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The Functional and Histological Effects of Clenbuterol on the Canine Skeletal Muscle Ventricle[1]

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Background. **We investigated the anabolic effects of the sympatho-mimetic drug clenbuterol upon pumping chambers constructed from latissimus dorsi muscle (LDM).**

Methods and results. **In control and treatment groups (n = 4 dogs each), skeletal muscle ventricles (SMVs) were constructed followed by a 3-week recuperative delay and 6–7 weeks of electrical conditioning at 2 Hz to induce phenotypic expression of fatigue resistant slow muscle fibers. The treatment group received oral administration of clenbuterol (8 μg/kg, 2x/day) during this period. The clenbuterol group increased significantly in body weight as compared with the control group ($P < 0.05$). In a terminal experiment, the SMVs were assessed with a mock circulation device to determine pumping performance and also were examined with regard to fiber type distribution and area in the SMVs and their contralateral *in situ* LDMs. Initially the clenbuterol group performed better than the control group, but by the end of a 60-min fatigue test, there were no significant differences. With regard to fiber type distribution and areas, the SMVs of the clenbuterol group exhibited a fast fiber distribution similar to unconditioned muscles (28% ± 4%), whereas the control group showed complete transformation (100%) to slow fibers. The fast fibers of the clenbuterol group were larger than control ($P < 0.05$), but the slow fibers were not significantly different.**

Conclusions. **At the dose given, clenbuterol does induce hypertrophy and preserves the normal percentages of fiber types, possibly by hyperplasia, but it does not affect chronic pumping performance of skeletal muscle ventricles in the canine model.**

***Key Words*: muscle; electrical stimulation; heart-assist device; hormones; catecholamines.**

INTRODUCTION

Pumping chambers constructed from skeletal muscle, termed skeletal muscle ventricles (SMVs), have been shown by our laboratory to be a potentially effective form of cardiac assistance. We have created skeletal muscle ventricles in dogs that have pumped blood in the circulation by contracting continuously for periods of months to years [1–3]. Yet improvements in SMV function and preservation of muscle tissue are always desirable. Clenbuterol is a potent β_2 agonist that has been described to have anabolic effects on skeletal muscle in animal studies [4, 5]. Increases in both muscle size and strength have been reported. Also, this agent has been shown to have thermogenic properties by increasing basal metabolic rate. The mechanism for these changes is incompletely elucidated, but these effects are most likely the result of adrenergic effects on skeletal muscle, although effects on atypical adrenoreceptors or nonreceptor-mediated pathways may also be involved [4]. Clenbuterol also has been shown to induce a slow-to-fast fiber type transition and increase force generation and twitch contraction speeds in the rat [6]. However, no such investigation of the effects of clenbuterol on the canine SMV exists to date.

The objective of our study was to evaluate clenbuterol as an agent that could potentially improve the function of canine SMVs. We tested the fatigue resistance and fiber type composition of SMVs in which clenbuterol was used as an adjunct to our standard

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protocol of electrical conditioning that induces fatigue resistance by total conversion of the muscle fibers to oxidative, slow twitch fibers.

METHODS

Experimental Design and Construction of Skeletal Muscle Ventricles

All aspects of the study were approved by the institutional Animal Investigation Committee and complied with the University guidelines and National Institutes of Health "Guide to the Care and Use of Laboratory Animals." [US DHSS Publication No. (NIH) 85-23, Rev. 1985]. Mongrel dogs (16–24 kg) were assigned to a control (n = 4) or clenbuterol treatment group (n = 4). In both groups, SMVs were constructed from the left latissimus dorsi muscle (LDM). Each dog underwent two operations, the first for SMV construction and the second was a terminal evaluation experiment. Anesthesia was induced using intravenous thiopental 20–25 mg/kg and was maintained after endotracheal intubation with 1.5% to 3.0% isoflurane inhalation. The SMV construction technique has been described previously [1]. Briefly, an incision from the left axilla to the tip of the eleventh rib was made, and the LDM was mobilized from the chest wall. The humeral attachment was maintained as was the neurovascular bundle that includes the thoracodorsal nerve, artery, and vein. A cuffed electrode was placed around the nerve and sutured in place. Next, the LDM was sutured to a polypropylene mandrel with a 1.0-cm Dacron cuff placed around the opening. The muscle was wrapped around the mandrel approximately two times and secured in place with 2-*O* polypropylene suture, creating the pumping chamber. The SMV was secured near the left axilla in the subcutaneous tissue. A pulse generator (Model 7421 Medtronic, Inc) was connected to the electrode and placed in the subcutaneous tissue of the left flank.

Muscle Conditioning

Immediately after the SMV constructive surgery, four dogs of the eight were given oral clenbuterol for 10 weeks (16 μg/kg/day, divided into twice-daily dosing). The control group received no clenbuterol. Both groups underwent a 3-week recuperative (vascular delay) period, and then the SMVs were electrically conditioned by turning on the pulse generators at a stimulation frequency of 2 Hz (0.21 mS pulse duration) for 7 weeks. This regimen has been previously shown to result in virtually complete conversion of fast-twitch muscle fibers (Type 2) to fatigue-resistant slow-twitch fibers (Type 1) [7–10].

Terminal Experiment

General anesthesia was induced as above, and an incision was made over the mouth of the SMV and the mandrel was removed. To determine the functional pumping capabilities of the SMVs, they were then connected to a mock circulation device built in our laboratory which has been previously described (Fig. 1) [11–13]. This device allows control of preload by infusion of saline into the SMV and control of afterload injection of air into the canister pressure port. Initially, the abilities of the control and treated SMVs to generate pressure and stroke volume were assessed against an afterload pressure 80 mmHg, as the preload was varied from 0 to 60 mmHg by continuous infusion of saline into the system via a port. The SMVs were stimulated at a frequency of 33 Hz (0.21 mS pulse width), with a duty cycle of 312 mS on and 812 mS off (54 bpm). The second set of measurements was obtained at the same contraction rate during a one hour (1-h) fatigue test with the preload maintained at 20 mmHg and the afterload at 80 mmHg. The system was allowed to run continuously for 60 min. All data were recorded with the Gould 4600 Series signal conditioners and Windaq data acquisition and analysis software (Dataq Instr., Akron, OH). Direct measures of systolic pressure, stroke volume, and mean pressure were obtained. Rate of pressure generation (dP/dT) was defined as the first derivative of the SMV pressure waveform. The tension-time index was calculated as the area under the SMV pressure curve from the first upward deflection of the pressure signal during systole to the maximum nega-

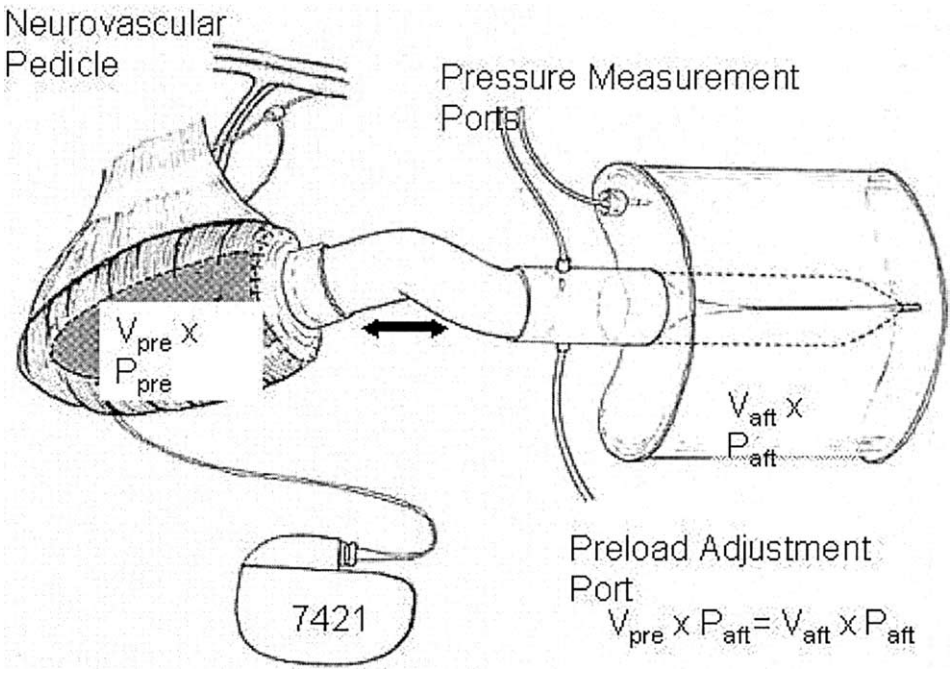

FIG. 1. An illustration of the mock circulation device, wherein saline is pumped by the SMV through a rigid conduit into a bladder within a pressurized canister. Preload and afterload can be varied by injection of saline or air into the SMV bladder and the canister respectively.