

EFFECT OF CIMATEROL ON GROWTH AND 3-METHYLHISTIDINE EXCRETION IN RATS

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Summary

Forty-two outbred female Sprague-Dawley rats weighing 145 g were used to study the effects of a beta-agonist, cimaterol, on growth, body composition and urinary excretion of 3-methylhistidine (MH) at 3, 6 and 18 d. Cimaterol (CIM) was administered in the feed at 10 mg/kg. The growth promoting effect of CIM was most evident during the initial part of the feeding period, followed by a gradual decrease in the magnitude of the response with no significant effect at 18 d. The action of CIM was confined to skeletal and cardiac muscles with no stimulating effect on other organs. The amount of urine excretion and urinary MH excretion was reduced ($p < .01$) at 3 d in the CIM group. No difference was found at 6 d, followed by an increased urine excretion ($p < .05$) and MH excretion ($p < .01$) at 18 d. An inverse relationship between growth rate and urinary MH excretion suggested that the increased growth rate of CIM-fed rats during the initial part of the feeding period is primarily attributed to the decreased protein degradation rate. It was further suggested that both fractional synthesis rate and fractional degradation rate increased during the later part of the feeding period.

(Key Words: Cimaterol, Growth, 3-Methylhistidine, Protein Degradation)

Introduction

Recent studies have consistently demonstrated that dietary administration of β -adrenergic compounds promote the deposition of body protein and reduce body fat gain (Baker et al., 1984; Emery et al., 1984; Beermann et al., 1986; Kim et al., 1987, 1989). A greater nitrogen retention and body protein gain in cimaterol-fed lambs was primarily attributed to a reduced nitrogen loss (30%) in urine of the treated animals (Kim et al., 1989). More recently, several investigators attempted to elucidate the mechanism of greater protein accretion and muscle hypertrophy in beta-agonists fed animals. Increased amino acid uptake (Deschaies et al., 1981), increased total RNA concentration in muscle (Beermann et al., 1987; Kim et al., 1988) and increased actin or myosin light chain mRNA concentrations (Smith et al., 1987; Babji and Booth, 1988) suggested the possibility

of increased protein synthesis in beta-agonists fed animals. Emery et al. (1984) reported increased fractional rate of protein synthesis in gastrocnemius muscle of rats injected with clenbuterol daily for 7 d. More recently, Bergen et al. (1989) and Claeys et al. (1989) concluded that the increased fractional synthesis rate (FSR) could account for the increased fractional accretion rate (FAR) of muscle protein in beta-agonists fed animals. They also observed an increase in estimated fractional degradation rate (FDR).

On the other hand, several other studies failed to demonstrate an increase in protein synthesis rate in beta-agonists fed animals (Klasing et al., 1985; Reeds et al., 1986; Bohorov et al., 1987; Morgan et al., 1988a). Reeds et al. (1986) further suggested that clenbuterol appeared to have a rapid, perhaps direct, inhibitory effect on protein degradation. Decreased proteolytic enzyme activities and higher calpastatin (protease inhibitor) activity in the muscles from beta-agonists fed animals (Kretchmar et al., 1988; Morgan et al., 1988b) also indirectly suggested a decreased protein degradation rate. Another indirect evidence of a possibly decreased protein degradation rate came from a study of Williams et al. (1987) who observed a decrease in urinary excretion of 3-methylhistidine in veal calves fed clenbuterol. 3-methyl-

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histidine excretion in urine has been widely used as a useful non-destructive technique for measuring *in vivo* protein degradation in rats (Young and Munro, 1978; Santidrian et al., 1981).

The present study was conducted to study the effects of cimaterol on the pattern of urinary excretion of 3-methylhistidine and its relationship to body weight gain in growing rats.

Materials and Methods

Forty-two outbred female Sprague-Dawley SPF rats weighing 145 g (40 d old) were purchased from Bantin and Kigman Inc.³ After 5 d of conditioning on a purified rat diet⁴, six animals were killed by decapitation for initial composition of body components. The remaining thirty-six animals were randomly divided into two treatment groups, control (CON) and cimaterol-fed (CIM). Cimaterol⁵ was mixed in the purified diet at 10 mg/kg. Three rats were housed in a cage with free access of diet and water. Body weight, body composition and urinary excretion of 3-methylhistidine were measured at 3, 6 and 18 d after initiation of experiment. Six control and six cimaterol-fed animals were used at each specified time period.

For the collection of urine, rats were transferred to individual metabolic cages at 72 h prior to the specified time period. Urine for the first 48 h was discarded and that for the next 24 h was collected into a flask containing 3 ml of 4 N HCl. Urine volume was measured. Following filtration, aliquots were hydrolyzed under vacuum in 6 N HCl at 110°C for 2 h. The hydrolysate was dried under a stream of air and resuspended in the lithium citrate buffer (Beckman's buffer) prior to analysis. 3-methylhistidine was separated and quantitated by ion-exchange chromatography on an automated amino acid analyzer, Beckman model 121-M.

After the collection of urine, the rats were killed by decapitation. Each rat was skinned, visceral contents were removed, and the plantaris and soleus muscles were dissected out. Weights of carcass, skin, liver, kidney, heart, plantaris and soleus muscles were recorded.

Statistical analysis was conducted using Student's t-test of Statistical Analysis System (SAS, 1982).

Results and Discussion

The effects of cimaterol on the growth and composition of body components at different time periods of administration are summarized in table 1. Cimaterol improved growth rate although this improvement was only evident during the initial part of the 18 d feeding period. The present study confirms the results of early studies by Reeds et al. (1986) and Kim et al. (1988), indicating the growth promoting effect of cimaterol and clenbuterol was effective only for a limited time period (about 10 d) after administration in rats. Kim et al. (1989) also observed that the enhanced growth rate was only evident during the first 42 d of the 90-d feeding trial in lambs. Carcass weight of CIM treated rats was greater than that of control group at 3 d ($p < .01$) and 6 d ($p < .05$). Cimaterol had no effect on weights of skin, liver and kidney throughout the experimental period. It did, however, increase the heart weights at 3 d (14.1%, $p < .05$) and 6 d (10.4%, $p < .05$) with no difference at 18 d. Weights of the plantaris muscle were also increased by cimaterol feeding at 3 d (10.6%, $p < .05$), 6 d (15.8%, $p < .01$) and 18 d (14.7%, $p < .01$). Though statistically not significant, mean weights of the soleus muscle in the CIM group were also greater than those of the control group.

It appears that the growth promoting effect of cimaterol is confined to skeletal and cardiac muscles in rats. Reeds et al. (1986) reported a similar result that the action of clenbuterol was confined to skeletal and cardiac muscles with no stimulating effect on the growth of liver, gastrointestinal tract and kidney. However, the increased growth of cardiac muscle was not observed in meat animals. Jones et al. (1985) and Kim et al. (1987) reported decreased heart weights in cimaterol-fed pigs and lambs. Explanation for the apparent differences among different animal species is not available at the present time.

The increase in muscle growth in response to cimaterol administration paralleled the increase in body growth. As previously reported (Kim et al., 1988), however, the extent of growth response is variable with different muscles. In the present

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CIMATEROL ON 3-METHYLHISTIDINE EXCRETION

TABLE 1. EFFECTS OF CIMATEROL ON GROWTH AND ORGAN WEIGHT

	0 d		3 d			6 d			18 d		
	Mean	SE	CON	CIM	SE	CON	CIM	SE	CON	CIM	SE
No. of animals	6		6	6		6	6		6	6	
Initial body wt (g)	165.7	2.3	167.8	164.7	2.5	166.7	166.5	2.0	168.7	169.7	2.1
Final body wt (g)			179.3	185.0	2.7	190.8	201.2*	4.0	212.8	224.3	6.5
Total wt gain (g)			11.5	20.3**	2.6	24.2	34.7**	2.4	44.2	54.7	4.9
Daily wt gain (g)			3.9	6.8**	.9	4.0	5.8**	.5	2.5	3.0	.3
Carcass wt (g)	85.0	1.3	95.5	100.1**	1.0	103.8	111.0*	2.4	118.1	126.7	3.7
Skin wt (g)	27.0	1.4	26.1	25.1	1.1	27.8	26.3	1.2	29.9	29.3	1.0
Liver wt (g)	9.3	.4	10.0	10.3	.4	10.4	11.0	.7	10.3	9.7	.9
Kidney wt (g)	1.73	.04	1.77	1.81	.05	1.72	1.80	.12	2.06	2.13	.11
Heart wt (g)	.70	.02	.78	.89*	.04	.77	.85*	.03	.81	.86	.07
Muscle wt (mg)											
Plantaris	144	3	169	187*	6	190	220**	6	224	257**	8
Soleus	68	2	75	83	4	82	93	4	96	110	6

* $p < .05$, differs from control.** $p < .01$, differs from control.

study, the plantaris muscle showed a greater growth response than the soleus muscle (table 1). Kim et al. (1988) reported a greater hypertrophy of type II fibers than type I fibers, thus explaining that the plantaris muscle (type II predominant) would have a greater hypertrophy than the soleus muscle (type I predominant).

Table 2 summarizes the effects of cimaterol on urine excretion and urinary excretion of 3-methylhistidine during the 18-d feeding period. The amount of urine excretion in the CIM group (2.1

ml/d) was significantly lower ($p < .01$) than that of the control group (4.1 ml/d) at 3 d. No differences were found at 6 d, followed by an increased ($p < .05$) urine excretion at 18 d in the CIM-fed animals (8.5 vs 6.6 ml/d). Cimaterol also significantly influenced the daily excretion of 3-methylhistidine, the pattern being similar to the amount of urine excretion. After 3 d of cimaterol feeding, urinary excretion of 3-methylhistidine was significantly ($p < .01$) decreased compared to the controls (1.26 vs 1.84 μ moles/d). Again, little differ-

TABLE 2. EFFECTS OF CIMATEROL ON URINE EXCRETION AND URINARY EXCRETION OF 3-METHYLHISTIDINE IN RATS

	0 d		3 d			6 d			18 d		
	Mean	SE	CON	CIM	SE	CON	CIM	SE	CON	CIM	SE
Urine excretion (ml/d)	4.55	.89	4.13	2.07**	.83	7.02	6.53	2.02	6.55	8.50*	.78
3-methylhistidine (μ moles/d)	1.54	.13	1.84	1.26**	.20	2.08	1.94	.23	2.30	3.28**	.21
3-methylhistidine (μ moles/g body wt)	9.3	.9	10.7	6.8**	1.2	10.9	9.6	1.3	10.8	14.7**	1.3
3-methylhistidine (μ moles/ml urine)	.34	.04	.48	.63	.09	.36	.47	.11	.37	.39	.06

* $p < .05$, differs from control** $p < .01$, differs from control

ences were found at d 6 and then 3-methylhistidine excretion was significantly increased ($p < .01$) in the CIM group at 18 d (3.3 vs 2.3 $\mu\text{moles/d}$).

When the daily weight gain and urinary excretion of 3-methylhistidine were plotted (figure 1), an inverse relationship was observed between the two parameters. The data suggested that the increased growth rate of CIM-fed rats during the early feeding period would be primarily, if not entirely, attributed to the decreased protein degradation rate.

Williams et al. (1987) reported that in clenbuterol-fed calves urinary creatinine excretion, as a measure of total muscle mass, was significantly increased, whereas N^7 -methylhistidine excretion was significantly ($p < .05$) decreased. They concluded that, based on estimates of muscle mass from urinary creatinine and protein degradation from N^7 -methylhistidine excretion, the fractional breakdown rate of muscle protein in clenbuterol-treated calves was only .66 of that in the controls when the calves weighed 120 kg.

In a separate study, Kim (1988) determined

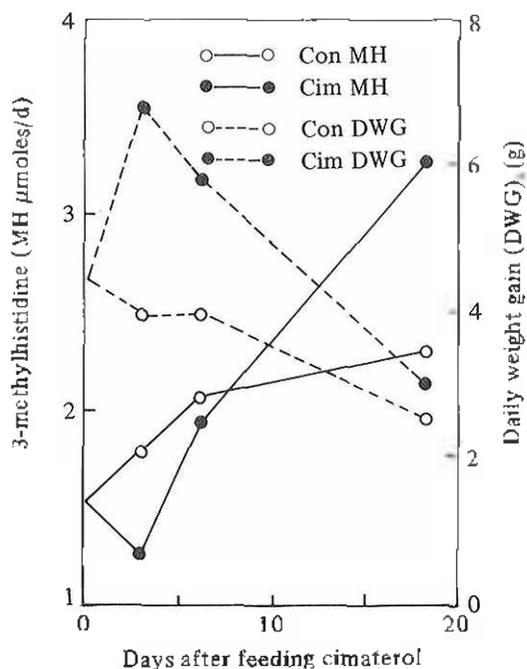


Figure 1. Changes of daily weight gain and urinary 3-methylhistidine excretion with the length of feeding period.

the effects of cimaterol on the fractional synthesis rate of protein. In the plantaris muscle but not in the soleus muscle, the protein FSR of the CIM-fed rats was 25% greater than that of the controls at 3 d. However, the difference was not significant ($p > .05$). Utilizing ^{14}C -AA continuous infusion in rats, Reeds et al. (1986) found no differences in FSR, whereas FAR were enhanced. Thus, they concluded that fractional breakdown rate was depressed. In a study with sheep fed N-free diet for 10d, Hovell et al. (1988) observed that clenbuterol administration for 5 d periods reduced the urinary endogenous nitrogen loss by 14%. They concluded that the drug acted by reducing body protein degradation. *In vitro* studies by Li and Jefferson (1977) also indicated that beta-agonists depressed degradation in skeletal muscle. Taken together, it appears that the rapidly increased growth rate and muscle accretion during the initial part of feeding period in beta-agonists fed animals can be primarily, if not entirely, attributed to the decreased FDR.

One interesting observation of the present study was that urinary excretion of 3-methylhistidine was gradually increased after d 6 (table 2) and yet the body weight gain in CIM-fed rats between d 6 and 18 was maintained at a level similar to the controls (table 1).

To maintain the increased body weight in spite of the apparently increased protein degradation, as evidenced by higher 3-methylhistidine excretion, it could be assumed that protein synthesis rate might also be increased after d 6. This suggests that the protein turnover rate is lower initially but greater during the later part of the feeding period. More definitive studies are needed to prove this hypothesis. However, Kim (1988) showed that feed/gain was lower in CIM-fed rats during the early feeding period, followed by a higher feed/gain ratio after d 14. A greater feed/gain ratio, decreased fat deposition (Berne et al., 1985) and higher daily heat production (MacRae et al., 1986; Kim et al., 1989) in animals fed beta-agonists for an extended period further suggest that additional energy is needed for a greater protein turnover during the later part of the feeding period.

When we closely examine the results of a study by Claeys et al. (1989), feed/gain ratio was lower in clenbuterol-fed lambs at 14 d but higher at 28 d, though none of these values was statistically

significant. In the meantime, the increase in muscle weights was greater in clenbuterol fed lambs. Both FSR and FDR determined at 28 d tended to be higher (though not significant) in treated animals. Similar results were also reported in pigs fed ractopamine for 21 and 35 d (Bergen et al., 1989). Bergen et al. stated that the sizable increase in protein accretion in semitendinosus muscle was accompanied by an increase in both synthesis and degradation. All these circumstantial evidences indicate that both FSR and FDR may be increased during the later part of the feeding period.

Further studies are warranted to unequivocally establish the mechanism for the increased skeletal muscle hypertrophy at different time points after the administration of beta-agonists. In addition, species and age (or weight) of animals, kinds of muscle to be examined and different beta-agonists may have further implications in the results and more fundamental biochemical processes including β_2 -receptors should be explored to explain any discrepancies among studies employing different experimental conditions.

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