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The effect of combination treatment with trenbolone acetate and estradiol-17β on skeletal muscle expression and plasma concentrations of oxytocin in sheep

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Abstract

Implantation of trenbolone acetate (TBA) in conjunction with estradiol-17β (E_2) increases growth, feed conversion efficiency, and carcass leanness in cattle. Our previous study in Brahman steers suggested that the neuropeptide hormone oxytocin (OXT) may be involved in increasing muscle growth after TBA-E_2 treatment. The present study aimed to determine whether *OXT* mRNA expression in the longissimus muscle (LM) is also up-regulated in TBA-E_2-implanted wethers as has been found in steers. Real-time quantitative PCR was used to measure the expression of the gene encoding the OXT precursor, three genes with increased expression in the LM muscle of TBA-E_2-treated steers, *MYOD1* (muscle transcription factor), *GREB1* (growth regulation by estrogen in breast cancer 1), and *WISP2* (Wnt-1 inducible signaling pathway protein 2), and two genes encoding IGF pathway proteins, *IGF1*, *IGFR*, in the LM of both untreated and TBA-E_2-treated wethers. The expression of *OXT* mRNA in wethers that received the TBA-E_2 treatment was increased ~4.4-fold ($P = 0.01$). TBA-E_2 treatment also induced a 2.3-fold increase in circulating OXT ($P = 0.001$). These data, together with the observation that untreated wethers had much higher baseline concentrations of circulating OXT than previously observed in steers, suggest that wethers and steers have quite different OXT hormone systems. TBA-E_2 treatment had no effect on the expression of *IGF1*, *IGFR*, and the muscle regulatory gene *MYOD1* mRNA levels in wethers ($P \geq 0.15$), but there was an increase in the expression of the two growth-related genes, *GREB1* ($P = 0.001$) and *WISP2* ($P = 0.04$). Both genes are common gene targets for both the estrogen and androgen signaling pathways. Consequently, their actions may contribute to the positive interaction between TBA and E_2 on additive improvements on muscle growth.

Keywords: Cattle; Estradiol; Gene expression; Oxytocin; Sheep; Trenbolone acetate

1. Introduction

Hormone growth promotant (HGP) implants containing a combination of the steroids trenbolone acetate (TBA) and estradiol-17β (E_2) are highly effective at improving growth rate and feed efficiency and increasing carcass leanness in cattle [1,2]. Previously, we investigated the effect of TBA-E_2 implants on the expression levels of genes in the longissimus muscle

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Table 1
Effects of TBA-E_2 implant on growth performance and carcass traits.

Trait	Treatment	
	Control	TBA-E_2
Initial BW, kg	37.1 ± 0.7[a]	37.1 ± 0.6
Final BW, kg	62.5 ± 2.2	69.2 ± 2.4[b]
ADG, kg	0.3 ± 0.02	0.38 ± 0.03[c]
HCW, kg	35.3 ± 0.9	36.8 ± 2.4
Loin weight, g	511.9 ± 28.3	515.7 ± 28.3
IMF, %	7.6 ± 0.3	6.9 ± 0.3
GR-fat[d], mm	29.1 ± 1.3	26.0 ± 1.4[e]

Abbreviations: HCW, hot carcass weight; IMF, intramuscular fat.
[a] Values are means ± SEM of nine lambs.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] Fat depth at 110 mm from the midline over the 12th rib.
[e] $P < 0.05$.

(LM) of Brahman steers [3]. The expression of the gene encoding the peptide hormone oxytocin (OXT) precursor in the LM was substantially increased (~97-fold) in TBA-E_2-treated steers. In addition, the concentration of circulating OXT was substantially increased (~50-fold) in treated steers compared with the untreated controls. Together with the observation that the expression of the gene encoding the OXT precursor is also increased in the LM of cattle in the late stages of fetal development, these results suggested that OXT may have a role in muscle growth both during fetal development and postnatally in TBA-E_2-treated animals [3]. In contrast, at the level of gene expression little evidence for a major role of intramuscularly synthesized IGF1 or changes in protein turnover in the muscle of TBA-E_2-treated steers was observed [3].

In this study, we investigated whether *OXT* mRNA expression in skeletal muscle (LM) and circulating OXT concentrations are as responsive to TBA-E_2 treatment in wethers as they are in steers.

2. Materials and methods

2.1. Animals, experimental procedures and tissue collection

All animal procedures in this study were approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Armidale Animal Experimentation Ethics Committee (Approval no. 09/29). Eighteen 7-mo-old, castrated male (wether) lambs (White Suffolk × Merino cross) with average initial BW of 37.1 kg (Table 1) and similar BCS were used in this study. During the experiment (85 d during May to August 2010), lambs were housed at F.D. McMaster Laboratory, Chiswick, animal house facility (30° S, 151° E and altitude of 1,046 m) and supplied with CSIRO sheep pellets (14% to 17% CP, 8.0 MJ/kg ME), wheaten chaff and water, ad libitum. On arrival at the animal house, the lambs were dosed orally with effective anthelmintics to remove helminth parasites and given 2 wk to acclimatize before the start of the experiment. On the first experimental day (0 d) animals were weighed individually, randomly assigned to one of the two treatment groups (nine animals per group), and tagged with unique numbered ear tags and group number. Groups included a nonimplanted control group and a treated group (implanted in the ear according to the manufacturer's instructions with ~60 mg TBA and ~6 mg E_2; Revalor-H [Coopers Animal Health]). The dose of the anabolic agent was adjusted accounting for the BW of lambs (compared with cattle) to give a similar metabolic effect. Three Revalor-H pellets (ten pellets are used for cattle) were repacked in the cassette and administered subcutaneously into the back of the ear with the use of an automatic implant gun. Immediately after implantation, the lambs were penned according to their treatment (one pen per treatment) and fed together in each pen. For this study the animals were pen-rotated every 3 wk to minimize pen effect. Individual BW was recorded every 21 d.

On day 83, the lambs were weighed (final BW) and on days 84 to 85 they were slaughtered at F.D. McMaster Laboratory with the use of conventional humane procedures. Carcasses were weighed, and ~50 g of tissues from the LM was collected within 30 min of slaughter. Strip loins (sixth to ninth rib) were collected from the right sides of the carcasses for meat quality analyses. The depth of fat was measured with a GR knife at the 12th rib and 110 mm perpendicular to the midline of the muscle and known as the GR site (GR-fat, mm). Percentage of intramuscular fat (IMF%) was determined on each sample in duplicate following established methods [4]. In addition, a transverse section of the longissimus muscle between the 12th and 13th rib was taken from each animal for gene expression analyses. We ensured that the sampling site was standardized for each muscle to avoid sampling-induced variation. The tissues were immediately frozen in liquid nitrogen and subsequently stored at −80°C.

2.2. Blood sampling

Blood samples (10 mL) were taken by jugular venipuncture on days 0, 21, 42, 63, and 83 and collected into lithium heparin anticoagulant Vacutainers (Becton Dickinson, Australia-New Zealand). Samples were immediately cooled in ice, and plasma was harvested by