

reducing equivalents (mean rate (\pm SE) of cytochrome c reduction= 2.8 ± 0.6 pM/mg muscle/min. at 37° C., n=25). Generation of reducing equivalents was blunted by NOS inhibition with NLA (Table 2), an effect abolished by the addition of NO donors to NLA-treated muscle (data not shown). In contrast, SOD and catalase did not affect basal production of reducing equivalents (Table 2). This contrasts sharply with results obtained from fatigued muscle in which cytochrome c reduction is increased 6-fold relative to passive muscle ($p<0.001$), 85 % of this increase being attributable to SOD-sensitive ROI (Table 2 below). These results indicate that reducing equivalents generated by unfatigued muscle are influenced by NO production (or that of a related redox form). Reduction of cytochrome c may result from the direct effect of NO (Ehrenberg, et al., 1960; Kucera, et al., 1987; Bell, et al., 1991) or from indirect effects on other enzymatic redox systems, for which there is precedent (Clancy, et al., *J. Clin. Invest.*, Vol. 90, pg. 1116 (1992)). Notwithstanding the precise mechanism, the production of NO under these conditions is supported by the several biochemical and functional studies reported above.

To obtain the data in Table 2 below, fiber bundles were isolated from rat diaphragm and incubated in oxygenated, buffered Krebs-Ringer solution containing cytochrome c, 5×10^{-5} M. After 60–90 min, solutions were removed and the absorbance at 550=was measured spectrophotometrically. Cytochrome c reduction rates (pmol/mg/min) were calculated and expressed relative to absorbance changes catalyzed by untreated (control) fiber bundles from the same muscle. Bath concentrations were NLA 1mM, SOD (superoxide dismutase) 10^3 U/ml, catalase 10^3 U/ml. Fatigue (used to assess effects during active contraction) was produced under isometric conditions using repetitive electrical field stimulation (supramaximal current density, 0.5 ms stimulus duration, 25 Hz, 250 ms train duration, 0.1 trains/s) for 1 hr. Differences from control: * $P<0.05$, ** $P<0.001$; difference from Fatigue: # $P<0.001$.

TABLE 2

Treatment	Cytochrome C Reduction % Control \pm SE
NLA	67 \pm 18*
SOD + Catalase	106 \pm 82
Fatigue	672 \pm 83**
Fatigue + SOD	188 \pm 41#

EXAMPLE 7

Oxyhemoglobin Improves Excitation-Contraction Coupling in Skeletal Muscle

We have shown that skeletal muscle produces nitric oxide (NO) and that endogenous NO depresses excitation-contraction coupling (Nature 372:546–548, 1994). We therefore

postulated that oxyhemoglobin (an NO scavenger) should improve excitation-contraction coupling. Fiber bundles were isolated from rat diaphragm, mounted isometrically in Krebs-Ringer solution, and incubated at 37° C. for 1 hour before being alternately exposed to oxygenated hemoglobin (50–500 μ M) or to Krebs-Ringer solution (control) at 18 minute intervals. Direct electrical stimulation ((0.2) ms pulses, 250 ms trains, supramaximal voltage) was used to produce submaximal (40 Hz) and maximal (200 Hz) tetanic contractions. Developed force was measured. Oxyhemoglobin produced dose-dependent increases in relative force (as % Po) developed during submaximal tetanic contractions ($p<0.05$). Maximal tetanic stress (Po, in N/cm²) was acutely depressed by oxyhemoglobin exposure ($p<0.05$ for 50 μ M and 250 μ M concentrations). All oxyhemoglobin effects reversed with washout. We conclude that oxyhemoglobin improves excitation-contraction coupling and also causes reversible depression of Po.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

What is claimed is:

1. A method for treating a mammal by stimulating the contraction of skeletal muscle of said mammal comprising: treating a mammal in need of treatment for amyotrophic lateral sclerosis, muscular dystrophies, congestive heart failure, or lung diseases by contraction of skeletal muscle by administering to said mammal a skeletal muscle contracting amount of a nitric oxide synthase inhibitor.

2. The method of claim 1 wherein said nitric oxide synthase inhibitor is selected from the group consisting of N^G-monomethyl-L-arginine; nitroarginine; N-nitro-L-arginine methyl ester; N-amino-L-arginine; N-methyl-L-arginine; ornithine; N-imino-ethyl-L-ornithine; methylene blue; trifluoropiperazine; heme binders; and methotrexate.

3. A process for enhancing skeletal muscle contraction in a mammal in need thereof comprising: treating the mammal in need of skeletal muscle contraction enhancement with an amount of a heme-containing compound effective to enhance skeletal muscle contraction in said mammal.

4. A method for stimulating the contraction of skeletal muscle in a mammal in need thereof comprising administering to said mammal in need thereof a skeletal muscle contraction enhancing amount of a nitric oxide scavenger.

5. The method of claim 4 wherein the nitric oxide scavenger is a heme-containing compound.

6. The method of claim 5 wherein the heme-containing compound is a heme-containing protein.

7. The method of claim 6 wherein the heme-containing protein is selected from the group consisting of hemoglobin and myoglobin.

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