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Muscle Wasting Associated with Endotoxemia in the Rat: Modification by the β_2-Adrenoceptor Agonist Clenbuterol

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A single injection of endotoxin (1 mg/kg, sc) in rats caused significant fever, body weight loss and reduction in gastrocnemius muscle mass, none of which was mimicked by pair-feeding. Infusion of endotoxin via osmotic minipump over five days caused transient fever and suppression of growth. Recovery of body weight was significantly enhanced by the administration of the β_2-adrenoceptor agonist clenbuterol (added to the diet at 4 mg/kg). In a separate experiment, injections of endotoxin (day 0 and day 2) caused significant reductions in body weight gain (42%), mass (9%) and protein content (13%) of gastrocnemius muscle over 3 days. Addition of clenbuterol to the diet reversed all of these effects but did not alter food intake or the febrile response to endotoxin. Clenbuterol caused large (20%) increases in the ratio of RNA to protein in muscle indicating that it may have stimulated protein synthesis. β_2-adrenoceptor agonists may therefore be of value in preventing *or* inhibiting muscle atrophy associated with infection or injury.

KEY WORDS: muscle atrophy; endotoxaemia; clenbuterol; β_2-adrenoceptor agonist; rat.

INTRODUCTION

Increased protein degradation and atrophy of skeletal muscle are common manifestations of bacterial infection and chronic sepsis.[1,2] This muscle wasting cannot simply be ascribed to reduced energy or protein intake and is generally considered detrimental, particularly if sustained over long periods. Since nutritional support alone is often not successful in preventing muscle loss,[3] pharmacological intervention may be of some value. Anabolic steroids have now been considered unsuitable as growth promoters in animal production due to their side effects, and are unlikely to be of value for treatment of muscle wasting associated with illness. β_2-adrenoceptor agonists, however, have considerably fewer side

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effects but exert potent anabolic actions on muscle in a number of species (see Ref. 4 for a review). The most effective of these, clenbuterol, stimulates muscle protein in several conditions including food restriction[5] and inherited muscular dystrophy in rodents.[6] The anabolic effects of clenbuterol have not been tested in man, but selective loss of body protein, a reduction in muscle protein and fever can be induced in experimental animals by injection or infusion of bacterial endotoxin (e.g. Refs 7, 8). In the present study we have investigated the effects of clenbuterol on body weight and muscle protein in rats treated with endotoxin.

MATERIALS AND METHODS

The animals used in this study were male, Sprague–Dawley rats (Charles River, Kent, UK) aged eight weeks, weighing 200 g. They were caged individually at an ambient temperature of 24°C (12 h light-dark cycle) with free access to commercially available diet (CRM, Labsure, Croydon, UK) in powdered form unless otherwise stated. Body weight and food intake were recorded daily. Colonic temperature was measured each morning (10.00–10.30) by insertion of a plastic-coated thermocouple, 5 cm beyond the rectum.

The endotoxin used in each experiment was *E. coli* 0127:B8 (Sigma, UK). Clenbuterol (Boehringer, W. Germany) was added to the diet at a final concentration of 4 mg/kg. At the termination of each experiment rats were killed by cervical dislocation and organs were removed and weighed. Protein and RNA content were determined by a dye reagent method (Bio-Rad, Watford, UK) and the modified method of Munro and Fleck,[9] respectively.

In the first experiment, three groups of animals were used: *ad libitum*-fed control group (injected with saline), *ad libitum*-fed endotoxin-treated (1 mg/kg body wt, sc), and pair-fed controls which were given the same amount of food as endotoxin treated rats. The effects of pair-feeding were studied 24 hr after those of endotoxin treatment so that each animal could be presented with exactly the same amount of food as one of the endotoxin treated rats. All animals were killed 20 h after the start of their treatment.

In the second experiment, rats were implanted with osmotic minipumps (0.2 ml) (Alzet, UK) subcutaneously under halothane anaesthesia (2% halothane in oxygen: nitrous oxide) containing either endotoxin or vehicle (0.9% saline) (8 animals). The dose of endotoxin administered was 0.34 mg/d. All animals were allowed free access to powdered stock diet, but half of those infused with endotoxin were presented with diet containing clenbuterol.

The third experiment also involved three groups of rats: control, endotoxin-treated and endotoxin plus clenbuterol. In this case clenbuterol was injected twice daily (1 mg/kg body wt, sc) and endotoxin on day 0 (1 mg/kg body wt, sc) and day 2 (0.5 mg/kg). The animals were killed on day 3.

Values are expressed as means ± SEM. The test of significance employed was analysis of variance and Student's *t*-for unmatched data, using two-tailed probabilities.

RESULTS

In the first experiment, a single injection of endotoxin caused a rise in body temperature of 0.7°C (data not shown), a fall in food intake (27%) and significant weight loss 24 h after injection (Table 1). These effects were associated with a reduction in the mass of gastrocnemius muscle (11%), an increase in liver weight (12%), but no change in the mass of heart, interscapular brown adipose tissue (BAT) or spleen. In contrast, animals pair-fed to exactly the same food intake as endotoxin-treated animals did not lose body weight, showed no reduction in the mass of gastrocnemius muscle, but did exhibit significant decreases in the mass of heart and BAT (Table 1).

Table 1. Acute effects of endotoxin[1] and food restriction on body weight gain and organ weight (Expt. 1)

	Control	Endotoxin	Pair-fed
Body weight gain (g)	8 ± 1	−6 ± 2*	5 ± 1†
Food intake (g/d)	23.3 ± 0.7	17.0 ± 0.9*	17.0 ± 0.9*
Gastrocnemius muscle weight (g)	0.88 ± 0.02	0.78 ± 0.02*	0.89 ± 0.02†
Heart weight (g)	0.74 ± 0.02	0.70 ± 0.01	0.68 ± 0.01*
Liver weight (g)	9.2 ± 0.3	10.3 ± 0.3*	8.4 ± 0.3†
Interscapular BAT weight (g)	0.31 ± 0.03	0.33 ± 0.02	0.24 ± 0.2*†
Spleen weight (g)	0.73 ± 0.05	0.78 ± 0.03	0.64 ± 0.03†

[1] *E. coli* endotoxin (1 mg/kg body weight) was injected subcutaneously and animals were killed after 20 hours.
Mean values ± SEM (n = 8)
* $P < 0.05$ vs control
† $P < 0.05$ vs endotoxin.

Endotoxin infusion (Expt. 2) induced a fever (0.6°C) which was sustained over the first 2 days (data not shown). Food intake was reduced by 26% on day 1 and by 16% on day 2 of endotoxin infusion, but thereafter returned to control values (data not shown). Total food intake over the 5 days experimental period was decreased by 9% in endotoxin-infused rats (Table 2). Body weight gain was also completely arrested for 48 h of endotoxin infusion, but showed rapid recovery thereafter, and by day 5 had almost returned to the control value (Fig. 2, Table 2). At this time gastrocnemius muscle mass, protein and RNA content were slightly, but not significantly, reduced in the endotoxin-infused group. Administration of clenbuterol to endotoxin-treated rats did not affect body temperature or food intake but significantly enhanced recovery of body weight (Fig. 1) which returned to control values by day 3. By day 5, gastrocnemius muscle mass and protein content in this group were almost identical to those of controls, but RNA content was significantly increased (Table 2). Thus the ratio of RNA to protein in muscle was 19% higher in clenbuterol-treated compared to control rats.

In the final experiment, injection of endotoxin had similar effects to infusion, causing significant but transient fever (0.7°C), a decline in food intake of 22% on day 1 (data not shown) and a reduction in body weight gain. Gastrocnemius muscle mass, protein and RNA content were all reduced by injection of endotoxin on day 3 (Table 3). Clenbuterol treatment did not affect body

Table 2. Effects of clenbuterol on body weight gain, food intake and composition of gastrocnemius muscle in rats treated with endotoxin by infusion[1] (Expt. 2)

	Control	Endotoxin	Endotoxin + Clenbuterol
Body weight gain[2] (g)	35 ± 1	30 ± 2*	35 ± 2
Food intake (g/d)	23.8 ± 0.5	21.8 ± 0.7*	22.0 ± 0.5*
Gastrocnemius muscle			
Weight (g)	0.98 ± 0.03	0.93 ± 0.02	0.99 ± 0.04
Protein content (mg)	163 ± 6	151 ± 7	163 ± 7
RNA content (mg)	2.4 ± 0.1	2.3 ± 0.1	2.9 ± 0.1*†
RNA/protein ($mg/mg \times 10^3$)	14.9 ± 0.3	14.7 ± 0.3	17.8 ± 0.2*†

[1] *E. coli* endotoxin was infused via osmotic minipumps, implanted subcutaneously for 5 days at the rate of 0.34 mg/d.
[2] Body weight gain was recorded over 5 days.
Mean values ± SEM (n = 8).
* $P < 0.05$ vs control.
† $P < 0.05$ vs endotoxin.

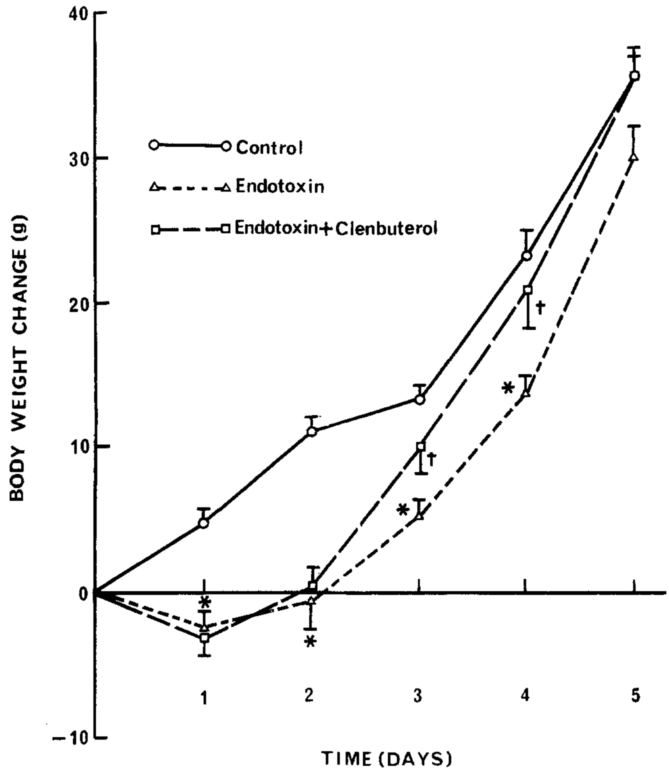

Fig. 1. Effects of clenbuterol on body weight change in rats treated with endotoxin by infusion (Expt. 2).

Mean values ± SEM (n = 8).
* $P < 0.05$ endotoxin vs control
† $P < 0.05$ endotoxin plus clenbuterol vs endotoxin

Table 3. Effects of clenbuterol on body weight gain, food intake and composition of gastrocnemius muscle in rats treated with endotoxin by injection[1] (Expt 3)

	Control	Endotoxin	Endotoxin + Clenbuterol
Body weight gain[2] (g)	17 ± 3	10 ± 2*	22 ± 3†
Food intake (g/d)	22 ± 0.9	18.3 ± 0.7	18.9 ± 1.6
Gastrocnemius muscle			
Weight (g)	0.91 ± 0.01	0.83 ± 0.03*	0.99 ± 0.02*†
Protein content (mg)	159 ± 4	139 ± 3*	162 ± 6†
RNA content (mg)	2.33 ± 0.07	2.00 ± 0.11*	2.84 ± 0.09*†
ENA/protein (mg/mg × 10^3)	14.6 ± 0.4	14.3 ± 0.6*	17.5 ± 0.4*†

[1] *E. coli* endotoxin was injected subcutaneously on day 0 (1 mg/kg body weight) and day 2 (0.5 mg/kg body weight) and animals were killed on day 3.
[2] Body weight gain was recorded over 3 days.
Mean values ± SEM (n = 6).
* $P < 0.05$ vs control.
† $P < 0.05$ vs endotoxin.

temperature or food intake, and body weight was again depressed on day 1. However, clenbuterol significantly enhanced the recovery of body weight, and weight gain was increased by twofold by day 3 compared to endotoxin-treated animals. Clenbuterol treatment also restored the mass and protein content of gastrocnemius muscle to control values and significantly increased the RNA content and RNA to protein ratio (Table 3).

DISCUSSION

The results of the present experiments demonstrate that injection or infusion of endotoxin both cause rapid reductions in food intake and decreases in muscle mass. In the first experiment, marked weight loss occurred 24 h after endotoxin administration and was accompanied by significant atrophy of gastrocnemius muscle (Table 1). These effects could not be ascribed simply to hypophagia since pair-fed rats given no endotoxin showed only a small decrease in body weight gain and no loss of muscle mass in spite of reduction in the mass of other organs (Table 1). The rapid loss of body weight following endotoxin injection presumably results in part from an increase in energy expenditure,[10] and the loss of muscle has been ascribed to both direct actions of endotoxin and to release of endogenous pyrogen (interleukin-1; 11).

The effects of endotoxin infusion (Ext. 2) on food intake, body weight and body temperature confirm our previous findings,[8] and also indicate that muscle mass was reduced. Clenbuterol significantly enhanced body weight gain over the latter period (days 2–5) of endotoxin infusion (Fig. 1) without affecting food intake, by producing a 16% increase ($P < 0.05$) in feed efficiency. Clenbuterol also caused small increases in gastrocnemius muscle mass and protein content, but the dramatic rise in RNA content indicates that the drug enhanced protein synthesis in muscle. Our previous studies have demonstrated an increase in muscle protein synthesis in response to clenbuterol as measured *in vivo*.[12]

Infusion of endotoxin caused only transient fever, hypophagia and suppression of body wieght. This tolerance is a common feature of responses to endotoxin in rodents. Recovery cannot be ascribed to degradation of the endotoxin or failure of the minipumps, since these elicited maximal responses when transplanted into naive rats (data not shown). The shorter protocol employed in the third experiment was used in an attempt to minimize the development of endotoxin tolerance and has been reported previously to cause muscle atrophy.[13] The results presented in Table 3 reveal significant loss of muscle and reduced body weight gain in rats injected with endotoxin. However tolerance to endotoxin had probably started to develop by the third day since body weight began to increase and body temperature had returned to normal. In this experiment, clenbuterol totally reversed the effects of endotoxin on body weight and muscle mass (Table 3). Weight gain of endotoxin-treated rats was increased by over two-fold by clenbuterol. This was again achieved without a change in food intake, resulting in a 114% rise in feed efficiency. Clenbuterol caused increases in gastrocnemius muscle mass and protein content of 14 and 17%, respectively, and stimulated RNA content by over 40%. This very large effect on RNA content is greater than we have usually observed (approximately 20%) in normal rats and supports the suggestion that clenbuterol stimulates muscle protein synthesis.

Overall, the present results demonstrate that clenbuterol significantly enhances body weight gain and muscle mass during endotoxaemia in the rat. The drug appears to be most effective during the recovery period, and has little effect on body weight over the early part of treatment. However, from the present data it is impossible to determine whether clenbuterol is active only during recovery, or whether it simply requires several days to exert its effects. It is difficult to resolve this problem in view of the rather short-lived effects of endotoxin in the rat. We are currently testing actions of clenbuterol in other models of wasting following bacterial and viral infections.

The mechanism of action of clenbuterol is unknown, but previous data suggest a direct action on muscle (see Ref. 4). In the present study, clenbuterol did not interfere with other actions of endotoxin, for example on fever or food intake, but may have modified hormonal responses to endotoxin. The results also suggest that β_2-adrenoceptor agonists may be of value in prevention of muscle wasting in human illness.

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