Suppression of Immune Response in Lambs During Treatment with the Beta-Adrenergic Agonist Clenbuterol

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ABSTRACT: The effect of the beta-adrenergic agonist clenbuterol on immune function was examined in sheep. Twenty ewe lambs were housed indoors, with food and water available on an ad libitum basis, and immunized against somatostatin (SRIF) using a SRIF-ovalbumin conjugate. Ten of the lambs were also treated with clenbuterol (400 µg/kg) each day; 10 controls were not treated. After 5 wk of treatment (with booster injections of the SRIF conjugate each fortnight), the lambs were bled and then slaughtered for carcass composition. The lambs that received immunization alone produced significant antibody titers against SRIF, whereas 9 of the 10 clenbuterol-treated lambs produced no significant, specific antibody response. There was no effect of clenbuterol treatment on liver, thymus, spleen, kidney, kidney fat, or biceps femoris weight compared with those lambs that were only immunized. These results indicate that treatment with clenbuterol may inhibit humoral antibody response to infection.

Key Words: Immunity, Immune Response, Clenbuterol, Beta-Adrenergic Agonists, Antibodies, Sheep

Introduction

Chronic stress is associated with increased susceptibility to infectious diseases (Kelley, 1980; Blecha, 1988; Gross and Seigel, 1988). Glucocorticoids have potent immunosuppressive effects (Claman, 1987; Munck et al., 1987; J efferies, 1991), and it has been assumed that it is the stress-induced release of glucocorticoids that causes the increased susceptibility to infection.

Elevation of glucocorticoids is preceded by, and accompanies, changes in sympathetic nervous system activity, in particular elevated secretion of adrenaline and noradrenaline. There is evidence that the immune system may be influenced by the autonomic nervous system. There is considerable autonomic innervation of lymphoid organs (Felten et al., 1985, 1987), and studies have indicated that adrenergic agonists may alter splenocyte function in vitro (Sanders and Munson, 1984, 1985), although the results of other studies conflict with this conclusion (Besedovsky et al., 1979).

Data on secondary (immunoglobulin G) humoral antibody response in vivo to sympathomimetics are lacking. For this reason we evaluated the effect of a popular beta-adrenergic repartitioning agent (clenbuterol) on immune function in sheep.

Materials and Methods

Twenty female Coopworth lambs were weaned and housed individually indoors. They were adapted to a complete pelleted diet (60% alfalfa, 30% barley, 5% linseed oil, and 5% molasses, providing 17.5% crude protein and 10 MJ/kg of DM metabolizable energy); lambs had free access to the diet and water. At 13 wk of age they were allocated to one of two treatment groups, such that the starting weights of the two groups were similar, and immunized with a somatostatin-ovalbumin conjugate prepared by glutaraldehyde condensation as described elsewhere (Spencer et al., 1983). The immunogen (1 volume) was emulsified with 2 volumes of Freund's Complete Adjuvant and injected in 250-µL aliquots at four subcutaneous sites in the axial region. Two weeks later, the lambs were given a similar secondary (booster) immunization in Freund's Incomplete Adjuvant. From this time, one group received 400 µg/kg of clenbuterol (Dong Whapharma, Seoul, Korea) by daily oral drench; controls received no other treatment. Further booster immunizations were given at fortnightly intervals.

Levels of antibody against somatostatin were measured in all lambs by co-incubation of ¹²⁵I-labeled somatostatin (Du Pont NEN Products, Wilmington, DE) with serial dilutions of plasma (Spencer et al., 1991) and separation with dextran-coated charcoal. Antibodies against the ovalbumin carrier were determined by ELISA using ovalbumin-coated microtiter plates and a rabbit anti-sheep IgG-horseradish peroxi-
dase conjugate following the same procedures as described for other antigens (Schurmann et al., 1995).

At 20 wk of age the lambs were slaughtered in a commercial abattoir, and carcass characteristics were measured. Spleen, thymus, liver, kidney, and biceps femoris were removed and weighed. White blood cells (WBC) were counted in all samples.

Antibody titer levels were expressed as the specific binding of radiolabeled SRIF (as a percentage of the total label added) at a 1:1,000 initial dilution of the plasma. The difference in antibody titers between the groups was assessed using the chi-square test. Other differences were analyzed by one-way ANOVA.

Results and Discussion

No gross differences in tissue responses at immunization sites were observed between treatment groups, but humoral antibody responses were different. Nine of the 10 control lambs immunized with the somatostatin-ovalbumin conjugate had significant SRIF antibody titers, as determined by their ability to bind radiolabeled SRIF, compared with untreated contemporaneous lambs. The mean binding at a 1:1,000 dilution was 26.8 ± 5.6%. In contrast, only one of the 10 lambs that received clenbuterol showed any significant binding (20.7%) of labeled SRIF at a 10 lambs that received clenbuterol showed any significant binding (20.7%) of labeled SRIF at a 1:1,000 dilution at 20 wk of age the lambs were slaughtered in a commercial abattoir, and carcass characteristics were measured. Spleen, thymus, liver, kidney, and biceps femoris were removed and weighed. White blood cells (WBC) were counted in all samples.

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Table 1. Growth rate, organ and tissue weights, and white blood cell counts in animals immunized against somatostatin either without (control) or with (treated) concurrent clenbuterol treatment

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Treated</th>
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<tbody>
<tr>
<td>Initial wt, kg</td>
<td>29.1 ± .89</td>
<td>29.7 ± .86</td>
</tr>
<tr>
<td>Weight gain, g/d</td>
<td>285 ± 17</td>
<td>305 ± 26</td>
</tr>
<tr>
<td>White blood cells, × 10^9/L</td>
<td>8.8 ± .6</td>
<td>9.9 ± .7</td>
</tr>
<tr>
<td>Thymus, g</td>
<td>34.3 ± 5.2</td>
<td>25.2 ± 2.2</td>
</tr>
<tr>
<td>Thymus, % of BW</td>
<td>.92 ± .15</td>
<td>.66 ± .06</td>
</tr>
<tr>
<td>Spleen, g</td>
<td>63.9 ± 9.7</td>
<td>53.3 ± 2.7</td>
</tr>
<tr>
<td>Spleen, % of BW</td>
<td>1.68 ± .24</td>
<td>1.38 ± .07</td>
</tr>
<tr>
<td>Liver, g</td>
<td>741 ± 28</td>
<td>664 ± 25.8</td>
</tr>
<tr>
<td>Liver, % of BW</td>
<td>19.5 ± .4</td>
<td>17.1 ± .5**</td>
</tr>
<tr>
<td>Kidney, g</td>
<td>123 ± 3.6</td>
<td>119 ± 5.2</td>
</tr>
<tr>
<td>Kidney, % of BW</td>
<td>3.2 ± .1</td>
<td>3.1 ± .1</td>
</tr>
<tr>
<td>Kidney fat, g</td>
<td>339 ± 30</td>
<td>297 ± 32</td>
</tr>
<tr>
<td>Kidney fat, % of BW</td>
<td>8.8 ± .6</td>
<td>7.8 ± .9</td>
</tr>
<tr>
<td>Biceps femoris, g</td>
<td>295 ± 12</td>
<td>343 ± 24</td>
</tr>
<tr>
<td>Biceps femoris, % of BW</td>
<td>7.8 ± .3</td>
<td>8.8 ± .5</td>
</tr>
</tbody>
</table>

*Values are means ± SE; **control vs. treated (P < .01).

These data indicate that clenbuterol treatment suppresses humoral immune response in sheep. However, Takahashi et al. (1993) could find no evidence of an effect of short-term (14 d) clenbuterol treatment on hemagglutination of sheep red blood cells in chickens. It is difficult to draw comparisons from the two studies because of differences in species, immunogens, length of treatment, and immune response measurements.

The weights of thymus and spleen (as indicators of lymphoid tissue growth) were not significantly different between treatment groups, neither was there any difference when the organ weights were expressed as a percentage of body weight (Table 1). However, the approximately 30% lower mean thymus weight and 10% lower mean spleen weight in the clenbuterol-treated lambs support the suggestion that elevation of beta-adrenergic status suppresses the immune system. We know of no published data to support or refute these findings on lymphoid tissue growth.

In the absence of specific antibodies to ovine lymphocyte subclasses in our laboratory, we were unable to evaluate the effect of clenbuterol on CD3⁺, CD4⁺, and CD8⁺ lymphocyte subpopulations, but such work is now indicated in laboratory rodents where these tools are more readily available. However, total WBC counts were not significantly different between treatment groups (Table 1).

Liver weight tended to be lower in the clenbuterol-treated lambs (P = .057) and was lower when expressed as a percentage of body weight (P < .01). Body weight gain itself was not significantly affected by clenbuterol treatment (Table 1). Clenbuterol treatment was also without effect on kidney, kidney and channel fat, or biceps femoris weights (Table 1), but when expressed as a percentage of body weight, biceps weight tended to be greater with clenbuterol treatment (P = .066).

Figure 1. Dilution curves for antibodies against somatostatin in lambs immunized against somatostatin either with (closed circles) or without (open circles) coin-treatment with clenbuterol. Values are means ± SE; n = 10 for each group.
The dose of clenbuterol used was comparable with that used in the majority of sheep studies reported in the literature, and the tendency toward a decrease in fat and increase in muscle weight is consistent with the re-partitioning effect reported in numerous studies (see review by Moloney et al., 1991). Thus the results suggest that the dose of clenbuterol was adequate and effective.

**Implications**

These results clearly show that clenbuterol treatment had a potent suppressive effect on the ability of lambs to raise specific humoral antibodies in response to a challenge to the immune system. This may have important, unacceptable implications for animal health and welfare. If beta-agonists are used as growth promoters or re-partitioning agents, treated animals may be more susceptible to infection and consequently exhibit poorer performance.

**Literature Cited**


