EFFECT OF BETA AGONISTS ON PROTEIN TURNOVER IN ISOLATED CHICK SKELETAL AND ATRIAL MUSCLE

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Abstract: Various β-adrenergic agonists were found to inhibit rates of protein degradation and net protein breakdown in isolated chick extensor digitorum communis (EDC) and atrial muscles. Rates of protein synthesis were not altered by these compounds. The β-agonist cimaterol inhibited rates of protein degradation in EDC muscles incubated with or without amino acids and insulin. Cimaterol also inhibited the increased proteolysis induced by injury to muscle or by incubating muscles at body temperature (42°C) versus 37°C. Thus, β-agonists may help promote skeletal muscle accretion *in vivo* even under conditions of severe negative nitrogen balance by slowing muscle proteolysis.

Introduction

In 1977, Li & Jefferson (1) showed that the β-agonist isoproterenol inhibited protein degradation in perfused rat hemicorpus by 20%, with no apparent effect on the rate of protein synthesis. Since then, administration of β-adrenergic agonists to domestic animals has been shown to promote growth of skeletal muscle (2,3).

For muscle to increase in size, the rate of protein synthesis must exceed that of protein degradation. In animals fed a diet supplemented with the synthetic β-agonists clenbuterol (4) or cimaterol (5), a decrease in the fractional rate of protein degradation (FDR) was observed. While these investigators reported no change in rates of muscle protein synthesis, other studies observed β-agonists to elevate the fractional rate of protein synthesis (FSR) in rat cardiac (6) and skeletal muscle (6-8) and in denervated rat soleus muscles (8,9). Hence, whether β-agonists increase muscle mass by stimulating protein synthesis and/or inhibiting protein degradation remains controversial. The studies presented here examine the effect of β-adrenergic agonists on protein turnover in isolated chick skeletal and atrial muscle.

Materials and Methods

Male Single-comb White Leghorn and Peterson-Arboracre chicks (Avian Services, Frenchtown, NJ) were provided with water and feed (Agway Country Broiler Maker; Flemington, NJ) *ad libitum*. Peterson-Arboracre chicks (2- to 3-wk old) were killed by cervical dislocation and intact beating hearts were removed and immediately submersed in saline to pump out excess blood within the chambers. Left atria were removed intact, bisected, blotted and weighed. White Leghorn chicks (2- to 3-wk old) were killed by cervical dislocation and intact extensor digitorum communis (EDC) muscles (15-30 mg) were dissected from tendon to tendon, blotted and weighed.

In some experiments, EDC muscles were incubated in a flaccid state or at resting length by pinning the tendons with stainless steel 30G 1/2 needles to Tygon tubing supports. After a 30-60 min preincubation at 37°C in 3 ml Hepes buffer equilibrated with O_2/CO_2 (19:1) (10), EDC muscles or atria were incubated for an additional 2h in fresh buffer. Except where indicated, muscles were preincubated and incubated in the presence or absence of β-agonist in buffer containing glucose (5 mM), insulin (0.1 U/ml) and all of the essential and nonessential amino acids (except tyrosine) at concentrations reported for rat plasma (11). Isoproterenol-HCl was purchased from Sigma Chemical Co. Cimaterol was a gift from C. Ricks, American Cyanamid Co. (Princeton, NJ). Clenbuterol was kindly provided by Dr. Karl Thomae of Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, CT).

Protein degradation was estimated from the amount of tyrosine released from muscle protein in the presence of cycloheximide (0.25 mM), an inhibitor of protein synthesis (12). Net protein breakdown was measured by the amount of tyrosine released from muscle protein in the absence of cycloheximide. Tyrosine in the medium and in the tissue (to determine tissue tyrosine pools) was measured by a standard fluorometric procedure (13). Protein synthesis was estimated by measuring the amount of [U-^{14}C] phenylalanine (ICN Radiochemicals, Costa Mesa, CA) (0.5 mM; 0.1 mCi/mmol) incorporated into muscle protein during the incubation period (12).

Results and Discussion

Effect of β-adrenergic agonists on protein turnover in skeletal muscle. Isolated EDC muscles were incubated under tension with 10 μM cimaterol, clenbuterol or isoproterenol in the presence of cycloheximide to determine the effect of these compounds on *in vitro* rates of protein degradation. These β-agonists signi-

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Table I. Effect of β-Agonists on Protein Degradation in Isolated Chick Skeletal Muscle[a]

Additions	Protein degradation (pmol Tyr/mg/2 hr)		
	None	+β-Agonist	Difference[b]
Cimaterol	194 ± 10	178 ± 10	−16 (−8%)
Clenbuterol	225 ± 10	201 ± 5	−24 (−11%)
Isoproterenol	203 ± 11	187 ± 14	−16 (−8%)

[a] Contralateral EDC muscles were incubated at 37°C under tension in buffer containing plasma concentrations (11) of all essential and nonessential amino acids (except for the omission of tyrosine and the addition of 0.5 m*M* phenylalanine) and insulin (0.1 units/ml) in the absence or presence of 10 μ*M* β-agonist. Data represent the means ± SE for six to eight muscles per incubation condition.
[b] The effect of the β-agonists were analyzed by the paired Student's *t* test where $P \leq 0.05$.

Table II. Effect of Cimaterol on Net Protein Breakdown and Protein Synthesis in Isolated Chick Skeletal Muscle[a]

Incubation additions	Net protein breakdown (pmol Tyr/mg/2 hr)	Protein synthesis (pmol Phe/mg/2 hr)
None	62 ± 7	167 ± 5
Cimaterol (1 μ*M*)	50 ± 7	164 ± 5
Difference	−12 (−20%)[b]	NS
None	65 ± 7	174 ± 7
Cimaterol (10 μ*M*)	49 ± 6	168 ± 6
Difference	−16 (−25%)[b]	NS
None	61 ± 5	167 ± 4
Cimaterol (100 μ*M*)	40 ± 4	162 ± 4
Difference	−21 (−34%)[b,c]	NS

[a] Contralateral EDC muscles were incubated in a flaccid state at 37°C in buffer containing amino acids (as described in Table I), but containing no insulin, in the absence or presence of cimaterol. Data represent the mean ± SE for eight muscles per incubation condition.
[b] The effects of the β-agonist were analyzed by the paired Student's *t* test, in which $P \leq 0.05$ and NS = not significant ($P > 0.05$).
[c] The effects due to the dose of cimaterol were compared by the unpaired Student's *t* test, in which $P \leq 0.05$ as compared with 1 μ*M*.

ficantly decreased the rate of tyrosine released into the incubation buffer by 8-11% (Table 1), but did not affect tissue tyrosine pools (data not shown). The decrease in tyrosine released into the medium therefore reflects an inhibition by β-agonists in the rate at which muscle proteins are hydrolyzed to amino acids.

To determine the effect of β-agonists on net protein breakdown and protein synthesis, EDC muscles were incubated in buffer supplemented with glucose and amino acids in the presence of varying concentrations of cimaterol. Insulin was excluded from the incubation media because it is known to stimulate rates of protein synthesis in muscle in vitro (12). Cimaterol (1-100 μM) had no affect on rates of protein synthesis, but cimaterol did inhibit rates of net protein breakdown by 20-34% depending on the concentration of cimaterol added to the buffer (1, 10 and 100 μM) (Table 2). The inhibition of net protein breakdown by 100 μM cimaterol was significantly ($p<0.05$) greater than the inhibition seen when EDC muscles were incubated in the presence of 10 μM cimaterol. Higher concentrations of cimaterol (200 and 400 μM) inhibited rates of protein degradation further (14.9 and 21.6%, respectively) as compared to the inhibition (10.4%) seen at 100 μM (data not shown). These results indicate that β-agonists affect the breakdown of muscle proteins in a dose-dependent manner.

To determine if β-agonists affect protein turnover in cardiac muscle, the left atria from Peterson-Arboracre chicks was bisected and incubated in the absence of presence of 100 μM cimaterol. Cimaterol inhibited rates of net protein breakdown by 21% (Table 3), while rates of protein synthesis remained unaffected (data not shown). In the presence of cycloheximide, cimaterol also inhibited rates of protein degradation in chick atria by 24% (Table 3). Intracellular tyrosine pools were unchanged following incubation of chick atria with cimaterol (Table 3). Thus, like its effect on isolated chick skeletal muscle, cimaterol inhibits the rate of breakdown of muscle proteins in isolated chick atria without affecting rates of protein synthesis in vitro.

Effect of incubation conditions, temperature, muscle injury and tension. Different incubation conditions are known to alter rates

Table III. Effect of Cimaterol on Protein Breakdown in Isolated Chick Atria[a]

	− Cimaterol	+ Cimaterol	Difference
Net protein breakdown (pmol Tyr/mg/2 hr)	109 ± 5	86 ± 4	−23 (−21%)[b]
Protein degradation (pmol Tyr/mg/2 hr)	139 ± 3	106 ± 3	−33 (−24%)[b]
Tissue tyrosine pool (pmol Tyr/mg)	54 ± 2	54 ± 2	NS

[a] Left atria from 10 chicks were bisected and preincubated at 37°C in buffer containing amino acids and insulin as described in Table I. After 30 min, atria were transferred to fresh media, but containing 100 μ*M* cimaterol. In another group of chicks (n = 10), atria were incubated in the above buffers containing 0.25 m*M* cycloheximide to measure protein degradation. The tissue tyrosine pool was determined after the 2-hr incubation.
[b] Data represent the means ± SE and the effects of cimaterol were analyzed by the paired Student's *t* test, in which $P \leq 0.05$ and NS = not significant ($P > 0.05$).

of muscle proteolysis in mammals. Insulin has been shown by many investigators to be an important factor for regulating and maintaining positive protein balance in heart and skeletal muscle (12,14,15). In isolated skeletal muscle, the branched chain amino acids together or leucine alone can stimulate protein synthesis and inhibit protein breakdown (12, 16). The addition of both amino acids and insulin to the incubation media has been shown to decrease rates of protein degradation in a variety of tissues. Our results also show that amino acids and insulin decrease rates of protein breakdown in chick EDC muscles by approximately 10% (Table 4). Cimaterol, when added in the presence of amino acids and insulin, inhibited rates of protein degradation by 16% as compared to a 17% inhibition by cimaterol when muscles were incubated without amino acids and insulin. Protein synthesis, which was stimulated by up to 2-fold in chick EDC muscles by the addition of amino acids and insulin, was not affected by cimaterol either in the absence or presence of amino acids and insulin (data not shown). Therefore, the inhibitory effects of β-agonists on protein breakdown in isolated muscle do not appear to be blocked by or require the presence of amino acids and insulin in the incubation buffer.

Cimaterol inhibited muscle proteolysis whether or not muscles were incubated in a flaccid state or at resting length (Table 4). In the absence of β-agonists, rates of protein breakdown in chick EDC muscles incubated in a flaccid state were not significantly different from rates of proteolysis in muscles incubated at resting length (Table 4). Incubation of chick EDC muscles for 2-3h in a flaccid state versus at resting length also did not appear to affect rates of protein synthesis (data not shown). Incubation of isolated rat skeletal muscle at approximate resting length has previously been reported to inhibit muscle proteolysis (15,17) and elevate rates of protein synthesis (17). Chick EDC muscle differs from rat muscle in that rat soleus muscles incubated in a flaccid state were found to contract by up to 25% of their normal resting length (17), while chick EDC muscles maintain their resting length following incubation in a flaccid state.

A rise in incubation temperature from 37°C to 40°C significantly increases rates of protein degradation in rat skeletal muscle (18,19). A 36% increase in the rate of proteolysis was also seen when chick EDC muscles were incubated at 42°C (average avian body temperature) versus 37°C (Table 4). Cimaterol, when added to the buffer, inhibited rates of protein breakdown by 13-16% in muscles incubated at either temperature.

Proteolysis increases in isolated muscles that have been injured by cutting after removal from the animal (20). In chick skeletal muscle, rates of protein degradation increased 60-62% when two cuts were made (without severing the entire muscle) on either side of the belly of each EDC muscle (Table 4). The addition of cimaterol to the incubation media inhibited rates of protein breakdown by 10% in uncut and 9% in cut muscles. These results demonstrate that cimaterol inhibits muscle protein breakdown under a variety of incubation and muscle conditions.

We have shown that β-agonists inhibit in

Table IV. Effect of Cimaterol on Protein Breakdown in Muscles Exposed to Insulin, High Incubation Temperature, Injury, and Stretch[a]

Incubation condition	Protein degradation (pmol Tyr/mg/2 hr)		
	− Cimaterol	+ Cimaterol	Difference
Additions			
None	253 ± 9	210 ± 7	−43 (−17%)
Amino acids + Insulin	226 ± 4	190 ± 3	−36 (−16%)
Difference	−27 (−11%)[c]	−20 (−10%)[c]	
Temperature			
37°C	163 ± 9	142 ± 9	−21 (−13%)
42°C	221 ± 6	186 ± 8	−35 (−16%)
Difference	+58 (+36%)[c]	+44 (+31%)[c]	
Muscle condition			
Uncut	264 ± 15	236 ± 15	−28 (−10%)
Cut	422 ± 12	383 ± 7	−39 (−9%)
Difference	+158 (+60%)[c]	+147 (+62%)[c]	
Flaccid	251 ± 9	226 ± 9	−25 (−10%)
Stretch	228 ± 9	198 ± 10	−30 (−13%)
Difference	NS	NS	

[a] Contralateral EDC muscles were incubated in the absence or presence of cimaterol (100 μM). The incubation temperature and stretch experiments were done in the presence of amino acids and insulin, as described in Table I. Intact muscles (without stretch) versus the injured muscles cut partially across the belly of the muscle were incubated in the absence of amino acids and insulin. Some muscles were incubated at 37°C (average human body temperature) vs 42°C (average avian body temperature). Data represent the means ± SE for seven to eight muscles per incubation condition.
[b] The effects of the β-agonist were analyzed by the paired Student's t test, in which $P \leq 0.05$.
[c] The effects due to changes in the incubation or muscle condition were compared using the unpaired Student's t test, in which $P \leq 0.05$.

vitro rates of protein degradation and net protein breakdown in chick skeletal and atrial muscle, with no apparent effect on rates of protein synthesis. Our results indicate that these compounds inhibit rates of protein breakdown even when the muscle is in a state of severe negative nitrogen balance. These observations may help to explain the increase in skeletal muscle mass and possibly the cardiac hypertrophy observed in animal species treated with β-agonists (4,6). β-Agonists may also be a valuable tool in the management of pathological states complicated by muscle wasting such as poor nutritional status, cachexia, and muscular dystrophy. Clearly more research needs to be done to determine the various actions of this group of compounds and the mechanisms by which β-agonists alter body composition in animals.

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References Cited

1. Li JB, Jefferson LS. Effect of isoproterenol on amino acid levels and protein turnover in skeletal muscle. Am J Physiol 232:E243-E249, 1977.
2. Hanrahan JP, Quirke JF, Bomann W, Allen P, McEwan JC, Kotzian JM, Roche JF. Recent Advances in Animal Nutrition. London: Butterworth, 1986, pp. 125-138.
3. Wallace DH, Hedrick HB, Seward RL, Daurio CP, Convey EM. Beta-Agonists and Their Effects on Animal Growth and Carcass Quality. London: Elsevier Applied Science, 1987, pp. 143-151.
4. Reeds PJ, Hay SM, Dorwood PM, Palmer RM. Stimulation of muscle growth by clenbuterol: lack of effect on muscle protein biosynthesis. Br J Nutri 56: 249-258, 1986.
5. Morgan JB, Jones SJ, Calkins CR. Muscle protein turnover and tenderness in broiler chickens fed cimaterol. J Anim Sci 67:2646-2654, 1989.
6. Deshaies Y, Willemot J, Leblanc J. Protein synthesis, amino acid uptake, and pools during isoproterenol-induced hypertrophy of the rat heart and tibialis muscle. Can J Physiol Pharmacol 59: 113-121, 1980.
7. Emery PW, Rothwell NJ, Stock MJ, Winter PD. Chronic effects of β_2-adrenergic agonists on body composition and protein synthesis in the rat. Biosci Rep 4:83-91, 1984.
8. Maltin CA, Hay SM, Delday MI, Lobley GE, Reeds PJ. The action of the β-agonists clenbuterol on protein metabolism in innervated and denervated phasic muscles. Biochem J 261:965-971, 1989.
9. Zeman RJ, Ludemann R, Etlinger JD. Clenbuterol, a β_2-agonist, retards atrophy in denervated muscles. Am J Physiol 252:E152-E155, 1987.
10. Baracos V, Greenberg RE, Goldberg AL. Influence of calcium and other divalent cations on protein turnover in rat skeletal muscle. Am J Physiol 250: E702-E710, 1986.
11. Mallette LE, Exton JH, Park CR. Control of gluconeogenesis from amino acids in the perfused rat liver. J Biol Chem 244: 5713-5723, 1969.
12. Fulks RM, Li JB, Goldberg AL. Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. J Biol Chem 250:290-298, 1975.
13. Waalkes TP, Udenfriend S. A fluorometric method for the estimation of tyrosine in plasma and tissue. J Lab Clin Med 50: 733-736, 1957.
14. Goldberg AL. Influence of insulin and contractile activity on muscle size and protein balance. Diabetes 28(S1): 18-24, 1979.
15. Rannels DE, Kao R, Morgan HE. Effect of insulin on protein turnover in heart muscle. J Biol Chem 250: 1694-1701, 1975.
16. Tischler ME, Desautels M, Goldberg AL. Does leucine, leucyl t-RNA or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle. J Biol Chem 257: 1613-1621, 1982.
17. Baracos VE, Goldberg AL. Maintenance of normal length improves protein balance and energy status in isolated rat skeletal muscles. Am J Physiol 251: C588-C596, 1986.
18. Fagan JM, Goldberg AL. The rate of protein degradation in isolated skeletal muscle does not correlate with reduction-oxidation status. Biochem J 227:689-694, 1985.
19. Baracos VE, Wilson EJ, Goldberg AL. Effects of temperature on protein turnover in isolated rat skeletal muscle. Am J Physiol 246: C125-C130, 1984.
20. Seider MJ, Japp R, Chen CP, Booth FW. The effects of cutting or of stretching skeletal muscle in vitro on the rates of protein synthesis and degradation. Biochem J 188:247-254, 1980.

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