Temporal Response of Rabbits to β-Adrenergic Agonist Feeding: Tissue Weight, Calpains and Calpastatin Activities, and Nucleic Acid and Protein Concentrations[1,2]

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ABSTRACT: Forty-eight crossbred rabbits were used in three replications of a 2 × 4 factorial arrangement to investigate the short-term responses of tissue accretion, calpains and calpastatin activity, and nucleic acid and protein concentrations to β-adrenergic agonist (BAA) feeding. Rabbits were fed a 17% CP diet with or without 7 ppm of $L_{644,969}$ and slaughtered after 1, 4, 8, or 16 d of treatment. Empty body dressing percentage and biceps femoris weight (as a percentage of empty body weight [EBW]) were significantly higher in the treated rabbits than in the controls after 16 d of treatment. Heart and liver weights (as a percentage of EBW) were higher ($P < .05$) after 1 d and liver weight (as a percentage of EBW) was lower ($P < .05$) after 16 d in treated vs controls. Except for an elevation of skeletal muscle m-calpain after 16 d, BAA-supplementation did not affect the calpain-calpastatin system. Muscle RNA concentrations and RNA:DNA ratios were higher ($P < .05$) in treated rabbits after 1 d and remained higher thereafter. Protein:RNA ratios were lower ($P < .01$) in treated than in control rabbits after 4 d and remained lower throughout the trial. Muscle DNA content was lower after 4 d and higher after 16 d; RNA content was higher after 4, 8, and 16 d; and protein content was higher after 16 d in treated vs control rabbits. Liver nucleic acid and protein concentrations were not affected by BAA treatment. Heart RNA:DNA ratios were higher ($P < .01$) after 1 d and protein:RNA ratios were lower ($P < .05$) after 4 d in the treated rabbits than in the controls. Collectively, these data imply that BAA-induced muscle growth in rabbits occurs through hyperplasia and seems to be related to elevated protein synthetic capacity.

Key Words: Rabbits, β-Adrenergic Agonist, Muscle, Liver, Heart, Calpain

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Introduction

β-Adrenergic agonists (**BAA**) are adrenomimetic compounds known to increase muscle growth under a variety of physiological conditions (for reviews see Mersmann, 1989; Yang and McElligott, 1989; Anderson et al., 1991). These compounds also affect other organs. Heart weight is increased in rats (Reeds et al., 1986) and rabbits (Forsberg et al., 1989), decreased in sheep (Kim et al., 1987) and cattle (Moloney et al., 1990), and remains unchanged in swine (Cromwell et al., 1988) treated with BAA. Conversely, liver weight is generally decreased by chronic BAA treatment (Reeds et al., 1986; Williams et al., 1989; Moloney et al., 1990; Muramatsu et al., 1991).

The mechanism for BAA-induced muscle growth has been studied extensively and may vary depending on the compound and species studied. The BAA-response in skeletal muscle growth has been partially attributed to decreased protein degradation (Reeds et al., 1986; Bohorov et al., 1987; Bardsley et al., 1992; Wheeler and Koohmaraie, 1992). This decrease seems to be related to alterations in the calpain proteinase system, particularly through elevation of calpastatin activity (Kretchmar et al., 1989; Koohmaraie et al., 1991; Bardsley et al., 1992; Wheeler and Koohmaraie, 1992; Pringle et al., 1993). This elevation occurs within 2 wk of treatment in lambs and is maintained with subsequent treatment (Pringle et al., 1993). Because the calpain system is found in both cardiac

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and hepatic tissues (Murachi et al., 1981), it is possible that the BAA-response of these tissues occurs through a mechanism similar to that in skeletal muscle. Therefore, this experiment was designed to investigate the mechanism(s) for the short-term responses of rabbit skeletal muscle, heart, and liver to BAA treatment.

Experimental Procedures

Experimental Design. Forty-eight doe rabbits (New Zealand White × California White; Elmwood Rabbit Production, Elmwood, NE) were assigned to three replicates of a 2 × 4 factorial arrangement, with two treatments (control and treated) and four slaughter end points (1, 4, 8, and 16 d). In addition, two rabbits were slaughtered at the initiation of each replicate to establish baseline values for the variables measured (n = 6). All housing, handling, and experimental procedures were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Upon delivery, the rabbits were ranked by weight, divided into groups of nine (including the initial slaughter group), and assigned to the nine treatments. The rabbits were individually housed and conditioned to their diets and surroundings for 10 to 14 d before initiation of each replicate, receiving ad libitum access to feed[3] and water containing 1.3% (vol/vol) of a coccidiostat (Albon; Roche, Nutley, NJ).

The control rabbits had ad libitum access to water and feed, along with 5 g of molasses, top-dressed daily on the diet. The treated rabbits received the same diet with the equivalent of 7 ppm of the β-adrenergic agonist $L_{644,969}$ added to the molasses. The rabbits were weighed on two consecutive days to start each replicate and once at assigned slaughter times (1, 4, 8, and 16 d). After weighing, the rabbits assigned to that slaughter end point were lightly anaesthetized with CO_2 and killed by decapitation. Blood was collected for determination of urea nitrogen. Within 30 min postmortem, a randomly selected hindlimb of each rabbit was skinned and the biceps femoris muscle was removed intact and weighed. The abdominal and thoracic cavities were opened and the liver and heart were removed and weighed. After weighing, tissues were minced and sampled fresh (5 g) for determination of calpains and calpastatin activities. The remainder of the tissue was frozen at −70°C and stored for measurement of nucleic acid and protein concentrations. Because of small size, hearts were pooled for the two rabbits in each treatment at each slaughter end point, before sampling. Because the rabbits had access to feed and water immediately before slaughter, empty body weight (**EBW**) was measured to minimize the variation caused by gastrointestinal fill. After tissue sampling, each full gastrointestinal tract was weighed, emptied, blotted dry, and reweighed. Empty body weight included the empty gastrointestinal tract. Within 10 h postmortem, carcasses were skinned, washed, weighed, and stored at −20°C.

Determination of Calpain and Calpastatin Activity. Activities of the calpains and calpastatin were determined on unfrozen, prerigor skeletal muscle, heart, and liver samples according to the procedures of Koohmaraie (1990) with the following modifications. Tissue samples (5 g) were homogenized in 2.5 volumes of extraction buffer (100 m*M* Tris, 5 m*M* EDTA, and 10 m*M* β-mercaptoethanol [**MCE**], pH 8.3), centrifuged, filtered, and dialyzed overnight (molecular weight cutoff 12,000 to 14,000) in buffer (20 m*M* Tris, 5 m*M* EDTA, and 10 m*M* β-MCE, pH 7.5). The dialyzed samples were centrifuged, filtered, loaded onto anion exchange (DEAE-Sephacel; Sigma Chemical, St. Louis, MO) columns (1.5 cm × 20 cm; Bio-Rad, Richmond, CA), and washed with three to five column volumes of elution buffer (20 m*M* Tris, .1 m*M* EDTA, and 10 m*M* β-MCE, pH 7.35). The samples were eluted using a linear NaCl gradient in elution buffer (0 to 350 m*M*, total volume 500 mL for skeletal muscle; and 0 to 400 m*M*, total volume 400 mL for heart and liver). For skeletal muscle 150 fractions (3 mL) and for heart and liver 130 fractions (3 mL) were collected.

Activities of skeletal muscle μ- and m-calpain and heart and liver m-calpain were determined using a casein solution (100 m*M* Tris, 1 m*M* NaN_3, 5 m*M* $CaCl_2$, 2 μL/mL of β-MCE, and 5 mg/mL casein, pH 7.5). One unit of proteinase activity was defined as the amount of enzyme necessary to cause an increase of 1.0 absorbance unit at 278 nm. Skeletal muscle calpastatin activity was determined using the same casein solution, following the procedures of Koohmaraie (1990). Because of the abundance of calpastatin in heart and liver samples, calpastatin and μ-calpain could not be adequately separated. Therefore, calpastatin activity was measured using the procedure of Koohmaraie et al. (1987). An aliquot (2 mL) from fractions thought to contain calpastatin was heated at 90°C for 4 min and centrifuged (2,000 × *g*) to precipitate the denatured material. The supernatant volume was recorded and the samples were assayed for calpastatin as described above with adjustment of calpastatin activity for changes in volume due to heating. One unit of inhibitor activity was defined as the amount of calpastatin necessary to inhibit one unit of DEAE-purified rabbit lung m-calpain activity. The various components of the calpain-calpastatin system eluted from our columns in a manner consistent with that reported by Wheeler and Koohmaraie (1991).

Nucleic Acid and Protein Concentrations. Tissue DNA was determined using the fluorogenic intercalating agent Hoechst 33258 (Sigma Chemical, St. Louis, MO; Labarca and Paigen, 1980). Tissue RNA was measured spectrophotometrically using the procedures

[3]Purina® Rabbit Chow® Performance Blend, a pelleted feed containing ≥ 17.0% CP, 2.0% crude fat, 18.0% crude fiber, and 8.0% ash.

of Munro and Fleck (1969), and tissue protein was determined using the biuret procedure of Gornall et al. (1949). Nucleic acid and protein contents of the tissues were calculated by multiplying the various concentrations in the tissues by the weights of the tissues.

Blood Urea Nitrogen. Blood was allowed to sit overnight at 4°C, centrifuged (2,500 × *g*) for 10 min, and the serum collected and frozen at –20°C for future analysis. Blood urea nitrogen was determined according to the procedures of Crocker (1967), using a Sigma urea nitrogen kit (Sigma Chemical; procedure #535).

Statistical Analyses. The data were analyzed using ANOVA for a completely randomized design with replication (Steel and Torrie, 1980). The treatment means of treated and control rabbits at specific slaughter end points, along with linear and quadratic effects, were compared with single df contrasts using the interaction of treatment and replication as the error term. The data are presented as means ± SEM. Different values for SE within a trait are due to differences in the number of observations for means.

Results

Animal Performance and Tissue Weights. Average daily gain was not affected ($P > .5$) by BAA treatment during the trial (Table 1). Empty body weight (Table 1) was higher in the control than in the BAA-fed rabbits after 4 d but was similar thereafter. Dressing percentage and biceps femoris weight (as a percentage of EBW, Table 1 and wet weight, data not shown) increased linearly in the BAA-fed rabbits and were higher than those in controls ($P < .05$) after 16 d. Liver weight (as a percentage of EBW; Table 1) increased initially and decreased thereafter in the BAA group and was higher after 1 d ($P < .05$) and lower after 16 d ($P < .05$) in the treated rabbits than in the controls. Heart weight (as a percentage of EBW; Table 1) was higher in the treated rabbits after 1 d ($P < .01$) and returned to control values thereafter. Blood urea nitrogen was numerically lower in the treated rabbits after 1 d and remained lower throughout the study. However, these differences were not significant ($P > .20$; Table 1).

Calpain-Calpastatin System. Except for the activity of skeletal muscle m-calpain, the calpain-calpastatin system was not affected ($P > .20$) by dietary BAA administration in the three tissues investigated (Table 2). Skeletal muscle m-calpain activity tended to be lower ($P < .10$) after 1 d and was higher ($P < .05$) after 16 d of BAA treatment. The ratios of the two proteinases and proteinase:inhibitor activities in muscle are consistent with other data in rabbits (Gopalakrishna and Barsky, 1985). The data for the various tissues are also comparable with earlier findings (Gopalakrishna and Barsky, 1985; Blomgren et al., 1989).

Table 1. Response of final empty body weight, average daily gain, dressing percentage, and tissue weights to β-adrenergic agonist (BAA) treatment

	Time on feed, d				
Trait	0	1	4	8	16
Empty body wt (EBW), g					
Control[a]	1,642.0 ± 47.3	1,804.7 ± 47.3	1,851.1 ± 47.3*	1,938.4 ± 47.3	2,182.1 ± 41.7
BAA[a,b]	1,642.0 ± 47.3	1,692.0 ± 47.3	1,692.0 ± 47.3	1,938.7 ± 47.3	2,196.1 ± 39.1
ADG, g					
Control[c]	—	82.4 ± 13.5	26.9 ± 13.5	27.6 ± 13.5	29.5 ± 12.7
BAA	—	60.4 ± 13.5	31.1 ± 13.5	39.0 ± 13.5	33.1 ± 11.9
EBW dressing percentage, %					
Control	58.7 ± .9	57.6 ± .9	59.5 ± .9	57.2 ± .9	58.5 ± .8*
BAA[c]	58.7 ± .9	57.3 ± .9	58.7 ± .9	58.6 ± .9	61.2 ± .8
Biceps femoris wt, % of EBW					
Control	1.49 ± .03	1.44 ± .03	1.51 ± .03	1.49 ± .03	1.50 ± .03**
BAA[ab]	1.49 ± .03	1.43 ± .03	1.50 ± .03	1.55 ± .03	1.74 ± .03
Liver wt, % of EBW					
Control	3.96 ± .27	3.50 ± .27*	3.66 ± .27	3.53 ± .27	4.15 ± .24*
BAA[c]	3.96 ± .27	4.53 ± .27	3.44 ± .27	3.37 ± .27	3.39 ± .22
Heart wt, % of EBW					
Control	.28 ± .01	.26 ± .01**	.28 ± .01	.26 ± .01	.26 ± .01
BAA	.28 ± .01	.31 ± .01	.28 ± .01	.27 ± .01	.27 ± .01
Blood urea nitrogen, mg/dL					
Control	19.4 ± 1.7	18.3 ± 1.7	17.2 ± 1.7	21.6 ± 1.7	19.3 ± 1.5
BAA	19.4 ± 1.7	15.2 ± 1.7	16.4 ± 1.7	19.2 ± 1.7	15.9 ± 1.4

[a]Linear effect ($P < .01$).
[b]Quadratic effect ($P < .01$).
[c]Linear effect ($P < .05$).
*,**Means within a trait, at a specific time, differ ($P < .05$ and .01, respectively).

Table 2. Response of biceps femoris (BF), liver, and heart calpains and calpastatin activities to β-adrenergic agonist (BAA) treatment

Trait	Time on feed, d				
	0	1	4	8	16
BF μ-calpain, units/g[a]					
Control	.53 ± .08	.53 ± .08	.41 ± .08	.42 ± .08	.52 ± .08
BAA	.53 ± .08	.39 ± .08	.31 ± .08	.42 ± .08	.46 ± .08
BF m-calpain, units/g					
Control	1.09 ± .10	1.15 ± .10†	1.01 ± .10	.96 ± .10	1.03 ± .10*
BAA[b]	1.09 ± .10	.88 ± .10	1.15 ± .10	1.19 ± .10	1.38 ± .10
BF calpastatin, units/g[a]					
Control	4.74 ± .60	4.14 ± .60	4.04 ± .60	4.12 ± .60	4.46 ± .60
BAA	4.74 ± .60	4.67 ± .60	5.32 ± .60	3.21 ± .70	3.75 ± .60
Liver calpastatin, units/g[a]					
Control	8.37 ± .60	6.59 ± .60	6.60 ± .60	6.95 ± .60	6.46 ± .60
BAA	8.37 ± .60	7.23 ± .60	7.78 ± .60	6.25 ± .60	6.38 ± .60
Heart calpastatin, units/g[a]					
Control	9.46 ± 1.12	9.26 ± 1.12	9.96 ± 1.12	9.78 ± 1.12	10.53 ± 1.12
BAA	9.46 ± 1.12	11.08 ± 1.12	9.71 ± 1.41	10.48 ± 1.12	12.41 ± 1.12

[a]No treatment effect ($P > .2$).
[b]Linear effect ($P < .01$).
†,*Means within a trait, at a specific time, differ ($P < .10$ and .05, respectively).

Nucleic Acids and Protein. Biceps femoris DNA and protein concentrations and protein:DNA ratios were not affected by BAA treatment ($P > .5$; data not shown). The RNA concentration and RNA:DNA ratio were higher in the BAA-fed rabbits than in controls after 1 d and remained higher throughout the feeding period (Table 3). The difference between treated and control rabbits increased to 4 d and then attenuated with continued BAA administration. Conversely, the muscle protein:RNA ratio (Table 3) in the BAA-fed rabbits was lower after 4, 8, and 16 d compared with controls. Primarily because of the advantage in muscle weights, DNA and protein contents were higher in the treated rabbit muscle than in the controls after 16 d (Table 4). The RNA content of the biceps femoris from BAA-treated rabbits was higher than in controls after 4, 8, and 16 d.

Unlike skeletal muscle, neither liver nor heart nucleic acid and protein concentrations were affected ($P > .20$) by BAA treatment (data not shown). Heart protein:DNA ratio was higher after 1 d ($P < .05$); RNA:DNA ratio was higher after 1 ($P < .01$) and 4 d ($P < .10$); and protein:RNA ratio was lower after 4 d ($P < .05$). All ratios were similar to controls within 8 d of treatment initiation (Table 3). Although liver protein content was not affected by BAA treatment ($P > .5$), both liver DNA and RNA contents were higher in the treated rabbits at the conclusion of the study.

Discussion

One of the most consistent responses to BAA treatment is enhanced muscle growth (Yang and McElligott, 1989), most prominently in muscles dominated by Type II (fast-twitch, glycolytic) fibers (Beermann et al., 1987). In our study, BAA-treated muscle weight began to diverge from controls after 8 d and was significantly higher than controls after 16 d of treatment.

It is commonly postulated that BAA-induced muscle growth occurs through a hypertrophic model (Beermann et al., 1987; Kim et al., 1987; Kretchmar et al., 1989; Koohmaraie et al., 1991; Pringle et al., 1993). This does not seem to be the case in rabbits. Forsberg et al. (1989) reported that DNA content was increased in the biceps femoris, extensor digitorium longus, and gastrocnemius muscles of cimaterol-treated rabbits and suggested a mechanism involving satellite cell incorporation to explain the changes in muscle growth. β-Adrenergic agonist treatment has also increased DNA in transplanted (Roberts and McGeachie, 1992) and denervated (Maltin et al., 1989) mouse muscle and caused increased proliferation of cultured chick embryo breast muscle satellite cells (Grant et al., 1990). However, further investigation of BAA-treated, denervated muscle showed no increase in DNA, recruitment of satellite cells, or mitosis (Maltin and Delday, 1992). These researchers suggested that the increases in DNA content of BAA-treated muscle may be due to proliferation of non-myofiber cells, as shown by Dumont et al. (1989). Unfortunately, our procedures for measuring DNA do not allow separation of myofiber from non-myofiber DNA. Thus, although biceps femoris DNA content was increased after 16 d of BAA treatment in this study, it is not obvious whether these changes were truly indicative of hyperplastic growth.

Previous work in lambs (Kretchmar et al., 1989; Koohmaraie et al., 1991; Pringle et al., 1993) and

Table 3. Response of biceps femoris (BF) and heart RNA concentration and RNA:DNA and protein:RNA ratios to β-adrenergic agonist (BAA) treatment

Trait	Time on feed, d				
	0	1	4	8	16
BF RNA concentration, μg/g					
Control	669.3 ± 27.3	641.9 ± 27.3*	639.1 ± 27.3**	640.7 ± 27.3**	662.8 ± 27.3*
BAA[ab]	669.3 ± 27.3	722.7 ± 27.3	873.2 ± 27.3	849.1 ± 27.3	763.5 ± 27.3
BF RNA:DNA, μg/μg					
Control	.56 ± .02	.53 ± .02*	.51 ± .02**	.52 ± .02**	.52 ± .02*
BAA[bc]	.56 ± .02	.61 ± .02	.74 ± .02	.68 ± .02	.62 ± .02
BF protein:RNA, mg/mg					
Control	351.3 ± 15.6	365.5 ± 15.6	373.5 ± 15.6**	365.5 ± 15.6**	359.0 ± 15.6*
BAA[bc]	351.3 ± 15.6	327.2 ± 15.6	268.8 ± 15.6	271.2 ± 15.6	312.0 ± 15.6
Heart RNA concentration, mg/g[d]					
Control	1.29 ± .10	1.25 ± .10	1.41 ± .10	1.34 ± .10	1.27 ± .10
BAA	1.29 ± .10	1.48 ± .10	1.57 ± .10	1.25 ± .10	1.30 ± .10
Heart protein:DNA, mg/mg					
Control[c]	74.6 ± 4.08	71.0 ± 4.08*	80.9 ± 4.08	88.6 ± 4.08	79.3 ± 4.08
BAA	74.6 ± 4.08	83.1 ± 4.08	74.5 ± 4.08	79.0 ± 4.08	79.3 ± 4.08
Heart RNA:DNA, mg/mg					
Control	.49 ± .03	.49 ± .03**	.52 ± .03†	.54 ± .03	.52 ± .03
BAA[e]	.49 ± .03	.61 ± .03	.60 ± .03	.50 ± .03	.52 ± .03
Heart protein:RNA, mg/mg					
Control	151.5 ± 8.6	145.1 ± 8.6	155.7 ± 8.6*	163.9 ± 8.6	154.3 ± 8.6
BAA	151.5 ± 8.6	138.3 ± 8.6	127.0 ± 8.6	159.1 ± 8.6	154.3 ± 8.6

[a]Linear effect ($P < .01$).
[b]Quadratic effect ($P < .01$).
[c]Linear effect ($P < .05$).
[d]No treatment effect ($P > .35$).
[e]Quadratic effect ($P < .05$).
†,*,**Means within a trait, at a specific time, differ ($P < .05$ and .01, respectively).

Table 4. Response of biceps femoris (BF) and liver total nucleic acid and protein contents to a β-adrenergic agonist (BAA) treatment

Trait	Time on feed, d				
	0	1	4	8	16
BF DNA, mg					
Control[a]	29.6 ± 1.7	31.5 ± 1.7	35.3 ± 1.7*	35.7 ± 1.7	38.7 ± 1.7**
BAA[ab]	29.6 ± 1.7	28.7 ± 1.7	30.0 ± 1.7	37.3 ± 1.7	46.9 ± 1.7
BF RNA, mg					
Control[c]	16.3 ± 1.2	16.6 ± 1.2	17.9 ± 1.2*	18.5 ± 1.2**	20.1 ± 1.2**
BAA[a]	16.3 ± 1.2	17.5 ± 1.2	22.2 ± 1.2	25.4 ± 1.2	28.5 ± 1.2
BF protein, g					
Control[a]	5.74 ± .38	6.06 ± .38	6.65 ± .38	6.74 ± .38	7.20 ± .38**
BAA[ab]	5.74 ± .38	5.66 ± .38	5.95 ± .38	6.87 ± .38	9.00 ± .38
Liver DNA, mg					
Control	73.2 ± 7.5	85.3 ± 7.5	91.3 ± 7.5	94.9 ± 7.5	90.6 ± 7.5**
BAA[ad]	73.2 ± 7.5	75.5 ± 7.5	76.7 ± 7.5	98.3 ± 7.5	121.1 ± 7.5
Liver RNA, mg					
Control[c]	127.8 ± 6.8	139.0 ± 6.8	153.4 ± 6.8†	151.1 ± 6.8	145.3 ± 6.8**
BAA[b]	127.8 ± 6.8	126.2 ± 6.8	134.2 ± 6.8	162.2 ± 6.8	190.1 ± 6.8
Liver protein, g					
Control[a]	7.31 ± .76	7.67 ± .76	8.09 ± .76	8.69 ± .76	11.10 ± .76
BAA[a]	7.31 ± .76	7.73 ± .76	7.54 ± .76	8.35 ± .76	11.12 ± .76

[a]Linear effect ($P < .01$).
[b]Quadratic effect ($P < .01$).
[c]Linear effect ($P < .05$).
[d]Quadratic effect ($P < .05$).
†,*,**Means within a trait, at a specific time, differ ($P < .10$, .05, and .01, respectively).

cattle (Wheeler and Koohmaraie, 1992) demonstrated a positive relationship between BAA-induced muscle growth and calpastatin activity. The majority of the BAA-induced alterations in lamb muscle weight and calpastatin activity occurs within 2 wk of treatment initiation (Pringle et al., 1993). Our data suggest that the enhanced muscle growth of BAA-treated rabbits does not require alteration of the calpain-calpastatin system. The only change in the calpain-calpastatin system was an increase in the activity of m-calpain, which is consistent with other reports (Kretchmar et al., 1989; Koohmaraie et al., 1991). However, it is difficult to conceive how this change could cause the increased muscle growth noted in this study. In agreement with the calpain-calpastatin findings, our blood urea nitrogen data also suggest that the proteolytic capacity of rabbit skeletal muscle is unchanged by BAA treatment. The calpastatin and μ-calpain data in this study do not agree with the findings of Forsberg et al. (1989), who fed BAA to rabbits for a prolonged period. Those researchers reported that cimaterol treatment decreased the activity of skeletal muscle calpastatin and μ-calpain. These discrepancies may be partially due to differences in the length of BAA treatment. Their data must also be interpreted with caution, because frozen samples were used to measure the calpain-calpastatin system and Koohmaraie (1990), Sainz et al. (1992), and Whipple and Koohmaraie (1992) have since reported that freezing of skeletal muscle decreased calpastatin and μ-calpain activities.

The BAA-induced increase in RNA concentration and content and RNA:DNA ratio are consistent with results of previous studies (Beermann et al., 1987; Forsberg et al., 1989; Williams et al., 1989; Koohmaraie et al., 1991; Pringle et al., 1993). Changes in muscle weight appear after the RNA concentration and RNA:DNA ratio begin to attenuate. It seems that the protein synthetic capacity is higher in the BAA-treated muscle, but it is unclear why the RNA measurements seem to peak before detectible changes in muscle weight. Collectively, these data suggest an alternative mechanism for BAA-induced muscle growth in rabbits. The muscle data suggest that BAA-induced muscle growth in rabbits occurs at least partially through a hyperplastic mechanism, whereby muscle protein synthetic capacity is elevated. This is contrary to the strictly hypertrophic mechanism reported in sheep and cattle, whereby protein degradation is generally lowered, and concurs with the ruminant-specific effect of BAA-supplementation mentioned by Bardsley et al. (1992).

To our knowledge, this is the first study to report the effects of BAA treatment on the calpain-calpastatin system in hepatic and cardiac tissues. The effects of BAA treatment on heart weight vary, with increased weight in rats (Reeds et al., 1986) and rabbits (Forsberg et al., 1989), decreased weights in sheep (Kim et al., 1987; Williams et al., 1989) and cattle (Moloney et al., 1990), and similar weights in swine (Cromwell et al., 1988). In our study, heart weight was higher after 1 d of BAA treatment and not different from controls thereafter. The change in weight was again independent of significant change in the calpain-calpastatin system. The increase in weight was accompanied by an increase in cell mass (protein:DNA; Table 3), indicative of hypertrophic growth in BAA-treated cardiac muscle. Heart RNA:DNA ratios were also significantly higher after 1 d of treatment, suggesting that an increase in the protein transcriptional efficiency and synthetic capacity of cardiac tissue may account for the slight changes in weight.

Commonly, chronic BAA treatment has decreased liver weight (Reeds et al., 1986; Kim et al., 1987; Williams et al., 1989; Moloney et al., 1990; Muramatsu et al., 1991). Our data agree, except that liver weight in the BAA-treated rabbits increased after 1 d of treatment (Table 1). According to the hypothesis, in muscle, that calpastatin activity is related to tissue size, then calpastatin activity in liver should decrease after 16 d of BAA treatment. However, in this study there were no BAA-induced responses in either liver m-calpain or calpastatin activities or in the concentrations of nucleic acids or protein, suggesting the lack of a clear relationship between any of the variables measured and liver size in BAA-fed rabbits. The changes in liver weight may simply be a reflection of the changes in tissue blood flow that accompany BAA supplementation. Eisemann et al. (1988) reported that heart rate and blood flow were increased by acute BAA treatment, whereas chronic treatment shunted blood flow to the musculature of the animal. Another possibility for the decreased liver weights is a decrease in the fractional protein synthesis rates, as reported by Muramatsu et al. (1991).

Implications

A better understanding of the regulation of skeletal muscle, heart, and liver growth could allow enhanced growth efficiency of meat animals through exogenous alteration of these tissues. It seems that rabbits fed a β-adrenergic agonist respond differently than do sheep, offering another avenue for study of the processes controlling tissue growth. Although these data are not conclusive as to a mechanism for β-adrenergic-agonist-induced tissue growth in rabbits, they offer direction for future considerations into the study of the mechanism of action of β-adrenergic agonists.

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