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The action of the β-agonist clenbuterol on protein metabolism in innervated and denervated phasic muscles

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1. Clenbuterol treatment in innervated and denervated phasic extensor digitorum longus, plantaris and gastrocnemius muscles from rats caused a significant increase in RNA and protein contents in all muscles except denervated extensor digitorum longus. 2. All muscles showed an increase in the fractional rate of protein synthesis (K_s) with clenbuterol, but the temporal response varied. 3. The data suggest that the effect of clenbuterol on protein metabolism in innervated muscles is muscle-type specific, and demonstrate the homology of response for denervated muscles.

INTRODUCTION

Dietary administration of the β-sympathomimetic, clenbuterol, has been shown to elicit a muscle-directed protein anabolic response in a variety of species (Ricks *et al.*, 1984; Jones *et al.*, 1985; Reeds *et al.*, 1986). In normal innervated rat muscles, of both predominantly slow- and fast-twitch fibre types, the response has been shown to be expressed as fibre hypertrophy (Maltin *et al.*, 1986*a*; Zeman *et al.*, 1988), resulting from a drug-mediated decrease in the fractional rate of protein degradation, with little or no change in the fractional rate of synthesis (Reeds *et al.*, 1986).

In contrast, clenbuterol has been shown to give rise to reversal or amelioration of denervation-induced atrophy in rat soleus muscles (Maltin *et al.*, 1986*b*; Zeman *et al.*, 1987), principally owing to a large increase in protein synthesis (Maltin *et al.*, 1987). This observation indicated that the responses of denervated and innervated muscles to clenbuterol are fundamentally different. These studies were carried out in denervated rat soleus muscles, which comprise predominantly slow-twitch fibres. Muscles comprising predominantly fast-twitch fibres, such as extensor digitorum longus (EDL), respond differently to a variety of stimuli, including clenbuterol treatment (Maltin *et al.*, 1986*a*), denervation (Cullen & Pluskal, 1977), immobilization (Booth, 1982), growth hormone (Prysor-Jones & Jenkins, 1980) and insulin (Preedy & Garlick, 1983) compared with soleus. Consequently, the present study was conducted to examine the effect of clenbuterol treatment on atrophy and protein metabolism in a group of denervated rat muscles which differ from soleus with respect to fibre-type composition, neural control and hormonal sensitivity.

MATERIALS AND METHODS

Animals and experimental protocol

Two identical experiments were carried out. The animals and experimental protocol were exactly the same as described previously (Maltin *et al.*, 1987). Briefly, male Hooded Lister weanling rats of the Rowett Research Institute strain were divided into 11 groups of 6 animals each. The rats were accustomed to the control diet (PW3: Pullar & Webster, 1977) before being subjected to unilateral removal of 0.7–1.2 cm of sciatic nerve under ether anaesthesia and aseptic conditions. On day 4 post-denervation, the rats were offered to appetite control diet (five groups) or diet containing clenbuterol (2 mg/kg: five groups). One group of rats was analysed as a zero-time control (i.e. 4 days post-denervation), and then one group from each of the control and clenbuterol-fed groups was analysed after 12, 24, 36, 72 and 168 h. The muscles analysed were extensor digitorum longus (EDL), plantaris (PL) and gastrocnemius (G) from both the innervated and denervated limbs. Samples were stored frozen according to the requirements of the subsequent analyses.

Expt. 1: muscle histochemistry

Muscle fibre-type assessment was based on the use of the Ca^{2+}-activated myofibrillar ATPase staining procedure from the method of Hayashi & Freiman (1966) as described previously (Maltin *et al.*, 1986*b*). Muscle samples were analysed at 0, 24, 36 and 72 h, and only from EDL and PL.

Expt. 2: determination of protein synthesis

The fractional rate of protein synthesis (K_s) was determined as described previously (Maltin *et al.*, 1987) by the methods of Garlick *et al.* (1980). Muscle protein and RNA contents were determined by the methods described by Reeds *et al.* (1986).

The fractional rate of degradation (K_d) at the measurement time was estimated as the difference between K_s and the fractional rate of protein gain (K_g). The latter was estimated from the difference in protein content between zero time and the experimental time (t), assumed to be linear, and expressed as a percentage of the muscle protein content at time t.

Absolute synthesis was calculated as the product of

Abbreviations used: EDL, extensor digitorum longus; G, gastrocnemius, PL, plantaris; K_s, fractional rate of protein synthesis (×100); K_d, fractional rate of protein degradation (×100); K_g, fractional rate of protein gain (×100); D.F., degrees of freedom; S.E.D., standard error of difference.

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protein content and fractional rate of protein synthesis at each time point.

Statistical analyses

Data on RNA and protein synthesis were collected from the EDL, PL and G muscles of 10 groups of 5 rats. The groups formed a 5×2 factorial, five times by control or clenbuterol treatment. Two sets of muscles were measured from each animal, one from the denervated left leg and the other from the innervated leg. For each variate the data were analysed by using a split-plot analysis of variance, comparisons of innervated versus denervated limbs used within animal error, and comparison of the effects of time and clenbuterol treatment used between animal error. The sums of squares for time were separated into linear and quadratic components. All analyses were carried out with the program Genstat V (Rothampstead Experimental Station, 1977) on a Prime 550 computer.

RESULTS

Effects of denervation

The changes in fibre size and protein mass after sciatic section were broadly the same as described for soleus muscle (Maltin *et al.*, 1987), and in agreement with many previous studies (e.g. Gutmann, 1963; Cullen & Pluskal, 1977; Goldspink, 1980). Denervation had a significant effect ($P < 0.001$) on all three muscles, with a slowing or cessation of growth over the period studied (Tables 1–3, Fig. 1). This was reflected in a decrease in both mean fibre cross-sectional area (Fig. 1) and total protein content (Tables 1–3). The atrophy was well progressed by day 4 post-denervation, i.e. at the start of clenbuterol administration, and protein contents were 82, 60 and 64% of innervated muscles for EDL, G and PL respectively, and there was a further decrease in protein content over the succeeding 7 days for G and PL. The RNA/protein ratio was elevated in denervated muscles (Tables 1–3), primarily owing to the decrease in protein content but also, in the case of EDL, because of increased RNA content.

Rates of protein synthesis in the three muscles responded differently to denervation. In G there was a transient reduction in K_s (Table 5), which had disappeared by day 3 (i.e. day 7 after denervation). In PL and EDL K_s had increased by day 4–5 after denervation (0–1 days in Tables 4 and 6) and remained significantly above that for the control limb. Absolute rates of synthesis were lower for denervated muscles at all times measured.

Effect of clenbuterol

Dietary administration of clenbuterol stimulated growth and protein gain significantly ($P < 0.05$) in both innervated and denervated PL and G (Tables 1–3, Fig. 1), similarly to that described previously for soleus (Maltin *et al.*, 1986*b*, 1987). In innervated muscles EDL again responded differently from G and PL. In EDL, protein and RNA contents increased in proportion, thereby maintaining the RNA/protein ratio, but in PL and G there was a significant but transient increase in the RNA/protein ratio. In the denervated muscles, higher RNA/protein ratios were maintained; again, in PL and G the drug appeared to accentuate these changes and to induce a significant further increase in the ratio, whereas in denervated EDL there was no significant difference from control denervated EDL muscles.

Table 1. Protein content and RNA/protein ratio in EDL

Male rats were subjected to unilateral sciatic section of the left leg. All animals were maintained on control diet for 4 days after denervation, at which time half the group were offered diet containing clenbuterol (2 mg/kg of diet) (see the Materials and methods section). Changes in mean protein content and mean RNA/protein ratio in innervated and denervated mucles from rats fed on control or clenbuterol (Clen)-containing diets from day 0 (4 days post-denervation) were measured. For effect of clenbuterol: †††$P < 0.001$. For effect of nerve status: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	Protein content (mg)			
	Innervated limb		Denervated limb	
Time (days)	Control	+Clen	Control	+Clen
0	4.9		4.0	
0.5	5.8	6.0	4.6**	4.5***
1.0	5.6	5.9	4.2**	4.9*
1.5	6.5	5.8	4.3***	4.9*
3.0	6.1	8.1†††	4.3***	4.8***
7.0	8.1	10.2†††	4.4***	5.2***

S.E.D. for effect of clenbuterol: 0.48 (40 D.F.)
S.E.D. for effect of nerve status: 0.42 (38 D.F.)

	RNA/protein (μg/mg)			
	Innervated limb		Denervated limb	
	Control	+Clen	Control	+Clen
0	16.2		21.0	
0.5	13.6	13.7	19.6***	20.9***
1.0	14.3	13.7	22.4***	21.0***
1.5	11.9	13.4	21.4***	23.6***
3.0	12.3	13.7	20.6***	21.8***
7.0	10.4	10.7	17.7***	17.1***

S.E.D. for effect of clenbuterol: 1.59 (40 D.F.)
S.E.D. for effect of nerve status: 1.05 (39 D.F.)

In innervated muscles, protein gain during 7 days of clenbuterol treatment was markedly increased (EDL 65%; G 41%; PL 72%) compared with untreated muscles. Most of the gain was achieved by day 3 and retained thereafter. Similar comparisons for the denervated limbs showed that clenbuterol administration did induce net protein anabolism in denervated muscles, i.e. the atrophy condition was partly reversed (Fig. 1; Tables 1–3), the absolute increases produced were equivalent to 26–38% of normal muscle growth and were smaller than for drug-treated innervated limbs. Improvements were more persistent over the 7 days treatment period for EDL and G than for PL. In both innervated and denervated muscles, the changes in protein content were closely paralleled by alterations in fibre area, treatment with clenbuterol producing amelioration of atrophy in denervated muscles as early as 36 h (Fig. 1).

Increases in protein gain induced by clenbuterol were mirrored by a stimulation of protein synthesis (Tables

Table 2. Protein content and RNA/protein ratios in gastrocnemius

All animals were treated as described in legend to Table 1. Changes in mean protein content and mean RNA/protein ratio in innervated and denervated gastrocnemius muscles from rats fed on control or clenbuterol (Clen)-containing diets from day 0 (4 days post-denervation) were measured. For effect of clenbuterol: †††$P < 0.001$. For effect of nerve status: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	Protein content (mg)			
	Innervated limb		Denervated limb	
Time (days)	Control	+Clen	Control	+Clen
0	48.1		29.1	
0.5	47.6	49.6	30.0***	28.4***
1.0	52.5	53.9	28.5***	28.4***
1.5	56.9	54.3	29.4***	30.7***
3.0	56.6	66.5†††	27.8***	30.8***
7.0	77.1	89.2†††	26.4***	36.6†††***

S.E.D. for effect of clenbuterol: 2.11 (40 D.F.)
S.E.D. for effect of nerve status: 2.13 (40 D.F.)

	RNA/protein (μg/mg)			
	Innervated limb		Denervated limb	
	Control	+Clen	Control	+Clen
0	15.9		17.0	
0.5	15.4	14.6	16.8	16.8*
1.0	15.2	15.5	17.2*	18.1**
1.5	13.8	15.8†	18.8***	19.7***
3.0	13.7	16.0†	16.3**	21.4†††***
7.0	12.7	13.5	15.9***	20.2†††***

S.E.D. for effect of clenbuterol: 0.89 (40 D.F.)
S.E.D. for effect of nerve status: 0.80 (40 D.F.)

Table 3. Protein content and RNA/protein ratio in plantaris

All animals were treated as described in legend to Table 1. Changes in mean protein content and mean RNA/protein ratio in innervated and denervated plantaris muscles from rats fed on control or clenbuterol (Clen)-containing diets from day 0 (4 days post-denervation) were measured. For effect of clenbuterol: †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$. For effect of nerve status: ***$P < 0.001$.

	Protein content (mg)			
	Innervated limb		Denervated limb	
Time (days)	Control	+Clen	Control	+Clen
0	11.1		7.1	
0.5	11.1	11.7	6.9***	7.7***
1.0	11.5	12.4	7.7***	7.4***
1.5	13.2	12.3	7.3***	7.3***
3.0	13.8	15.7†††	7.0***	8.6††***
7.0	15.8	19.2†††	6.7***	8.4†††***

S.E.D. for effect of clenbuterol: 0.60 (40 D.F.).
S.E.D. for effect of nerve status: 0.46 (30 D.F.)

	RNA/protein (μg/mg)			
	Innervated limb		Denervative limb	
	Control	+Clen	Control	+Clen
0	12.2		16.5	
0.5	12.2	11.8	17.2***	17.1***
1.0	12.6	12.0	16.8***	19.1††***
1.5	10.9	12.8†	17.0***	20.1†††***
3.0	12.4	14.0	16.0***	19.0†††***
7.0	10.7	12.3	16.6***	17.4***

S.E.D. for effect of clenbuterol: 0.82 (40 D.F.)
S.E.D. for effect of nerve status: 0.64 (38 D.F.)

4–6). In innervated muscles increases in K_s were 29–36% ($P < 0.05$) at 3 days, but had returned to control values by 7 days. No change in the 'translational efficiency' (K_s/RNA, i.e. g of protein synthesized/day per g of total RNA) occurred (Tables 4–6), and the extra synthesis appeared to be associated with the increase in total RNA content (from Tables 1–3).

In denervated muscles the drug induced generally earlier and more persistent increases in K_s (Tables 4–6); responses of EDL and PL were maximal at 1.5 and 3 days, but K_s was still elevated at 7 days. For G the increase in K_s was not maximal until 3 days, and was still pronounced at 7 days. In all three muscles improved rates of protein gain coincided with the period of elevated K_s (Tables 4–6).

The 'translational efficiency' was also increased in the denervated muscles from drug-treated animals, such that it was almost restored to that found in normal innervated muscles from untreated animals.

Calculation of K_d by difference assumes linear protein gain; in the present study this assumption is invalid certainly after day 3 of clenbuterol treatment. Only for the first 24 h of drug treatment was there a consistent effect for innervated muscles, when the increases in K_s were insufficient to account alone for the change in protein mass; these values necessitate accurate determination of small differences, however, and should be treated with caution. For most circumstances, in denervated muscles changes in K_s exceeded increases in the rate of protein gain.

DISCUSSION

It is now well established that clenbuterol can elicit a protein anabolic response in both innervated (Emery *et al.*, 1984; Reeds *et al.*, 1986) and denervated (Maltin *et al.*, 1986*a*; Zeman *et al.*, 1987) rat muscle. The protein anabolic responses in denervated muscle appear to arise either from improvements in K_s, as for the phasic muscles reported in the present study, or from decreases in K_d, observed in companion data from tonic soleus muscle (Maltin *et al.*, 1987). These differences reflect the current confusion and controversy which surround the effect of β-agonists on muscle protein metabolism.

The initial experiments of Emery *et al.* (1984) showed a 34% increase in K_s in normal gastrocnemius after 7 days of twice-daily injections of large doses of clenbuterol (2 mg/kg body wt. per day), but Reeds *et al.* (1986),

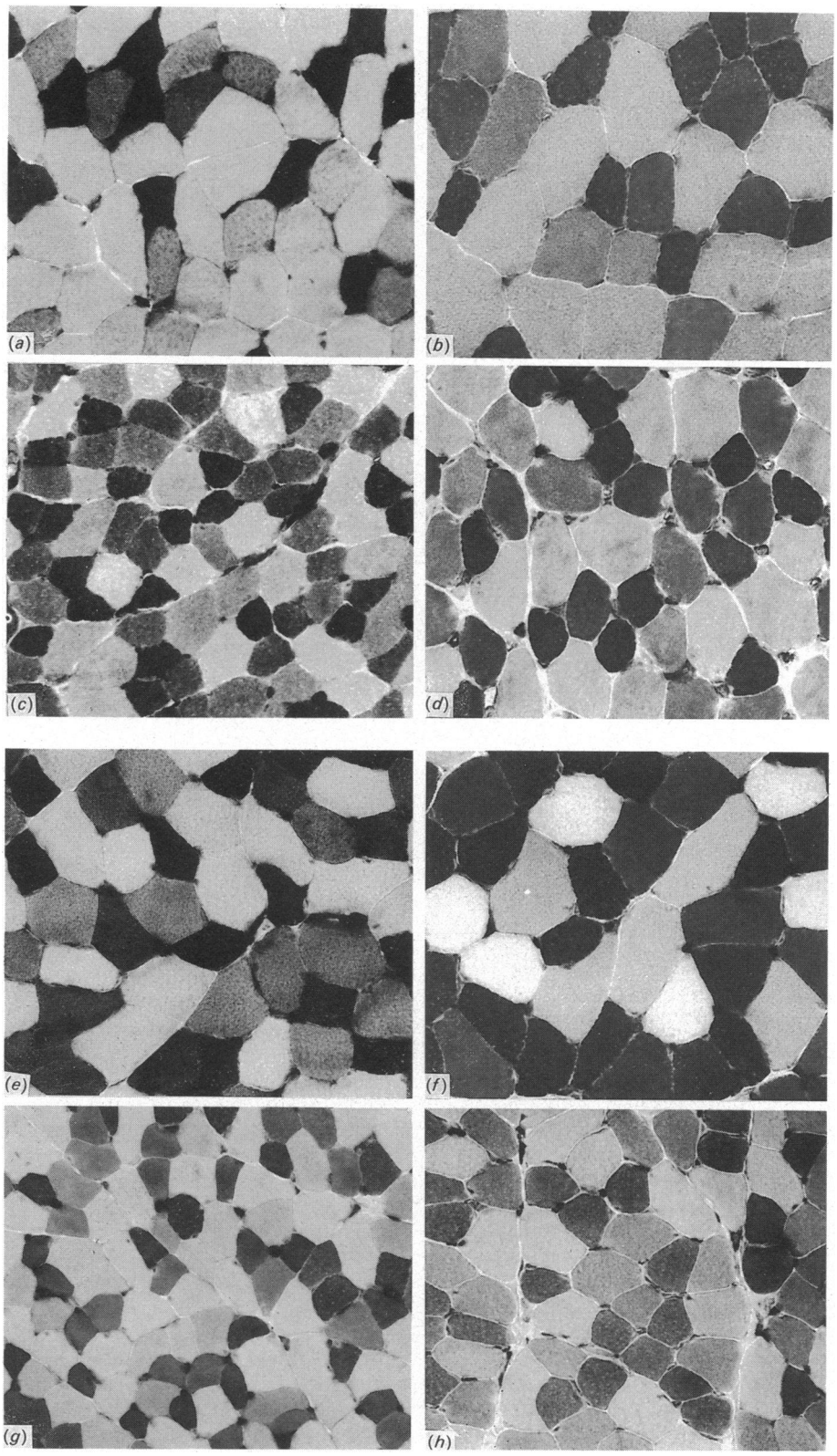

Fig. 1. Transverse cross-sections of muscles treated to demonstrate the activity of Ca^{2+}-activated myofibrillar ATPase

(*a*) and (*b*), Innervated EDL from rat fed on (*a*) control diet or (*b*) clenbuterol-containing diet for 36 h. (*c*) and (*d*), 4-days-denervated EDL contralateral from rat as in (*a*) and (*b*) respectively. (*e*) and (*f*), Innervated PL from rat fed on (*e*) control diet or (*b*) clenbuterol-containing diet for 36 h. (*g*) and (*h*), 4-days denervated PL contralateral from rat as in (*e*) and (*f*) respectively. Bar = 50 μm.

Table 4. Protein metabolism in EDL

All animals were treated as decribed in legend to Table 1. Changes in mean K_s and mean K_s/RNA in innervated and denervated EDL muscles from rats fed on control or clenbuterol (Clen)-containing diets from day 0 (4 days post-denervation) were measured. For effect of clenbuterol: †$P < 0.05$, ††$P < 0.01$. For effect of nerve status: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	K_s (%/day)			
	Innervated limb		Denervated limb	
Time (days)	Control	+Clen	Control	+Clen
0	17.1		19.1	
0.5	16.3	15.6	22.6***	28.9††***
1.0	17.0	18.1	22.0**	25.9***
1.5	16.8	19.8	22.3**	28.2††***
3.0	15.6	20.2†	20.4**	22.6
7.0	15.0	14.4	20.0**	25.4†***

S.E.D. for effect of clenbuterol: 2.03 (39 D.F.)
S.E.D. for effect of nerve status: 1.75 (38 D.F.)

	K_s/RNA (g of protein synthesized/day per g of RNA)			
	Innervated limb		Denervated limb	
	Control	+Clen	Control	+Clen
0	11.0		10.9	
0.5	12.6	12.5	11.6	13.9
1.0	12.1	13.5	9.9	12.9
1.5	14.2	14.9	10.7**	12.5*
3.0	12.9	14.9	10.0*	10.4***
7.0	14.5	13.5	11.4**	14.9†

S.E.D. for effect of clenbuterol: 1.64 (39 D.F.)
S.E.D. for effect of nerve status: 1.14 (38 D.F.)

Table 5. Protein metabolism in gastrocnemius

All animals were treated as described in legend to Table 1. Changes in mean K_s and mean K_s/RNA in innervated and denervated gastrocnemius muscles from rats fed on control or clenbuterol (Clen)-containing diets from day 0 (4 days post-denervation) were measured. For effect of clenbuterol: ††$P < 0.01$, †††$P < 0.001$. For effect of nerve status: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	K_s (%/day)			
	Innervated limb		Denervated limb	
Time (days)	Control	+Clen	Control	+Clen
0	17.1		13.8	
0.5	17.2	18.0	14.0**	16.7
1.0	18.6	19.4	15.1**	19.3††
1.5	18.1	20.9	16.9	22.2††
3.0	16.1	21.0†††	15.9	23.4*†††
7.0	15.9	15.5	16.3	23.2***†††

S.E.D. for effect of clenbuterol: 1.64 (39 D.F.)
S.E.D. for effect of nerve status: 1.15 (39 D.F.)

	K_s/RNA (g of protein synthesized/day per g of RNA)			
	Innervated limb		Denervated limb	
	Control	+Clen	Control	+Clen
0	10.9		8.2	
0.5	11.3	12.4	8.5**	10.0**
1.0	12.3	12.5	8.8***	10.6*
1.5	13.1	13.3	9.2***	11.2*
3.0	11.7	13.2	9.8**	11.0*
7.0	12.6	11.3	10.2**	11.4

S.E.D. for effect of clenbuterol: 1.10 (39 D.F.)
S.E.D. for effect of nerve status: 0.84 (39 D.F.)

using dietary administration of the drug (equivalent to 200 μg/kg body wt. per day), observed no change in K_s at various times from 4 to 25 days, and also none at 20 h (P. J. Reeds & S. M. Hay, unpublished work), although the protein anabolic effect persisted until at least day 11. Reeds *et al.* (1986) concluded that the major control was through inhibition of protein degradation. In a subsequent study with particular emphasis on the early time points, this contention remained true for innervated soleus (Maltin *et al.*, 1987). This conclusion appeared consistent with two previous observations. Garber *et al.* (1976) showed that adrenaline and the β-agonist isoprenaline inhibited the release of glutamine and alanine from isolated rat epitrochlearis muscle. Similarly, Li & Jefferson (1977) found that isoprenaline had no effect on K_s, but decreased K_d in the isolated rat hemicorpus. More recently, in their studies on myoblasts treated with the β-agonist cimaterol, Forsberg & Merrill (1986) again found no change in K_s but an 18% decrease in K_d. Similarly, Wang *et al.* (1988) reported a decrease in a Ca^{2+}-dependent proteinase in the muscle of cimaterol-treated lambs. Hence, from these and other pieces of evidence it was concluded that the action of β-agonists, which caused muscle protein anabolism, such as clenbuterol and cimaterol, was mediated through regulation of protein breakdown.

However, in contrast, the Snell dwarf mouse, which has a pituitary defect, responds to clenbuterol with an increase in K_s in gastrocnemius but no change in K_d (Pell *et al.*, 1987). Similarly, when the β-agonist ractopamine was given to pigs, it was reported to increase the synthesis of muscle α-actin by 50% and to increase the cellular concentration of α-actin mRNA by 2–3-fold (Helferich *et al.*, 1988).

The present study showed that in innervated phasic muscles there was a rapid initial (after 36–72 h treatment) increase in K_s caused by clenbuterol. Hence it might be suggested that this early and apparently transient increase in K_s had been missed by Reeds *et al.* (1986). However, Reeds *et al.* (1986) used non-denervated animals, and it is possible that the innervated muscles in a unilaterally denervated animal used in the present study may have been subjected to increased work load, and this in itself has been shown to increase protein synthesis and protein

Table 6. Protein metabolism in plantaris

All animals were treated as described in legend to Table 1. Changes in mean K_s and mean K_s/RNA in innervated and denervated plantaris muscles from rats fed on control or clenbuterol (Clen)-containing diets from day 0 (4 days post-denervation) were measured. For effect of clenbuterol: †$P < 0.05$, †††$P < 0.001$. For effect of nerve status: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	K_s (%/day)			
	Innervated limb		Denervated limb	
Time (days)	Control	+Clen	Control	+Clen
0	17.7		16.9	
0.5	17.5	19.5	19.5*	22.1*
1.0	18.9	20.5	22.3**	25.0***
1.5	18.8	22.1†	21.9**	29.8†††***
3.0	17.0	23.1†††	21.5***	24.7†
7.0	16.5	15.8	22.6***	24.5***

S.E.D. for effect of clenbuterol: 1.56 (40 D.F.)
S.E.D. for effect of nerve status: 0.99 (40 D.F.)

	K_s/RNA (g of protein synthesized/day per g of RNA)			
	Innervated limb		Denervated limb	
	Control	+Clen	Control	+Clen
0	14.5		10.3	
0.5	14.5	16.6	11.4**	13.0
1.0	15.1	17.1	12.7*	13.1**
1.5	17.6	17.4	12.9***	14.8*
3.0	14.1	16.6	13.4	13.9**
7.0	15.6	14.1	13.6	14.1

S.E.D. for effect of clenbuterol: 1.26 (40 D.F.)
S.E.D. for effect of nerve status: 1.07 (38 D.F.)

and RNA contents (Laurent *et al.*, 1978; McMillan *et al.*, 1987). The mechanisms by which RNA accretion (and thus the potential for protein synthesis) is stimulated under conditions of work hypertrophy appear to be augmented by the use of clenbuterol (Maltin *et al.*, 1987). In the present study the protein anabolism was expressed predominantly within the first 3 days, which contrasts with the more prolonged action of clenbuterol described by Reeds *et al.* (1986). The joint stimuli of work and clenbuterol may restrict extension of the myotrophic response in phasic muscles.

In innervated muscles the basis for differences in the response of EDL, PL and G to clenbuterol compared with that of innervated soleus is of particular interest. It is well established that different muscles show different responses to a variety of stimuli (Rannels & Jefferson, 1980; Deshaies *et al.*, 1981; Odedra & Millward, 1982; Preedy & Garlick, 1983; Turinsky, 1987), and in general it is soleus which appears to stand out as unusual. For example, phasic muscles (e.g. EDL, PL and G), dominated in composition by fast-twitch glycolytic and fast-twitch oxidative glycolytic fibres, show responses of protein metabolism to insulin administration, but have lower rates of synthesis than do tonic muscles (e.g. soleus), which are insulin-insensitive and are dominated by slow-twitch oxidative fibres. A difference in sensitivity to clenbuterol has previously been observed between innervated soleus and EDL (Maltin *et al.*, 1986*b*); soleus showed a more rapid and extensive response to the drug than did EDL. The RNA concentration, and hence the capacity to alter K_s, may also be near maximal in tonic muscles, so anabolic responses can only be achieved in soleus through a decrease in K_d. In contrast, the potential to augment RNA content does exist in phasic muscle, as shown by metabolic and compositional changes induced by work hypertrophy (Maltin *et al.*, 1987), allowing part, at least, of any response to involve stimulation of K_s. These effects may also be accompanied by a depression in K_d, analogous to soleus, but this may be masked by the rapid nature of the changes and the technical difficulties associated with estimation of K_d by difference.

After denervation, the changes entrained in muscle are well documented. The anabolic response of denervated phasic muscles to clenbuterol is associated with a rapid and fairly persistent increase in K_s (Maltin *et al.*, 1987). In innervated soleus, however, the anabolic response appeared to be mediated by decreases in K_d. As discussed previously (Maltin *et al.*, 1987), these response differences between neural states could be attributed to different contents of receptors (Banerjee *et al.*, 1977), although it is unlikely that these would be β-adrenoceptors (Reeds *et al.*, 1988). Alternatively, clenbuterol might be mimicking or exerting its effect through a myotrophic growth factor (see, e.g., Davis & Kiernan, 1980), the receptors for which are increased in number after denervation. The effect may be analogous to the restoration of K_s/RNA to innervated-muscle control values induced by insulin action when rats are refed after an overnight fast (Garlick *et al.*, 1983).

Thus at present it is not known how the anabolic response of clenbuterol is mediated in either innervated or denervated muscle, or which receptors are involved; direct involvement of β-adrenergic receptors seems unlikely. If clenbuterol mediates or mimics the action of a local or systemic factor, the mechanism may still involve receptors which show differential sensitivity between muscle types and nerve status.

In conclusion, the data demonstrate that in the innervated state response of a muscle to clenbuterol in terms of protein kinetics is dependent on the specific muscle in question. In contrast, denervated muscles of a variety of types show a homology of response not evident in innervated muscles.

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