Attenuating effects of clenbuterol, β2-agonist, on immobilization-induced atrophy of rat hindlimb muscle fibers

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Abstract In this review, we discuss the morphologic influence of clenbuterol on muscle atrophy with particular focus on cast-immobilization used in surgical care. β2-agonists induce muscle hypertrophy, particularly in fast muscles. Similar to these anabolic actions, the β2-agonist clenbuterol attenuates immobilization-induced atrophy in fast muscles. Furthermore, the attenuating effects of this agonist on muscle atrophy may be related to its preventative effect in fast muscle fibers.

Keywords: clenbuterol, immobilization, hindlimb muscle, atrophy, β2-agonist, attenuating effects

Introduction

Skeletal muscles are heterogeneous and are composed of slow- and fast-twitch fibers that differ in the composition of contractile proteins, oxidative capacity, and substrate preference for ATP production1-4). Slow-twitch fibers display low fatigability, high oxidative capacity, and a preference for fatty acids as substrates for ATP production4). Fast-twitch fibers have higher fatigability, greater contractile strength, lower oxidative capacity, and a preference for anaerobic glycolysis4). Thus, the fiber-type composition of skeletal muscle profoundly affects energy consumption.

The β2-agonist clenbuterol [CLE; 4-amino-α-(t-butylamino)methyl-3, 5-dichlorobenzyl alcohol], has been used as a non-steroidal anabolic drug for sport doping5). CLE is also used as a therapeutic agent in patients with asthma, where it acts as a bronchodilator that exerts a relaxing effect on bronchial smooth muscles6). In addition to bronchodilation, CLE has many physiological and pharmacological actions such as lipolysis, glycolysis, glycogenolysis, vasodilatation, and cardiac effects1-3,6-9). According to the “Clenbuterol Information (2014)” of the World Anti-Doping Agency (WADA), the need for athletes to exercise extreme caution while consuming meat when traveling to competitions in certain parts of the world has been emphasized. The reason for this is that in the livestock industry in certain parts of the world, CLE has also been utilized to increase lean muscle mass and reduce fat content, and consuming these meats may result in doping. CLE therefore causes an increase in skeletal muscle mass in both livestock and humans.

To investigate the attenuating effects of CLE on muscle atrophy in catabolic conditions such as denervation, unweighting, and joint immobilization, many studies have been conducted using laboratory animals10-20). Furthermore, the effects of CLE on the recovery of muscle strength and area in humans have been investigated after open medial meniscectomy21). Thus, CLE is expected to have significant therapeutic actions in rehabilitation. However, these effects of CLE have not been clarified with respect to atrophied slow and fast muscles under various conditions. Therefore, in this paper, the morphologic influence of CLE on muscle atrophy is discussed, with particular focus on cast-immobilization used in surgical care.

Attenuating effects of CLE on skeletal muscles in various catabolic conditions

As shown in Table 1, previous reports have focused on the attenuating effects of CLE on morphological properties of muscle fibers. Our report was the first study that investigated attenuating effects of CLE on both fast and slow muscles in the cast-immobilized hindlimbs of rats20). Under the various conditions listed in Table 1, the preventative action of CLE was seen in the fast muscle mass. These results suggest that CLE might attenuate muscle atrophy of fast muscle regardless of varying catabolic conditions. On the other hand, the preventative action of CLE on the slow muscle mass was seen under denervation and unweighting conditions, but the same actions were not seen in these two studies under the immobilized condition19,20). From these results, it was thought that the
Table 1. Responses of weights and fiber cross-sectional areas of skeletal muscle to $\beta_2$-agonist clenbuterol.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Species</th>
<th>Skeletal muscle</th>
<th>Weight, Relative weight (RW)</th>
<th>Cross-sectional area (% area)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Denervation + Clenbuterol</strong></td>
<td>Rat</td>
<td>SOL</td>
<td>SO †, FOG † (% area SO †, FOG ↓)</td>
<td>Maltin et al. 1986 [10]</td>
<td></td>
</tr>
<tr>
<td>Clenbuterol (2mg/kg/day, 4 days)</td>
<td>23 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clenbuterol (~1.6mg/kg/day, 2-3 weeks)</td>
<td>Rat, Infant</td>
<td>EDL †, TA †, SOL †, GAS †</td>
<td>EDL → SOL →</td>
<td>Zeman et al. 1987 [11]</td>
<td></td>
</tr>
<tr>
<td>Clenbuterol, diet (2mg/kg/day, 3 days)</td>
<td>Rat</td>
<td>PLA †</td>
<td>PLA n.d. (%area PLA n.d.)</td>
<td>Maltin et al. 1989 [12]</td>
<td></td>
</tr>
<tr>
<td>Clenbuterol (1, 10, 200μg/kg/day, 3 days)</td>
<td>Rat, Infant</td>
<td>SOL; 1μg →, 10μg ↑, 200μg ↑</td>
<td>SOL; FOG ↑, SO ↑ (%area SO ↑, FOG →)</td>
<td>Maltin et al. 1992 [13]</td>
<td></td>
</tr>
<tr>
<td><strong>Unweighting + Clenbuterol</strong></td>
<td>Rat</td>
<td>SOL →, SOL(RW) ↑</td>
<td>ST †, FT †</td>
<td>Ricart-Firinga et al 2000 [14]</td>
<td></td>
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<tr>
<td>Clenbuterol (0.6mg/day, 2 weeks)</td>
<td>Adult</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Clenbuterol (10μg/kg/day, 3 weeks)</td>
<td>Rat, 3, 38mo</td>
<td>PLA; young ․, old ․, SOL; young ․, old ․</td>
<td>PLA; young ․, old ․</td>
<td>Chen et al. 2000 [15]</td>
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<tr>
<td>Clenbuterol, diet (2mg/kg/day, 2 weeks)</td>
<td>Rat, 12, 30mo</td>
<td>EDL; 12mo †, 30mo †</td>
<td>EDL; 30mo Type I †, II †</td>
<td>Herrera et al. 2001 [16]</td>
<td></td>
</tr>
<tr>
<td>Clenbuterol, diet (1.0mg/kg/day, 2 weeks)</td>
<td>Rat, 8wk</td>
<td>EDL; EDL(RW) †</td>
<td>EDL; Type I †, II †</td>
<td>Yamazaki et al. 2005 [17]</td>
<td></td>
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<tr>
<td>Clenbuterol, diet (1.0mg/kg/day, 2 weeks)</td>
<td>Rat, 7wk</td>
<td>SOL †, SOL(RW) †</td>
<td>SOL; Type I †, II †</td>
<td>Yamazaki et al. 2009 [18]</td>
<td></td>
</tr>
<tr>
<td><strong>Immobilization + Clenbuterol</strong></td>
<td>Rat</td>
<td>SOL †</td>
<td>SOL †</td>
<td>Cancelliero et al. 2008 [19]</td>
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<tr>
<td>Clenbuterol (10μg/kg/day, 7 days)</td>
<td>3, 4mo</td>
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<tr>
<td>Clenbuterol, diet (1mg/kg/day, 9 days)</td>
<td>Rat</td>
<td>EDL †</td>
<td>EDL; Type I †, IIa ․, IIb →</td>
<td>Suzuki et al. 2014 [20]</td>
<td></td>
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</tbody>
</table>

SOL, soleus muscle; EDL, extensor digitorum longs muscle; TA, tibialis anterior muscle; GAS, gastrocnemius muscle; PLA, plantaris muscle; SO, slow-twitch oxidative fiber; FOG, fast-twitch oxidative glycolytic fiber; ST, slow-twitch fiber; FT, fast-twitch fiber; Type I, type I fiber; Type II, type II fiber; Up arrow, increase; lateral arrow, no change; n. d., no data.
attenuating effects of CLE on slow muscles in denervation and unweighting conditions were more remarkable than in the immobilized condition.

Effects of CLE on immobilized fast muscles

We administered CLE (1 mg/kg body weight per day) to cast-immobilized rats (IMM) for 9 days. As shown in Fig. 1A, CLE attenuated 87.6% of the decrease in weight of the immobilized fast muscle, extensor digitorum longus (EDL). On the other hand, many studies have reported the anabolic effects of CLE administration on fast muscles under normal conditions. Therefore, it is thought that the anabolic actions of CLE contributed to its attenuating effects on the reduction of weight in the immobilized EDL muscle.

As shown in Fig. 2, the cross-sectional areas of all types of fibers of the EDL were 20% greater in the CLE+IMM group than in the IMM group. Type IIa (12.7%) and IIb (85.7%) fibers were predominant in the fiber type composition of EDL muscle. Therefore, the attenuating effects of CLE on immobilization-induced atrophy of the EDL may be related to the preventative effect on atrophic Type II fibers. These results are consistent with the results of previous studies. On the other hand, the cross-sectional area of Type I fibers in the EDL did not differ between the control and IMM groups, suggesting that Type I fibers in the EDL muscle were recruited for movement during immobilization. The toes of the hindlimbs were free from immobilization; because the contraction of the EDL extends to the toes, the Type I fibers in this muscle may have been recruited during immobilization. However, these results have not been independently confirmed.

Effects of CLE on immobilized slow muscles

As shown in Fig. 1B, CLE attenuated only 11.5% of the decrease in weight of the immobilized-slow muscle, soleus (SOL). Cancelliero et al. have reported a significant preventative effect on the immobilized SOL muscle in a similar study.

Furthermore, several studies have examined the anabolic effects of CLE in the normal SOL muscle; however, these results were not consistent. On the other hand, most studies have reported the attenuating effects of CLE on atrophic SOL muscle in catabolic conditions such as denervation and unweighting. Therefore, these results may suggest that the anabolic effect on the SOL muscle is stronger under catabolic conditions than under normal conditions.

As shown in Fig. 2, we did not observe a significant ef-
Effect of CLE on the cross-sectional area of SOL muscle fibers. However, the cross-sectional area of Type IIa fibers of the SOL in the CLE+IMM group was 9.5% greater than that in the IMM group. This result suggests that the attenuating effect of CLE on immobilization-induced atrophy of the SOL may be related to the preventative effect in atrophic Type II fibers.

Differences in the effects of CLE between fast and slow muscles

We have reported that the administration of CLE induced hypertrophy and decreased β2-adrenergic receptor (AR) mRNA expression of the fast muscle without inducing these changes in the slow muscle26). Furthermore, many studies have suggested that β2-AR signaling pathways of synthesis and degradation of protein are different between fast and slow muscles, and that this agonist induces hypertrophy in fast muscles rather than in slow muscles30-34). These results suggest that the effects of β2-AR stimulation with CLE differed between fast and slow muscles, and that the hypertrophy induced by this agonist occurred more clearly in fast muscles than in slow muscles. Similarly, in our study, the immobilization-induced atrophy was attenuated to a greater extent in the EDL than in the SOL. It is thought that the attenuating effects of CLE on immobilized muscles were reflected in the responses of β2-AR signaling pathways to CLE administration in each fiber type.

Furthermore, we reported that cast-immobilization-induced muscle disuse down-regulated glucocorticoid receptor (GR) expression in the slow muscles35). These results suggest that muscle disuse suppresses glucocorticoid signals such as muscle protein breakdown and transcription of the β2-AR gene via down-regulation of GR expression in slow muscles. Therefore, it was thought that cast-immobilization had a different influence on the β2-AR signaling pathways of the fast and slow muscles.

In our study, the attenuating effects of CLE on muscle fiber atrophy were different between the fast and slow fibers in each type of skeletal muscle. However, there have been no biochemical reports that examined the preventative effects of CLE on each type of muscle fiber. Further studies are required to clarify these issues.

Conclusion

It is thought that the β2-agonist CLE attenuates immobilization-induced atrophy to a greater extent in the fast muscle than in the slow muscle and that the attenuating effect of this agonist on muscle atrophy may be related to its preventative effect on fast muscle fibers. In almost all of the studies that investigated the attenuating effects of CLE on muscle atrophy, a dose of CLE beyond the physiological level was used. Therefore, to confirm the therapeutic actions of CLE in rehabilitation, studies performed under various conditions, including a physiological dosage, are required.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

References


