S-Nitrosation of Skeletal Muscle Contractile Proteins Reduces Ca^{2+} Sensitivity and Inhibits Myosin Activity *In Vitro*

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Nitric oxide (NO) is produced in skeletal muscle by neuronal NO synthase and induces S-nitrosation (-SNO) in proteins that alter contractile function, but its effect on myofibrils is not known. Here we investigated protein S-nitrosation in physiological conditions *in vitro* and in exercise. Sprague-Dawley rats were subjected to high-intensity swimming for 4 min with 10% of body weight added to the tail and protein–SNO content was analyzed by the Griess/Saville method in EDL and soleus muscles. In EDL, -SNO formation doubled ($P<0.05$; $n=9$) compared to controls. To mimic nitrosative stress in myocytes, we incubated skinned fibers with 0-1000 µM S-nitrosoglutathione (GSNO) for 30 min and processed the proteins by the biotin-switch method. Myosin and actin were the main targets for -SNO. To detect NO effects on contraction, we incubated single skinned fibers with GSNO for 1 h at 15°C and measured maximal (P_o) and submaximal tension (pCa 6). GSNO had no effect on P_o but decreased the Ca^{2+} sensitivity by ~50%, an effect that was reversible on incubation with ascorbic acid ($P<0.05$; $n=4$). *In vitro*, GSNO (10 µM, 1 h) inhibited MgATPase activity of isolated myosin by 50%, regardless of pH (pH 6 – 8). Taken together, these results suggest that myosin is an important target for S-nitrosation in skeletal muscle and that -SNO formation inhibits contractile function during nitrosative stress.

Keywords: skeletal muscle, nitric oxide, GSNO, exercise, calcium sensitivity

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