

Stimulation of muscle growth by clenbuterol: lack of effect on muscle protein biosynthesis

BY PETER J. REEDS, SUSAN M. HAY, PATRICIA M. DORWOOD
AND ROBERT M. PALMER

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

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1. Young rats were offered to appetite a semi-synthetic diet either alone or containing the β_2 -selective agonist clenbuterol (4-amino- α -[*t*-butylamino] methyl]-3,5-dichlorobenzyl alcohol).

2. In female rats (starting weight 116g) the presence of the drug at daily doses greater than 10 $\mu\text{g}/\text{kg}$ body-weight per d increased the growth of skeletal and cardiac muscle but had no stimulatory effect on the growth of the liver, gastrointestinal tract and kidney.

3. Male rats (starting weight 53 g) received clenbuterol at a daily oral dose of 200 $\mu\text{g}/\text{kg}$ body-weight per d. Animals were slaughtered after 0, 4, 8, 11, 18, 21 and 25 d of treatment. At 4, 11, 21 and 25 d muscle protein synthesis was measured by the method of Garlick *et al.* (1980). Although clenbuterol increased the rate of protein and RNA accretion in gastrocnemius and soleus muscles, protein synthesis was not increased.

4. The results suggested that the drug had a rapid, perhaps direct, inhibitory effect on protein degradation. It is concluded that the growth-promoting effect of clenbuterol may be specific to muscle and that the drug may act in a novel manner which circumvents the physiological mechanisms responsible for the control of muscle growth.

The use of selective (Rothwell *et al.* 1983*a*; Dulloo & Miller, 1984) and non-selective (Rothwell *et al.* 1983*b*) sympathomimetic agents in the manipulation of growth and body composition, and particularly in effecting a reduction in body fat mass, has received a great deal of attention in recent years. Agonists with α or β receptor specificity have proved effective in increasing daily energy expenditure and in lowering the rate of fat deposition. Although the effects of these agents on body protein mass have been variable, in general they reduce the rate of protein deposition (Dulloo & Miller, 1984). Recent work, however, has shown that some compounds with a marked degree of specificity for β_2 receptors, as defined by Lands *et al.* (1967), not only reduce body fat gain but also promote the deposition of body protein (Baker *et al.* 1983; Dalrymple *et al.* 1983; Rothwell *et al.* 1983*a*; Emery *et al.* 1984; Ricks *et al.* 1984). In this respect the action of these drugs and in particular clenbuterol is similar to that of some anabolic steroids (Vernon & Buttery, 1976, 1978; Loblely *et al.* 1982, 1983) but the growth-promoting effects of this drug show less species or sex specificity (Ricks *et al.* 1984) than steroidal growth-stimulating agents.

One characteristic of previous results of experiments on the action of clenbuterol has been an increase in muscle growth. It is likely that any significant increase in body protein will also involve some degree of muscle hypertrophy and it is not certain whether the effect of the drug is confined to muscle. Furthermore, with the exception of a single report (Emery *et al.* 1984), the changes in protein synthesis and degradation, which must underlie any change in protein deposition, have not been investigated. The measurement of these changes is a necessary first step in the investigation of the mechanism of action of clenbuterol. The present paper reports the results of such an investigation in immature rats.

MATERIALS AND METHODS

The reagents for the assays were purchased from Sigma Chemical Co. (Poole, Dorset). L-[2,6- ^3H]phenylalanine was obtained from Amersham International (Amersham, Bucks). Clenbuterol was kindly donated by Boehringer-Ingelheim (Bracknell, Berks).

Animals and feeding schedule

The animals were male or female Hooded-Lister rats of the Rowett strain. They were weaned at 19 d after birth and immediately divided into groups of six animals of equal mean weight. Animals that differed from the mean weight by more than 1 standard deviation were excluded. In a preliminary experiment only female rats were used, in the main experiment only male rats were used. The animals were housed initially in groups of six and offered stock rat diet *ad lib*. Their body-weights were measured daily over this time and after 4 d they were, if necessary, redivided so that the groups had equal mean weights and hence equal mean daily growth rates. They were then housed separately in racks containing six cages per row and offered a semi-synthetic diet (PW3; Pullar & Webster, 1977) *ad lib*. for 3 d. Although one group of rats occupied a row of cages, the position of a group between experiments was random. In the preliminary experiment, designed to establish an appropriate dose of clenbuterol, female rats were allowed to attain a body-weight of approximately 110 g (actual weight 116 g) before the start of the experimental period. In both experiments clenbuterol was administered orally by adding the powdered drug (as the hydrochloride) to the diet. Throughout the experiment the animals were housed in wire-mesh-floored cages at 23° in a room with a 12 h light–12 h dark cycle (lights on at 06.00 hours). Food intake, adjusted for spillage, and body-weights were measured daily between 09.00 and 10.30 hours

Dissection and the measurement of tissue protein synthesis

At various times after the start of clenbuterol administration, six animals from each group were killed by cervical dislocation and both hind-limbs were removed. The abdomen was opened, the gastrointestinal tract excluding the stomach but including contents, the whole liver, both kidneys and the whole heart were removed and weighed. The heart was opened and any adhering blood was allowed to drain out and the heart was blotted before weighing. The hind-limbs were then skinned and the gastrocnemius, soleus, plantaris and extensor digitorum longus muscles from both limbs were excised and weighed. All subsequent analyses were made on pooled samples of each muscle from both hind-limbs.

Protein synthesis was measured by the method of Garlick *et al.* (1980) at between 09.30 and 10.30 hours. All animals had full stomachs. Animals were injected, via a lateral tail vein, with 1.5 mmol L-phenylalanine/kg body-weight and 50 μ Ci (1.85 MBq) L-[2,6-³H]phenylalanine. The animals were killed exactly 10 min after the injection, the hind-limbs were removed, skinned and plunged into ice water (+ 30 s) and the liver (+ 50 s) and heart (+ 70 s) were removed, blotted and immediately frozen in liquid nitrogen. Blood was washed from the heart before it was frozen. The gastrocnemius, soleus and plantaris muscles were then excised, blotted thoroughly and frozen. The tissues were stored at –20° until analysed. The measurements of protein and RNA contents and the specific radioactivity of free- and protein-bound phenylalanine were as described previously (Garlick *et al.* 1980; McNurlan *et al.* 1982). The radioactivity in the samples was measured by scintillation counting using NE 265 (Nuclear Enterprises, Edinburgh). Protein synthesis was calculated as described by Garlick *et al.* (1980).

Calculation of protein degradation

The rate of muscle protein degradation was calculated from the difference between the fractional rate of protein synthesis and the fractional rate of protein deposition. The main problem in using this approach is the calculation of rates of protein deposition and hence degradation in individual animals. The rate of body-weight gain is unsatisfactory as in immature animals muscle protein is deposited at a faster fractional rate than is body-weight. There are, moreover, some small differences in fractional growth rates of individual muscles. We reasoned that if it could be demonstrated that, at a given age, the ratio of muscle protein

to some non-destructive measure, such as body-weight, was constant or had a low variability then this ratio could be used to predict the amount of protein in individual muscles. Thus, the rate of protein deposition between two times (t_1 and t_2) could be calculated from the difference between the predicted value at t_1 and the measured value at t_2 .

In the event we found that the coefficient of variation of gastrocnemius protein content:body-weight was 2.1% and for soleus protein content:body-weight 3.3%; each less than the coefficients of variation of body-weight (about 5%) and of muscle protein (gastrocnemius 5.0 and soleus 6.2%). Treatment with clenbuterol did not increase the variability of the ratio. The rate of protein deposition in the soleus and gastrocnemius muscles of the experimental animals was estimated as follows. Further groups of animals offered either the control or the clenbuterol diet were killed at the start of the experimental period, and at 8 and 18 d of treatment. Full dissection was carried out, muscle protein and RNA were measured and the protein mass of the muscles was expressed as a proportion of the body-weight. These animals were killed at the same time of day at which synthesis was measured in the other animals. From these results, as well as from those obtained from the animals killed at 4, 11, 21 and 25 d, the predicted muscle protein content at 0, 8 and 18 d in the experimental animals was calculated and used to predict the rate of protein gain between 0–4, 8–11, 18–21 and 21–25 d. The fractional rate of protein deposition was then calculated from the slope of the line of log protein content *v.* time by linear regression analysis including the preceding and succeeding time points.

Two points should be emphasized. First that this approach requires particularly close attention to matching animals for weight and growth rate in a preliminary period and second that although body-weight is the base measurement, rates of weight gain form no part of the calculation.

Statistics

The statistical significance of the differences in mean values was assessed by unpaired two-tailed *t* tests.

RESULTS

In a preliminary experiment, groups of female rats (starting weight 116 g) were offered diet PW3 either containing no clenbuterol (control) or clenbuterol at doses ranging from 0.1 to 50 mg/kg diet for 15 d. The results (Table 1) show the marked effect on the growth of the leg muscles and heart at daily doses greater than 10 μ g/kg body-weight. The maximum effect occurred at a dose of 100–1000 μ g/kg body-weight per d and in the subsequent experiment animals were offered diets containing the drug at 2 mg/kg diet. At doses of less than 50 mg/kg diet the drug had no effect on daily food intake, other than a reduction on day 1.

Changes in body-weight and tissue weight in young male rats at various times of clenbuterol administration are shown in Table 2. At every time point the same qualitative effects were noted; the weight of the soleus, plantaris and gastrocnemius muscles and the heart were significantly higher in the treated groups ($P < 0.01$) but the growth of the extensor digitorum longus muscle was affected to a lesser degree. The weight of the gastrointestinal tract (results not shown), liver and kidneys were not increased. After 11 d of treatment with clenbuterol, liver and kidney weights were significantly lower than those from control animals.

The changes in muscle weights were matched by increases both in protein and total RNA (Table 3) and after 4 d the concentrations of both protein and RNA were significantly higher in the muscles of animals that had received clenbuterol than those of control animals so that, at the earliest time-point, muscle protein accretion was increased to a slightly greater

Table 1. *Body- and tissue weights of groups of six female rats offered to appetite for 15 d diet PW3 (Pullar & Webster, 1977) containing no clenbuterol or clenbuterol at different concentrations*
(Mean values with their standard errors for six animals per group)

| Group... | 1 | | 2 | | 3 | | 4 | | 5 | |
|-----------------------------------|------|-----|--------|-----|--------|-----|--------|-----|------|-----|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Clenbuterol (mg/kg diet)... | 0 | | 0.1 | | 1.0 | | 10 | | 50 | |
| Body-wt (g): | | | | | | | | | | |
| Initial | 116 | 2 | 117 | 2 | 116 | 2 | 116 | 2 | 119 | 2 |
| Final | 151 | 3 | 168*** | 4 | 172*** | 3 | 156 | 3 | 141 | 4 |
| Food intake† (g/d) | 11.6 | 0.4 | 11.4 | 0.5 | 11.2 | 0.4 | 11.7 | 0.4 | 11.2 | 0.5 |
| Liver wt (mg) | 6035 | 142 | 6080 | 146 | 6601 | 150 | 5854 | 76 | 4785 | 130 |
| Kidney wt (mg) | 1880 | 73 | 1890 | 75 | 1932 | 72 | 1792 | 70 | 1718 | 77 |
| Heart wt (mg) | 703 | 26 | 832* | 22 | 908*** | 32 | 870*** | 16 | 778 | 23 |
| Gastrocnemius wt (mg) | 1431 | 22 | 1572* | 28 | 1630** | 29 | 1589* | 13 | 1510 | 96 |
| Soleus wt (mg) | 176 | 6 | 201** | 7 | 210** | 6 | 192* | 7 | 180 | 6 |
| Plantaris wt (mg) | 316 | 6 | 343** | 6 | 363*** | 8 | 350* | 6 | 333 | 7 |
| Extensor digitorum longus wt (mg) | 157 | 7 | 179* | 6 | 183** | 6 | 180* | 7 | 169 | 6 |

Mean values were significantly different (df 10) from those of group 1: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Average for whole 15 d period.

Table 2. *Body- and tissue weights of young male rats (starting age 4 weeks post partum) offered to appetite diet PW3 (Puller & Webster, 1977) or the same diet containing clenbuterol (2 mg/kg diet)*

(Mean values with a common estimate of variance, pooled standard deviation (PSD))

| Day of treatment... | | 0† | 4 | 11 | 21 | 25 | PSD | df |
|-----------------------------------|-------------|------|--------|---------|--------|--------|-----|----|
| No. in each group... | | 10 | 12 | 6 | 12 | 6 | | |
| Initial body-wt (g) | Control | 53.7 | 53.4 | 53.2 | 53.7 | 52.9 | 3.6 | 41 |
| | Clenbuterol | — | 53.2 | 53.3 | 53.2 | 52.9 | 3.2 | 41 |
| Daily food intake (g) | Control | — | 9.5 | 11.3 | 14.6 | 15.2 | 0.9 | 41 |
| | Clenbuterol | — | 9.4 | 11.8 | 14.5 | 15.2 | 0.9 | 41 |
| Final body-wt (g) | Control | — | 73.1 | 114.4 | 170.3 | 180.0 | 6.7 | 82 |
| | Clenbuterol | — | 76.9* | 121.9* | 175.0 | 185.3 | | |
| Liver wt (mg) | Control | 2700 | 3580 | 5710 | 8370 | 8580 | 320 | 82 |
| | Clenbuterol | | 3520 | 5410 | 7720* | 7910* | | |
| Kidney wt (mg) | Control | 870 | 908 | 1430 | 1900 | ND | 84 | 73 |
| | Clenbuterol | | 870 | 1370 | 1790* | ND | | |
| Heart wt (mg) | Control | 356 | 396 | 635 | 840 | 900 | 42 | 82 |
| | Clenbuterol | | 486*** | 736*** | 988*** | 1035** | | |
| Gastrocnemius wt (mg) | Control | 357 | 566 | 972 | 1442 | 1703 | 51 | 82 |
| | Clenbuterol | | 634** | 1228*** | 1641** | 1808** | | |
| Soleus wt (mg) | Control | 42 | 64 | 138 | 195 | 220 | 9 | 82 |
| | Clenbuterol | | 86*** | 153*** | 230*** | 256*** | | |
| Extensor digitorum longus wt (mg) | Control | 38 | 55 | 106 | 157 | ND | 13 | 73 |
| | Clenbuterol | | 63 | 147** | 179* | ND | | |
| Plantaris wt (mg) | Control | 76 | 123 | 198 | 358 | 417 | 21 | 82 |
| | Clenbuterol | | 158*** | 233** | 399* | 439* | | |

ND, not determined.

Mean values were significantly different from the control values at a time point (22 df at 4 and 21 d, 10 df at 11 and 25 d): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Initial slaughter group.

extent than muscle weight gain. RNA: protein was unaltered by clenbuterol treatment.

At no time-point was the rate of protein synthesis (either expressed as a fractional rate or against total RNA) increased by clenbuterol, even when the fractional growth rate of muscle protein had been increased by 40% (Table 4). At later time-points (21 and 25 d of treatment) there was a significant reduction in muscle protein synthesis in the clenbuterol-treated animals. Because the fractional muscle growth rate was significantly increased (particularly after 4 d of treatment) with no change in the rate of protein synthesis, it appeared that clenbuterol produced a marked and highly significant reduction in the rate of muscle protein degradation. It was clear that the effect of the drug on both fractional growth rate and protein degradation reduced with time and in the gastrocnemius was no longer significant by 25 d of treatment.

DISCUSSION

The present results confirm a number of previous reports of a stimulation of growth in mammals treated with oral clenbuterol (Baker *et al.* 1983, 1984; Ricks *et al.* 1984). They extend these observations by demonstrating that the effect can occur in very young animals whose growth was not nutritionally restrained and hence were presumably growing to their genetic potential. Although an effect on bone growth has not been excluded, the action of the drug appeared to be confined to muscle (both skeletal and cardiac). In fact rather than

Table 3. Protein (mg per muscle) and RNA (μg per muscle) contents and the ratio, muscle protein (mg): body-weight (g), of the gastrocnemius and soleus muscles of young male rats (starting age 4 weeks post partum) offered to appetite diet PW3 (Pullar & Webster, 1977) or the same diet containing clenbuterol (2 mg/kg diet)

(Mean values with their standard errors)

| Day of treatment... No. in each group... | 4 | | 8 | | 11 | | 18 | | 21 | | 25 | |
|---|-------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Protein content (mg) | | | | | | | | | | | | |
| Control | 55 | 2 | 85 | 4 | 122 | 5 | 163 | 2 | 237 | 7 | 256 | 7 |
| Clenbuterol | | | 103** | 4 | 156*** | 5 | 220*** | 5 | 269*** | 5 | 293*** | 5 |
| RNA content (μg) | | | | | | | | | | | | |
| Control | 814 | 23 | 1122 | 33 | 1163 | 23 | 1358 | 22 | 1806 | 57 | 1864 | 28 |
| Clenbuterol | | | 1377*** | 40 | 1369*** | 13 | 1783*** | 24 | 2121** | 66 | 2152*** | 56 |
| Muscle protein: body-wt (mg/g) | | | | | | | | | | | | |
| Control | 1.024 | 0.011 | 1.162 | 0.007 | 1.243 | 0.008 | 1.424 | 0.006 | 1.515 | 0.011 | 1.503 | 0.010 |
| Clenbuterol | | | 1.339 | 0.010 | 1.543 | 0.008 | 1.804 | 0.015 | 1.726 | 0.022 | 1.674 | 0.009 |
| Protein content (mg) | | | | | | | | | | | | |
| Control | 6.27 | 0.27 | 8.30 | 0.20 | 13.4 | 0.6 | 22.8 | 0.6 | 28.4 | 0.6 | 32.3 | 0.8 |
| Clenbuterol | | | 12.5*** | 0.20 | 16.6*** | 0.7 | 29.1*** | 1.0 | 34.2*** | 1.0 | 40.2*** | 1.0 |
| RNA content (μg) | | | | | | | | | | | | |
| Control | 103 | 3 | 123 | 4 | 176 | 7 | 242 | 8 | 262 | 8 | 265 | 12 |
| Clenbuterol | | | 195*** | 7 | 216*** | 6 | 309*** | 5 | 333** | 14 | 316* | 8 |
| Muscle protein: body-wt (mg/g) | | | | | | | | | | | | |
| Control | 0.116 | 0.001 | 0.114 | 0.001 | 0.136 | 0.002 | 0.199 | 0.002 | 0.182 | 0.002 | 0.190 | 0.002 |
| Clenbuterol | | | 0.162 | 0.001 | 0.164 | 0.001 | 0.238 | 0.003 | 0.219 | 0.002 | 0.229 | 0.002 |

Mean values were significantly different from control values at a time point (22 df at 4 and 21 d, 10 df at 8, 11, 18 and 25 d): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The increase in muscle protein:body-weight was significant at the 0.1% level at all time points.

† Initial slaughter group.

Table 4. Muscle protein synthesis rates (k_s %/d and k_{RNA} g protein/g RNA per d), fractional protein deposition rates (k_g %/d) and calculated degradation rates (k_d %/d) in gastrocnemius and soleus muscles of control rats and rats given clenbuterol (200 μ g/kg body-wt per d)

(Mean values with their standard errors for six animals per group)

| Day of treatment | | Gastrocnemius | | | | Soleus | | | |
|------------------|-----------|---------------|-----|-------------|-----|---------|-----|-------------|-----|
| | | Control | | Clenbuterol | | Control | | Clenbuterol | |
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 4 | k_s | 16.1 | 0.9 | 16.9 | 0.4 | 17.9 | 0.5 | 18.3 | 0.6 |
| | k_{RNA} | 12.2 | 0.4 | 12.3 | 0.2 | 12.1 | 0.8 | 11.7 | 0.3 |
| | k_g | 10.1 | 0.8 | 14.2*** | 0.5 | 9.7 | 0.6 | 14.6*** | 0.6 |
| | k_d | 6.0 | 0.7 | 2.7** | 0.7 | 8.2 | 0.4 | 3.7*** | 0.1 |
| 11 | k_s | 13.6 | 0.5 | 11.7 | 0.6 | 17.7 | 0.6 | 17.9 | 0.7 |
| | k_{RNA} | 16.4 | 0.6 | 14.4 | 0.6 | 16.7 | 0.4 | 16.9 | 0.4 |
| | k_g | 8.2 | 0.3 | 8.4 | 0.3 | 11.1 | 0.3 | 12.9* | 0.4 |
| | k_d | 5.4 | 0.5 | 3.3 | 0.4 | 6.7 | 0.8 | 5.0 | 0.3 |
| 21 | k_s | 11.9 | 0.6 | 9.7** | 0.2 | 14.6 | 0.4 | 11.7** | 0.6 |
| | k_{RNA} | 16.3 | 0.6 | 13.2*** | 0.2 | 17.8 | 0.4 | 14.8*** | 0.6 |
| | k_g | 4.6 | 0.6 | 3.8 | 0.3 | 5.0 | 0.4 | 5.0 | 0.3 |
| | k_d | 7.3 | 0.5 | 5.9* | 0.3 | 9.6 | 0.4 | 6.7* | 0.8 |
| 25 | k_s | 9.3 | 0.8 | 7.3 | 0.9 | 13.4 | 0.9 | 10.0** | 0.3 |
| | k_{RNA} | 16.3 | 1.2 | 12.4* | 1.1 | 20.3 | 0.8 | 16.6** | 0.8 |
| | k_g | 4.6 | 0.4 | 4.1 | 0.5 | 4.2 | 0.3 | 3.2 | 0.4 |
| | k_d | 4.7 | 0.8 | 3.2 | 0.3 | 9.2 | 0.8 | 6.8* | 0.2 |

Mean values were significantly different from control values (10 df): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

being increased, the growth of the liver and kidney was reduced in animals that had been exposed to the drug for some time. The effect of clenbuterol on muscle growth was rapid, being evident 4 d after administration, and the drug did not merely increase muscle weight but also increased the rate of muscle protein and RNA accretion.

The growth-promoting effect of clenbuterol became less with time. By 21 d, although the absolute rate of protein gain remained slightly elevated in the treated animals, the fractional rate of muscle growth was similar in both groups. Whether this reduction in the effect represented a true tachyphylaxis or whether it reflected an eventual limitation of muscle growth imposed by an unaltered rate of skeletal growth is not known.

Although the present results are in general agreement with those reported by Emery *et al.* (1984), they differ in a number of respects.

First, in our hands, clenbuterol had only a small effect on the rate of body-weight gain and, second, clenbuterol consistently increased heart mass and protein content. The first of these differences may relate to the facts that in the present experiments the animals received the drug by the oral route and at a much lower dose (approximately 200 μ g/kg body-weight per d) than that given subcutaneously (2 mg/kg body-weight per d) by Emery *et al.* (1984) and, perhaps associated with this, there was a lack of effect on food intake. Because clenbuterol has opposite effects on the deposition of both body protein and fat (Baker *et al.* 1984; Emery *et al.* 1984; Ricks *et al.* 1984), its effects on weight gain will depend critically on the relative degrees to which these components of body mass are affected. We have recently confirmed that body fat is decreased by clenbuterol at this low dose (P. J. Reeds and S. M. Hay, unpublished results). Also, there may be an interaction between the actions of clenbuterol on protein and fat deposition and age, as it appeared that in older female rats body-weight gain in clenbuterol-treated rats was increased to a greater extent

than in young male rats. At this stage we can offer no explanation for the difference in the response of heart mass except to note that repeated administration of the mixed β agonist isoprenaline increases the mass of the heart (Hill & Malamud, 1974; Deschaies *et al.* 1981).

Apart from the previous results reported by Emery *et al.* (1984), there have been no reports of systematic investigations of the changes in protein turnover that underlie the considerable increase in muscle protein accretion associated with clenbuterol treatment. In the present experiments it was clear that at no time was there an increase in either the fractional rate of muscle protein synthesis or in the rate of protein synthesis per unit total RNA. Indeed at later times both these estimates of protein synthesis were reduced significantly in animals receiving clenbuterol. Although the results suggest strongly that clenbuterol treatment reduced the rate of muscle protein degradation, some caution has to be exercised in the interpretation. The rate of protein synthesis was measured over a short period at between 09.30 and 10.30 hours and it is possible that protein synthesis was stimulated at some other time of day. This seems unlikely. Garlick *et al.* (1978) found no diurnal variation in muscle protein synthesis in normal rats fed *ad lib.* and although the animals ingest less diet during the light period than in the dark, all the animals in the present study had considerable amounts of food in their stomachs at the time of death. Furthermore, because the drug was administered in the diet, it seems unlikely that there would have been a marked variation in the rate of delivery of clenbuterol at different times of day. The lack of effect on muscle protein synthesis does, however, contrast with the previous observation of a significant (30%) stimulation of protein synthesis in gastrocnemius muscle of older rats injected with subcutaneous clenbuterol (Emery *et al.* 1984). Although there is no sure explanation for this difference it may relate to the somewhat different approaches that were adopted in the two experiments. Emery *et al.* (1984) measured muscle protein synthesis 1 h after a single subcutaneous injection of clenbuterol. As they pointed out, this time-interval was chosen to match the point at which the thermogenic action of clenbuterol was at a maximum, and it is possible that the increase in muscle protein synthesis (at a time when, in their experiment, muscle protein accretion apparently had not been increased) related to the thermogenic effect and not directly to the mechanism whereby the drug increases muscle growth. Indeed, Deschaies *et al.* (1981) have also observed a transient (1–2 h) increase in muscle protein synthesis following isoprenaline injection.

The apparent changes in muscle protein degradation in clenbuterol-treated animals are particularly interesting. The fact that this drug was able to stimulate muscle protein accretion in entire male rats that were growing at 10%/d, and did so without increasing their food intake, suggests that clenbuterol may, in some way, circumvent a mechanism that normally limits muscle growth, even in well-nourished animals. Potential for increased muscle growth clearly exists in these young rats as their hind-limb muscles will hypertrophy when subjected to an increased work-load (Goldspink *et al.* 1983). Because clenbuterol might alter activity or basal muscle tonus, it is possible that the mechanism of action of clenbuterol is similar to that of work-induced hypertrophy. This seems unlikely as after 3–4 d of overload hypertrophy (Goldspink *et al.* 1983), protein synthesis and degradation are both increased and clenbuterol treatment for 4 d stimulates neither process.

A second point of interest is the apparent similarity of the effect of clenbuterol to some aspects of the effect of trenbolone, a steroidal growth promotor which increases muscle growth and which also appears to reduce the rate of muscle protein degradation (Vernon & Buttery, 1976, 1978; Lobley *et al.* 1983; Sinnett-Smith *et al.* 1983). Taken at face value this similarity of effect suggests a similarity in mechanism but there are important differences in the actions of the two growth-promoting agents. Clenbuterol is quite clearly more generally active than trenbolone, which appears to be effective only in castrates and females and is, moreover, ineffective in some species. Furthermore, it appears that steroidal growth

promoters increase the protein mass of most organs (Vernon & Buttery, 1976, 1978) while the present results suggest that the action of clenbuterol is specific to muscle.

It is not certain whether the action of clenbuterol is direct or whether it interacts with some other hormonal system. The present results show that the effect of the drug is very rapid and, although the earliest measurements of muscle mass and composition were made after 4 d of treatment, there was a significant increase in body-weight gain on day 1 of treatment (control 4.3 (SEM 0.1) g/d, clenbuterol 4.6 (SEM 0.1) g/d; df 94; $P < 0.05$) despite a somewhat lower food intake in the treated animals on the 1st day of exposure to clenbuterol. This rapid response argues in favour of a direct action and this is supported by the fact that Emery *et al.* (1984) were unable to find changes in insulin, growth hormone and 3,5,3'-triiodothyronine levels in animals repeatedly injected with clenbuterol. An interaction with adrenal glucocorticoid action or secretion has, however, not been excluded. Furthermore there is evidence that the mixed β agonist isoprenaline reduces the rate of protein degradation in the perfused rat hemi-corpus (Li & Jefferson, 1977). It remains to be demonstrated that clenbuterol has a similar direct effect *in vitro*.

Although further comments on the mechanism of action of clenbuterol are premature, it is interesting that the drug is a potent bronchodilator and has been used in the control of premature labour (Kern, 1977). Both of these actions suggest that it may interfere with arachidonic acid metabolism, and there is evidence that this is so (Yamazaki *et al.* 1984). In view of recent work implicating the synthesis of prostaglandin E_2 in the control of muscle protein degradation (Rodemann & Goldberg, 1982; Palmer *et al.* 1985), it is tempting to speculate that clenbuterol may be acting by inhibiting the synthesis of this prostanoid.

Clenbuterol is, as pointed out by Ricks *et al.* (1984), an attractive candidate as an anti-obesity and growth-promoting agent. However, the large, apparently specific and potentially novel actions of the drug on skeletal muscle protein metabolism and deposition make it an important tool in the attainment of a greater insight into the mechanisms which lie at the basis of the control of muscle protein turnover and hence of the growth of this major component of body protein.

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