, accounted for 75.57 ± 3.31% of the total nuclei (or %fusion). Treatment media consisted of Ractopamine (10 μM in H_2O) or volume equivalent vehicle control (H_2O) in serum-free differentiation media.

After 72 hours in treatment media, total RNA was harvested with TRIzol reagent and used for first-strand cDNA synthesis Quantitative PCR analysis was conducted with a Smart Cycler (Cepheid, Sunnyvale, CA) and SYBR Green I dye (Applied Biosystems, Foster City, CA) in order to determine the relative expression levels of skeletal α-actin and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) in primary porcine myotubes.

In order to evaluate the transcriptional regulation of porcine skeletal α-actin we used a reporter construct that contained 1929 bp of 5' flanking sequence, the first exon and 188 bp of the first intron fused to the reporter gene luciferase. Transient transfection procedures were utilized to introduce luciferase constructs into primary cell cultures. Transfected cells were stimulated to differentiate and were treated with ractopamine (10 μM) in serum-free media as described above.

Data was analyzed by one- or two-way analysis of variance (ANOVA) with JMP software (Release 5.0, 2001; SAS Institute Inc., Cary, NC). When main effects were significant, least squared means (LSM) differences were determined by Tukey's HSD procedure, *post hoc*. Least squared means and the standard error of the mean are reported. Comparisons were significantly different at the level of $P < 0.05$.

Results.

After 72 hours in SFDM supplemented with ractopamine, skeletal α-actin mRNA abundance increased more than five-fold (5.374 ± 1.230 vs. 1.000 ± 0.1146; $P < 0.05$) in porcine myotubes relative to untreated control cultures (Figure 1). In contrast, the skeletal α-actin promoter was unresponsive to ractopamine (Figure 2).

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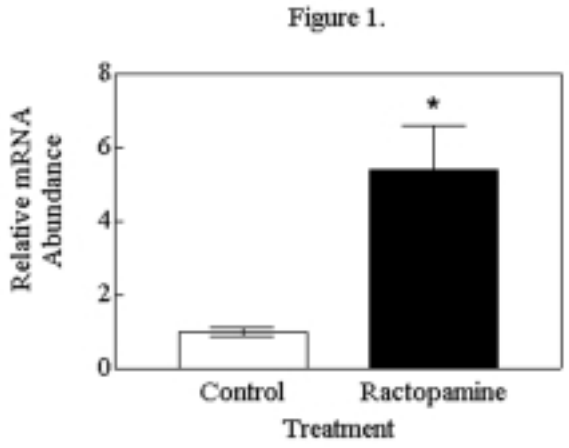

Figure 1. Detection of relative skeletal-α-actin mRNA in ractopamine stimulated (_) and control (_) porcine myotube cultures as determined by real-time quantitative RT-PCR. For skeletal α-actin, mRNA transcript abundance was quantified by the comparative threshold cycle (Ct) method as described in materials and methods. Data represent lsmeans ± SEM relative to the control mean. Asterisk indicates a ractopamine effect ($P < 0.05$)