

Beta-Adrenergic receptor agonist modulation of skeletal muscle growth

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ABSTRACT: Mechanisms by which ractopamine and other beta-adrenergic agonists stimulate skeletal muscle growth are discussed. Oral administration dose-response studies in surgically altered laboratory animals provide evidence that indirect endocrine-mediated effects are not an essential component of efficacy. Results from age-comparison studies in laboratory animals and livestock species provide evidence that metabolic maturity of skeletal muscle may be a critical factor with regard to efficacy, suggesting that receptor presence and density are important. Temporal studies demonstrate the rapidity of responses associated with protein and lipid metabolism changes, and that progressive decline in rate of anabolic response in skeletal muscle results from chronic administration. Associated results that demonstrate progressive beta-adrenergic receptor density reductions are observed and suggest, likewise, that protein accretion rate and muscle growth rate responses are receptor-mediated. Measurement of in vivo

metabolic effects resulting from continuous systemic infusion has been conducted in relatively few experiments. Detailed blood flow and hind limb net flux data are available for a single beta-agonist, cimaterol. Kinetics studies and close arterial infusion of cimaterol in the hind limb of growing cattle demonstrate large transient increases in amino acid extraction from the circulation and similar patterns of net uptake when compared with the contralateral control saline-infused hind limb. Predictions of differential net effects on protein accretion using integration of essential amino acid net flux measurements are corroborated by quantitative documentation of protein mass differences in individual muscles from treated and control hind limbs. Definitive descriptions of specific pathway mechanism(s) of action for increasing protein synthesis have not as yet been reported. Therefore, additional research is required for elucidation of cellular and intracellular components of mechanism(s) of action.

Key Words: Beta-Adrenergic Agonists, Growth, Muscles

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Introduction

Rapid preferential increase in skeletal muscle protein mass is the most striking response to oral administration of select phenethanolamines in laboratory and farm animal species. Dose-dependent increases in weight of individual muscles have been observed in rapidly growing rats, mice, rabbits, lambs, cattle, pigs, chickens, and turkeys (Anderson et al., 1991; Moloney et al., 1991). Although up to 40% greater weight of muscles has been observed in treated sheep and cattle, magnitude of response varies greatly among phenethanolamines studied and is influenced by age, species, sex, diet, breed, time to reach plateau, and other factors (Beermann, 1993; Mersmann, 1998). Percentage of weight or protein mass increase also varies somewhat from muscle to muscle, and in a few instances a significant response was not observed in a small number of muscles. Because these compounds reduce body fat

without altering organ or bone mass, they are also referred to as repartitioning agents. These repartitioning phenethanolamines are classified as beta-adrenergic receptor (β AR) agonists because they share structural similarities and pharmacological properties with the endogenous catecholamines epinephrine and norepinephrine, and many of their metabolic or physiological responses can be reduced or blocked by β AR antagonists (Barnes, 1995). The objectives of this paper are to highlight our current state of knowledge regarding the mechanisms underlying β AR agonist effects on skeletal muscle growth and to identify key areas in which new research efforts are needed. Results from studies in several species provide clarification of effects on muscle fiber histology and histochemistry and patterns of RNA, DNA, and protein accretion. Inferences are drawn from these results for effects on cellularity and cellular metabolic changes. Influences on aspects of protein synthesis and degradation, as they pertain to protein accretion in muscle growth, are also described.

Indirect vs Direct Actions

The complexity of hormonal influences on skeletal muscle growth make it necessary to separate possible

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indirect, endocrine-mediated actions of β AR agonists from direct, β AR-dependent mechanisms of action. Indirect modes of action of β AR agonists include possible perturbations in circulating concentrations, sensitivity, and(or) responsiveness to hormones known to influence skeletal muscle growth. Surgically altered animal models that exhibit lack of specific metabolic hormones have provided additional insight. Assessment of direct β AR-mediated effects on skeletal muscle includes close (direct) arterial infusion of β AR agonists and saline in contralateral hind limbs, pretreatment or cotreatment with general and β AR subtype-specific receptor antagonists in vivo, and in vitro incubation of agonists and antagonists in myoblast, satellite cell, and myotube cultures. Variation in results between in vitro and in vivo studies has provided equivocal results, however. Several lines of evidence support involvement of β AR in mediation of muscle growth responses. Selectivity and binding kinetics of phenethanolamines for β AR subtypes have been demonstrated (Colbert et al., 1991; Spurlock et al., 1994; Smith et al., 1990). Temporal changes in down-regulation or desensitization of beta-adrenergic receptors is observed with chronic β AR agonist administration (Smith, 1989). Myoblast and satellite cell culture studies provide demonstration of activation of β AR signal transduction pathways (Shappell et al., 2000), and blockage of responses with nonselective and selective antagonists implicate β AR dependence (Reeds et al., 1988; MacLennan and Edwards, 1989). Use of more recent technologies to create knockout and receptor-modified animal models provides strong evidence for receptor-mediated mechanisms.

Effects on Muscle Cellularity

The anabolic effects of β AR agonists on muscle include muscle fiber hypertrophy, changes in muscle fiber type frequency, and differential rates of muscle RNA, DNA, and protein accretion. No evidence of muscle fiber splitting or increase in fiber number has been reported. Percentage increases in average fiber cross-sectional area are similar to percentage increases in muscle weight in lambs (Beermann et al., 1986; Kim et al., 1987) and rats (Maltin et al., 1986), although some changes in fiber type distribution have been observed. In general, the increase in hypertrophy of type II fibers accounts for the increase in muscle mass without any quantitative change in muscle length. Similar changes have been observed in pigs fed 20 ppm ractopamine (Aalhus et al., 1992). No in vivo increase in myotube or muscle fiber number has been reported in β AR agonist-treated animals. Lack of response in very young nursing lambs (Williams, 1989) and other species suggests that either muscle fibers lack sufficient β AR number or function to respond early in life or that fractional growth rate in very young muscle fibers is at the maximum.

Results of temporal studies show that 25% increases in RNA concentration and 85% increase in total mass of RNA occurred in parallel with 30% increases in the

total weight and protein content of hind limb muscles in lambs fed 10 ppm cimaterol for 3 wk (O'Connor et al., 1991b). During this same time interval DNA concentration was reduced by 42% and total DNA content of the same muscles was unchanged. After 6 wk of treatment, differences in RNA concentration and content disappeared, but muscle weight and protein content remained 25% higher in treated lamb muscle. DNA concentration remained 25% less. These results suggest that stimulation of satellite cell proliferation and incorporation into growing muscle fibers is not an essential component of fiber hypertrophy. Although ractopamine has been shown to elicit a 30% increase in proliferation rate, and in protein and DNA concentrations in cultured mouse C₂C₁₂ myoblasts (Shappell et al., 2000), this in vitro effect is lost after successive passages of the cells, and ractopamine failed to increase DNA or protein in myotubes derived from the C₂C₁₂ cells. Grant et al. (1990) observed an increase in proliferation, but not fusion, of chick breast muscle satellite cells incubated with ractopamine. These results were confirmed in studies using turkey satellite cells (McFarland et al., 1995; Shappell et al., 2000) and porcine satellite cells (Cook et al., 1995). Shappell et al. (2000) concluded "that in turkeys, ractopamine may not work directly through the β -adrenergic receptor of muscle cells (and c-AMP) to stimulate changes in muscle carcass traits, but possibly through extramuscular effects, such as increased blood flow and amino acid uptake by muscles." In summary, in vitro studies fail to provide substantial evidence in support of β AR-mediated stimulation of satellite cells as a means by which muscle hypertrophy is initiated by feeding β AR agonists in growing animals.

Indirect Effects of β AR Agonists

Stimulation of skeletal muscle growth by β AR agonists may involve modulation of normal endocrine influences on growth and metabolism. Many studies were conducted to investigate these possibilities. Feeding cimaterol to growing lambs increased growth hormone and T₄ concentrations and decreased IGF-I concentrations after 6 wk (Beermann et al., 1987). In subsequent studies, no change in IGF-I, decreased T₄, and markedly decreased (50%) insulin concentrations were observed after 3 wk of treatment (O'Connor et al., 1991a). Young et al. (1995) also reported no change in IGF-I following chronic administration of clenbuterol to sheep. In cattle fed cimaterol, an acute decrease in growth hormone concentration was followed by a chronic increase in growth hormone and a decrease in IGF-I (Chikhou et al., 1991). Dawson et al. (1993) observed no significant differences in growth hormone or IGF-I concentrations in steers fed cimaterol. The normal episodic pattern of growth hormone secretion was absent in the steers fed cimaterol, however. A similar result was observed in lambs fed a high-energy diet with or without cimaterol administration (Beermann, unpublished data).

Anderson et al. (1991) suggested that the repartitioning effects of phenethanolamines may be due in part to opposing effects on insulin sensitivity in adipose tissue vs skeletal muscle. Ractopamine reduced sensitivity to insulin of adipocytes from rats (Hausman et al., 1989) and pigs (Liu and Mills, 1990). Budohoski et al. (1987) observed increased sensitivity to insulin in soleus muscle receiving chronic treatment with β AR agonists. Beermann (1987) noted no change in sensitivity to insulin in lamb hindquarters, using insulin challenges, despite a 50% reduction in circulating insulin concentrations. More refined studies using the hyperinsulinemic and euglycemic clamp techniques in cattle demonstrated a transient (Eisemann and Bristol, 1998) and chronic (Sternbauer et al., 1998) decrease in insulin sensitivity with clenbuterol administration. Although results available to date do not support significant changes in insulin sensitivity as the mechanism by which skeletal muscle growth is stimulated by β AR agonists, more definitive studies are required to clarify these relationships in muscle.

The anabolic response of muscle in endocrine-altered animals to β AR agonist administration adds support for mechanism(s) independent of hormone modulation. Muscle growth is significantly increased in animals with genetic growth hormone deficiency (Bates and Pell, 1991), in hypophysectomized rats (Thiel et al., 1987), and in diabetic (McElligot et al., 1987), adrenalectomized (Buttery and Dawson, 1987), and hypothyroid rats (Beermann, unpublished data). Additive effects of clenbuterol and growth hormone have been demonstrated in veal calves (Maltin et al., 1990). Likewise, cotreatment of pigs with salbutamol and growth hormone (Hansen et al., 1997) and ractopamine and growth hormone (Jones et al., 1989) resulted in additive responses, suggesting separate mechanisms of action account for the independent effects. In summary, indirect effects of β AR agonists, especially effects on endocrine-mediated influences on muscle growth, appear to be negligible in accounting for the hypertrophic responses. Evidence in support of this conclusion is found in analysis of results from studies in which direct β AR-mediated effects were investigated.

Direct Effects of β AR Agonists

Close arterial infusion of cimaterol is a novel and appropriate model for differentiating systemic and local (direct) effects of cimaterol on skeletal muscle metabolism and protein accretion in the hind limb of cattle (Byrem et al., 1998). Close arterial infusion is the direct, continuous infusion of a compound into the arterial circulation of an organism to achieve a local elevated concentration of the compound relative to its concentration in the systemic circulation. Pharmacokinetic analysis of cimaterol administration in steers demonstrated that a 10-fold elevation in concentration in the hind limb is theoretically possible (Byrem et al., 1992), and an appropriate dose was identified in a dose-response

study (Byrem et al., 1996). Close arterial infusion of cimaterol for 21 d increased the rate of blood flow and extraction of essential amino acids from the circulation in the hind limb in a transient manner. Uptake of amino acids by the hind limb exhibited a large acute increase, followed by a gradual continuous increase to a maximum of 160% of controls on d 14, and then a decline during the last 7 d of infusion. Fractional rates of protein accretion were 61 and 130% higher in cimaterol-infused hind limbs on d 7 and 14, respectively. Calculation of cumulative net protein accretion rates in the treated and contralateral saline-infused hind limb predicted 10 to 15% differences in protein mass of the semitendinosus and semimembranosus muscles, respectively. These differences were confirmed with proximate analysis of the muscles taken on d 21 of the experiment. Close arterial and systemic infusion of clenbuterol in sheep provided similar responses (Aurousseau et al., 1993) and confirm results obtained from cattle fed clenbuterol in which net flux across the portal-drained viscera, the liver, and hindquarters was studied (Eisemann and Huntington, 1993).

The other approach taken by many to assess direct β AR-mediated effects of beta agonists is to demonstrate dependence on β AR functionality in relation to a variety of response variables (Barnes, 1995). At present there are three subtypes of β AR, designated β_1 , β_2 , and β_3 (Liggett and Raymond, 1993). Distribution and density of β AR subtypes in mammalian tissues is known for several laboratory animal species (Minneman et al., 1979), but as mentioned earlier, few studies provide information for livestock species (Smith, 1989; Spurlock et al., 1994; Bridge et al., 1998).

Pretreatment and cotreatment of animals with general or selective β AR antagonists proved effective. Reeds et al. (1988) used propranolol, a nonselective β_1 and β_2 antagonist, and alenolol (β_2 -specific) in combination with clenbuterol feeding in rats. Dosages of the antagonists were 10 and 100 times the concentration of clenbuterol. Effects of clenbuterol on heart weight, fat deposition and energy expenditure were reduced with the antagonists, but the muscle hypertrophy response was not. Similar results were obtained by MacLennan and Edwards (1989) when similar doses of clenbuterol and propranolol were orally administered to rats. Muscle growth response was reduced in an additional treatment group that received intraperitoneal injection of propranolol with subcutaneous administration of clenbuterol. Blockage of the muscle growth response provided evidence for involvement of the β AR. Unfortunately, these experiments do not reveal which cell types the β AR are active in. Therefore, these results do not directly differentiate direct β AR-mediated and indirect actions or effects.

Choo et al. (1992) conducted similar studies in which the β_2 -specific antagonist ICI-118,551 blocked the anabolic responses of clenbuterol in rats. To date, similar studies have not been reported for livestock species, but other evidence for β AR dependence is available.

These include quantitative measurement of cAMP response in skeletal muscle slices, cultured myoblasts, and myotubes (Sillence and Matthews, 1994; Shappell et al., 2000) and documentation of desensitization or down-regulation of β AR in muscle tissue following chronic exposure to β AR agonists (Smith, 1989; Spurlock et al., 1994).

Effects of β AR Agonists on Protein Synthesis and Degradation

Early investigations failed to provide evidence for increased fractional rates of protein synthesis during significant elevations in protein synthesis rates in rats (Reeds et al., 1986) or sheep (Bohorov et al., 1987) fed clenbuterol, leading to the suggestion that reduction in protein degradation rates accounted for the muscle growth observed. Similar conclusions were reported by MacRae et al. (1988), but the possibility exists that increases in fractional protein synthesis rates are transient. This is supported by the steady increase from 1 to 14 d, and then a dramatic decline during the subsequent 7 d in net amino acid flux across the hind limb in cattle treated by the close arterial infusion of cimaterol (Byrem et al., 1998). Using the Snell Dwarf mouse as a sensitive model, Bates and Pell (1991) demonstrated that clenbuterol and growth hormone both increased whole-body and muscle fractional protein synthesis rates. They noted, however, that growth hormone treatment exhibited a larger response. Bergen et al. (1989) observed an increase in fractional protein synthesis rate from 4.6 to 6.7%/d in muscle of pigs fed 20 ppm ractopamine. Similar results were observed by Culham et al. (1990) using labeled lysine instead of tyrosine.

Alternative measures that support an increase in protein synthesis include measurement of mRNA abundance for myofibrillar proteins such as actin, myosin, and so on. Several investigators using this approach observed significant increases in response to feeding phenethanolamines. α -Actin mRNA abundance was increased by feeding ractopamine to pigs (Bergen et al., 1989; Helferich et al., 1990; Grant et al., 1993). Increased α -actin mRNA abundance was also observed in muscle of sheep fed the beta-agonist L-644,969 (Koochmaraie et al., 1991). Smith and co-workers observed elevated myosin mRNA abundance in cattle fed clenbuterol and ractopamine (Smith et al., 1989; 1995). These results are interpreted as evidence for increased rate of transcription in muscle or as increased stability of RNA that would lead to greater rates of mRNA synthesis in muscle fibers. Whether these events are linked to protein kinase A activation via cAMP is not known.

Protein degradation is usually determined by differences between measured fractional protein accretion and synthesis rates, rates of urinary 3-methyl histidine excretion, or by measurement of protease activities in muscle tissue (Goll et al., 1998). Common among results of many studies is the indication that muscle protein degradation may be reduced or not affected in animals

administered phenethanolamines that enhance muscle growth rate. Examples include total body and urinary excretion of 3-methyl histidine, indicative of a 25% reduction in fractional degradation rates in rats fed cimaterol for 7 d (Eadara et al., 1989); a 27% reduction in fractional degradation rate of skeletal muscle proteins in steers fed L-644,969 (Wheeler and Koochmaraie, 1992), increased calpastatin mRNA in muscle of steers receiving close arterial infusion of cimaterol (Sun et al., 1994), and elevated calpastatin activity in sheep and bovine muscle of animals fed the beta-agonist L-644,969 (Kretchmar et al., 1990; Koochmaraie et al., 1991; Wheeler and Koochmaraie, 1992; Pringle et al., 1993; and Killefer and Koochmaraie, 1994). Similar effects were reported by Bardsley et al. (1992) and Parr et al. (1992). Ractopamine-fed pigs fail to exhibit increased calpastatin activity or mRNA, however (Ji et al., 1991). Because accurate direct methods for measurement of protein degradation rates are not available, definitive conclusions regarding effects of repartitioning phenethanolamines cannot be made at this time.

Implications

A rapid increase in fractional rate of muscle protein synthesis occurs with oral administration of β -agonists, and some, but not all (ractopamine), may reduce fractional protein degradation rates. This appears to be a receptor-mediated response, rather than an endocrine-dependent one. A critical area for research is the need to identify specific β -receptor subtypes in muscles of livestock species and determine their role in growth enhancement. Subtype-specific agonists and antagonists must be used to investigate mechanism(s) of regulation for these phenethanolamines. New technologies such as creation of β -receptor subtype gene knockout animals, use of specific antibodies to β -receptor subtypes, and use of transgenic approaches to alter their function, or their expression, will reveal the details of the mechanisms by which these repartitioning phenethanolamines enhance muscle growth and reduce fat accretion.

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