Skeletal muscle force and actomyosin ATPase activity reduced by nitric oxide donor

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Perkins, William J., Young-Soo Han, and Gary C. Sieck. Skeletal muscle force and actomyosin ATPase activity reduced by nitric oxide donor. J. Appl. Physiol. 83(4): 1326–1332, 1997.—Nitric oxide (NO) may exert direct effects on actin-myosin cross-bridge cycling by modulating critical thiol groups on the myosin head. In the present study, the effects of the NO donor sodium nitroprusside (SNP; 100 µM to 10 mM) on mechanical properties and actomyosin adenosinetriphosphatase (ATPase) activity of single permeabilized muscle fibers from the rabbit psoas muscle were determined. The effects of N-ethylmaleimide (NEM; 5–250 µM), a thiol-specific alkylating reagent, on mechanical properties of single fibers were also evaluated. Both NEM (≥25 µM) and SNP (≥1 mM) significantly inhibited isometric force and actomyosin ATPase activity. The unloaded shortening velocity of SNP-treated single fibers was decreased, but to a lesser extent, suggesting that SNP effects on isometric force and actomyosin ATPase were largely due to decreased cross-bridge recruitment. The calcium sensitivity of SNP-treated single fibers was also decreased. The effects of SNP, but not NEM, on force and actomyosin ATPase activity were reversed by treatment with 10 mM DL-dithiothreitol, a thiol-reducing agent. We conclude that the NO donor SNP inhibits contractile function caused by reversible oxidation of contractile protein thiols.

METHODS

Permeabilized rabbit psoas single fiber preparation. Permeabilized single fibers were prepared from glycerinated rabbit psoas muscle (Ward’s Natural Scientific Establishment). A 1-cm strip of muscle tissue was excised and placed in a relaxing solution maintained at room temperature (22°C) containing (in mM): 7.0 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 1.0 free Mg2+, 2.0 ATP, 20.0 imidazole, 15 creatine phosphate, 1 mg/ml creatine phosphokinase, and sufficient KCl to adjust the ionic strength to 150 mM. The pH was adjusted to 7.0 with KOH. The negative log free Ca2+ concentration (pCa) of the relaxing solution was 9. Single fibers were then dissected with fine forceps under a microscope and immersed for 25 min in a skinning solution that was of the same composition as the relaxing solution except that 1% Triton X-100 and 10 mM DL-dithiothreitol (DTT) were added. The skinning solution was kept at 15°C to thermoequilibrate the fibers before mechanical measurements. DTT was added to the skinning solution to prevent oxidation of cysteine thiols on contractile proteins.

Activating solutions had the same composition as the relaxing solution, except that the pCa was adjusted to range between 7.0 and 3.8 with the following steps: 7.0, 6.0, 5.8, 5.5, 5.2, 5.0, 4.0, and 3.8. The final concentrations of Ca2+ ([Ca2+]i), ligand, and Ca2+-ligand complexes were calculated by using a computer program with the algorithm described by Fabiato and Fabiato (11) and the formation constants reported by Godt and Lindley (12). In preliminary studies, using aequorin luminescence to measure [Ca2+], we verified that SNP did not have an effect on the pCa of the solutions. All reagents, except aequorin, were purchased from Sigma Chemical. Aequorin was purchased from Dr. John Blinks at Friday Harbor Laboratory, University of Washington.

Measurement of fiber mechanical properties. Segments of single permeabilized fibers, 3–4 mm in length, were mounted in a Göttingen Scientific Instruments Muscle Research System for measurements of isometric force, maximum unloaded shortening velocity (V0) and actomyosin ATPase activity (14). The fiber was placed in a 1-cm-long quartz cuvette with a 1-mm2 cross-sectional area (10 µl volume) through which relaxing or activating solutions were perfused at 15°C. The ends of the single fiber were attached via stainless steel microforceps to a force transducer on one end and a servo-controlled displacement motor at the other end. This provided a stable and noncompliant attachment of the fiber during mechanical
measurements. In this system, sarcomere length was visually monitored by using a monocular microscope, and a video image of the fiber was digitized by using an image-processing system calibrated for morphometry (MegaVision 1024XM). Sarcomere length was adjusted to 2.5 µm and maintained at this length throughout the isometric mechanical measurements. The relative uniformity of sarcomere spacing along the length of the fiber was verified and taken to indicate that the arrangement of contractile proteins was not disrupted by the skinnning procedure and that there was no significant compliance at the end attachments of the fiber. A stretch-release/restretch conditioning program (5) was used to increase the stability of the preparation. In addition to sarcomere length, total muscle fiber length (L_m) and fiber diameter were also measured with the use of the imaging system. Fiber cross-sectional area was estimated on the basis of the diameter measurements.

During mechanical measurements, temperature of the perfusate was maintained at 15°C with a water-bath cooler. The efficacy of the permeabilization procedure was established by the lack of any contractile response to the addition of 10 mM caffeine to the relaxing solution. Isometric force was then determined after exposure to varying pCa in the activating solution. In all fibers, maximum isometric force was obtained at a pCa of 4.

At a pCa of 4 and in the presence of 5 mM MgATP^2- , V_o was determined by using the "slack" test (10), in which the muscle was quickly released after developing maximal force and shortened to different fractions of L_m (8, 10, 12, and 14% of L_m). The time required to redevelop force was measured, and the slope of the line relating this time to the change in length was calculated as a measure of V_o and expressed as muscle fiber length per second (L_m/s).

Measurement of actomyosin ATPase activity. Isometric actomyosin ATPase activity was measured at 15°C by using a fluorescence-coupled enzyme assay (14). The ATPase assay solution contained relaxing solution, plus 5 mM phosphoenolpyruvate (PEP), 0.2 mM reduced β-nicotinamide adenine dinucleotide (NADH), 100 U/ml pyruvate kinase (PK), and 140 U/ml lactate dehydrogenase (LDH). The fluorescence-coupled enzyme assay involves the following reactions

\[
\text{ATPase} \quad \begin{align*}
\text{ATP} & \rightarrow \text{ADP} + P_i \\
\text{ADP} + \text{PEP} & \rightarrow \text{pyruvate} + \text{ATP} \\
\text{LDH} & \rightarrow \text{pyruvate} + \text{NADH} \\
\text{lactate} + \text{NAD}^+ & \rightarrow \text{pyruvate} + \text{NADH}
\end{align*}
\]

where, in reaction 1, ATP is hydrolyzed by the actomyosin ATPase to ADP and inorganic phosphate (P_i) during detachment of the myosin head from the myosin binding domain of actin (15). In reaction 2, ATP is regenerated from ADP and PEP by PK. In reaction 3, the resulting pyruvate is converted to lactate by LDH, which results in stoichiometric conversion of fluorescent NADH to nonfluorescent NAD^+. For each mole of ADP produced by the actomyosin ATPase, 1 mole of NADH is converted to NAD^+. Therefore, the amount of ADP produced by the actomyosin ATPase is determined by measuring the decrease in NADH fluorescence after stopping the flow in the quartz cuvette. We also determined the effect of 5 mM MgATP^2- on actomyosin ATPase activity. Calibration involved photometric measurements of fluorescence in the presence of known amounts of NADH. Previous work on permeabilized single fibers has shown that mitochondrial ATPases and sarcoplasmic reticulum ATPase make no detectable contribution to the observed ATPase activity (19). The light source was a mercury lamp, and excitation and emission wavelengths were set by using a 340-nm bandpass filter and 450-nm interference cutoff filter, respectively. The photomultiplier was positioned perpendicular to the axis of excitation, and the gain was fixed during the actomyosin ATPase measurements. In control experiments, we found that exposure to 50 µM NEM or 10 mM SNP did not interfere with NADH fluorescence.

Isometric actomyosin ATPase activity was determined at a pCa of 9 (relaxing conditions) and 4 (maximal activating condition). During the actomyosin ATPase measurements, flow through the cuvette was stopped for 15 s, and the rate of extinction of the NADH fluorescence signal was recorded. Thereafter, flow through the cuvette was reinitiated, and the activating solution was exchanged. This cycling of flow every 15 s through the cuvette was regulated by using a computer-controlled peristaltic pump.

Sensitivity of permeabilized fibers to thiol modification. Single fibers were initially perfused with relaxing solution (pCa 9) to establish baseline force. The fibers were then perfused with pCa 4 activating solution, and maximum isometric force was measured once a stable plateau was reached and maintained for 5 min. This was taken as the reference or initial force. The muscle fiber was then reperfused with relaxing solution until the force returned to baseline. After ~1 min, the fiber was perfused for 20 min with NEM (5–250 µM) in the relaxing solution. Thereafter, the fiber was once again perfused with the pCa 4 activating solution, and the force response was remeasured. Finally, after perfusing the fiber for another 20 min with relaxing solution containing DTT, force responses to the pCa 4 activating solution were measured again. Rundown in the contractile response was assessed in separate fibers in which the same sequence of events was followed except that the fibers were not exposed to NEM or DTT.

The effect of exposing fibers to 50 µM NEM on actomyosin ATPase activity was also evaluated. The same protocol was followed as described above for determining the effect of NEM on isometric force.

Effects of SNP on permeabilized fibers. The effect of SNP (100 µM to 10 mM) on maximum isometric force and V_o of single psoas fibers was determined by using the same protocol as described above for determining the effects of NEM. In addition, the effect of varying concentrations of SNP on isometric actomyosin ATPase activity of fibers was measured. In separate fibers, the effect of 5 mM SNP on the force-pCa relationship of permeabilized fibers was also determined. The reversibility of the effects of SNP on force, actomyosin ATPase, and V_o by DTT was also evaluated. In each experiment, rundown in the force and actomyosin ATPase responses were evaluated in separate fibers by using the same sequence of events except that the fibers were not exposed to SNP.

Data acquisition and statistical analysis. Force, length, and photometric data were digitally acquired at a 1-kHz sampling rate with the use of a Pentium personal computer equipped with a National Instruments AT-MIO-16 digital acquisition board and LabView software. In addition, the Guth Scientific Instruments Muscle Research System was computer controlled. The 50% effective concentration of pCa (pCa_{50}) of the force-pCa relationship was determined by using a sigmoid regression analysis in SigmaPlot. An analysis of variance for repeated measures was used to compare forces in control and agent-treated fibers. When appropriate, an unpaired Student's t-test was used for post hoc analysis of
RESULTS

Characterization of mechanical and actomyosin ATPase responses of permeabilized fibers. Maximum isometric force of permeabilized single fibers was generated at pCa 4. The dependence of force on pCa of the activating solution displayed a sigmoidal relationship with a pCa50 of 5.42 ± 0.04. During maximal activation at 15°C, the V0 of psoas fibers was 1.28 ± 0.21 L/cm²/s. At a pCa of 4, isometric actomyosin ATPase activity was 2.14 ± 0.11 nmol·mm⁻³·s⁻¹, whereas resting ATPase activity at pCa 9 was 0.13 ± 0.09 nmol·mm⁻³·s⁻¹. Isometric actomyosin ATPase activity at pCa 4 decreased significantly over 20 min from 2.14 ± 0.11 to 1.81 ± 0.18 nmol·mm⁻³·s⁻¹ (P < 0.05; see Fig. 4). Addition of 10 mM DTT to the skinned fiber perfusate had no significant effect on either initial or subsequent maximal force and actomyosin ATPase activity.

NEM effects on force and actomyosin ATPase activity. The isometric force generated during maximal activation at pCa 4 was significantly reduced, in a concentration-dependent fashion, by exposure to NEM for 20 min (Table 1). This effect of NEM on maximal isometric force was not reversed by 20-min exposure to DTT. Exposing the permeabilized fiber to DTT alone had no effect on either the initial maximum force or the NEM related reduction in force (Table 1).

In parallel with the reduction in isometric force generated at pCa 4, there was a 44% reduction in actomyosin ATPase activity after treatment with 50 µM NEM compared with the initial control value for the same fiber (P < 0.05; Table 1). The actomyosin ATPase activity after treatment with 50 µM NEM was also significantly lower than that of time-matched controls (P < 0.01; Table 1) and lower by 37% compared with time-matched controls (P < 0.01). In the relaxing solution (pCa 9), exposure to 50 µM NEM also reduced actomyosin ATPase activity by 53% compared with initial values in the same fibers (P < 0.01; Table 1) and by 45% compared with time-matched controls (P < 0.05; Table 1).

The isometric actomyosin ATPase activity determined at pCa 9 and pCa 4 in the presence of 5 mM MgATP²⁻ (0.54 ± 0.16 and 2.09 ± 0.19 nmol·mm⁻³·s⁻¹, respectively) was not different from that obtained at pCa 9 and pCa 4 in the presence of 2 mM MgATP²⁻ (0.60 ± 0.13 and 2.14 ± 0.11 nmol·mm⁻³·s⁻¹, respectively).

SNP effects on force, actomyosin ATPase activity, and V0. The maximal isometric force generated at pCa 4 was significantly reduced, in a concentration-dependent fashion, by 20-min exposure to SNP but only at concentrations >0.5 mM (Figs. 1 and 2). Exposing fibers to SNP for times >20 min did not affect the extent of force reduction. At 5 mM SNP, there was a 35% reduction in

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Table 1. Effects of NEM on isometric force and actomyosin ATPase activity

<table>
<thead>
<tr>
<th>Condition</th>
<th>Force, N/cm²</th>
<th>Actomyosin ATPase, nmol·mm⁻³·s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pCa 4</td>
<td>pCa 9</td>
</tr>
<tr>
<td>Control</td>
<td>12.5 ± 1.6</td>
<td>0.60 ± 0.13</td>
</tr>
<tr>
<td>20 min</td>
<td>10.5 ± 1.3*</td>
<td>0.53 ± 0.11</td>
</tr>
<tr>
<td>Control + DTT</td>
<td>12.9 ± 1.5</td>
<td>0.69 ± 0.15</td>
</tr>
<tr>
<td>20 min + DTT</td>
<td>11.1 ± 1.2</td>
<td>0.55 ± 0.12</td>
</tr>
<tr>
<td>NEM, 25 µM</td>
<td>7.0 ± 0.8**</td>
<td>0.29 ± 0.10**</td>
</tr>
<tr>
<td>NEM, 50 µM</td>
<td>4.3 ± 1.2***</td>
<td>0.35 ± 0.13**</td>
</tr>
<tr>
<td>NEM, 100 µM</td>
<td>3.8 ± 1.1**</td>
<td>0.08 ± 0.10**</td>
</tr>
<tr>
<td>NEM, 250 µM</td>
<td>0.5 ± 0.3**</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 experiments. NEM, N-ethylmaleimide; ATPase, adenosinetriphosphatase; pCa, negative log free Ca²⁺ concentration; DTT, dithiothreitol. *Significantly different from control, P < 0.05; †significantly different from time-matched control (20 min), P < 0.05.
isometric force at pCa 4, which was significantly less (P < 0.001) than the 15% reduction observed in control fibers.

Addition of CN− (10 mM), a breakdown product of SNP, or NO2− (5 mM), the product of NO autoxidation, resulted in no significant reduction in force. The SNP effect on force reduction at pCa 4 was reversible after exposure of the SNP-treated fiber with DTT (Fig. 3). Treatment of fibers with DTT in the absence of SNP and/or after rundown resulted in no significant change in force at pCa 4.

The actomyosin ATPase activity at pCa 4 was decreased 35% after exposure to 5 mM SNP (see Figs. 1 and 4). A significant reduction in the pCa 9 actomyosin ATPase was not observed. The reduction in the pCa 4 actomyosin ATPase activity was fully reversed by exposure of SNP-exposed skinned single fibers to 10 mM DTT for 20 min.

The effects of 5 mM SNP on the isometric force-pCa relation were determined (Fig. 5). Both the maximal force and Ca2+ sensitivity were decreased by SNP. The pCa50 values for initial, timed-control, and SNP-treated fibers were 5.42, 5.27, and 5.1, respectively. The pCa50 for SNP-treated fibers was significantly lower than that in both initial and timed-control fibers.

The V0 for the rabbit psoas single fibers treated with 25 µM NEM for 20 min was decreased 43% from 1.29 ± 0.16 to 0.74 ± 0.09 L/min (P = 0.0004). Exposure of the skinned single fiber to 5 mM SNP for 20 min resulted in a smaller (17%) but significant reduction in V0 (1.14 ± 0.09 vs. 0.95 ± 0.09 L/min, P = 0.002; Fig. 6). There was no significant change in V0 in 20-min timed controls (1.26 ± 0.15 vs. 1.38 ± 0.17 ml/s; P = 0.15). Treatment of the 20-min control and NEM-treated fibers with DTT for 20 min resulted in no significant change in V0. There was no significant difference between V0 in control vs. SNP-treated fibers after 20 min exposure to DTT.

**DISCUSSION**

In single permeabilized rabbit psoas muscle fibers, exposure to the NO donor SNP inhibited isometric force, Ca2+ sensitivity, and actomyosin ATPase activity. Similarly, exposure of permeabilized fibers to NEM, which selectively modifies thiols, reduced both isometric force and actomyosin ATPase activity in a concentration-dependent manner.

The NEM-mediated reduction in force is consistent with previous work in which a maleimide spin label (6, 31) and the thiol-specific reagent 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; Ref. 36) were found to decrease force and stiffness in permeabilized single fibers. In these previous studies (6, 36), it was concluded that inhibition of fiber contractile properties was the result of direct modification of cysteine residues on contractile proteins. In rabbit psoas muscle fibers, two highly reactive and ATPase-critical thiols, SH-1 and SH-2, are present on the myosin head (30, 32). In the present study, permeabilized fibers were exposed to thiol-modifying reagents under relaxing conditions because this increases the reactivity of cysteine thiols on the myosin head, particularly SH-1, relative to the cysteine thiols of other contractile proteins (9, 35). The specificity of SH-1 modification has been verified by extraction of the myosin from treated single fibers and subsequent determination of ATPase activities, thiol titrations, and radiolabeling experiments (6, 31). We suggest that the effect of the NO donor SNP in reducing isometric force in permeabilized fibers was also mediated by reversible oxidation of critical thiols on the myosin head.
SNP was selected as the NO donor in the present study for several reasons. It is a widely used NO donor in biological studies and is also used in the medical treatment of hypertension. It is also relatively stable in aqueous solution. Although it is typically thought to work by releasing NO and activating soluble guanylate cyclase (16), SNP also reacts with biological thiols and formed the basis of one of the earliest assays for the detection of thiols in tissue extracts. SNP reacts with free thiols to give rise to a compound with a red color and an absorption maximum at 510 nm (18), in all likelihood an S-nitrosothiol or protein. The efficiency and speed with which SNP participates in S-nitrosylation reactions (33) with protein thiols, however, is unknown.

After NEM treatment, actomyosin ATPase was reduced in parallel with the reduction in maximal isometric force at pCa 4. The basal ATPase activity of permeabilized fibers under relaxing conditions (pCa 9) was also reduced. Although there is a possibility that there is some loss of myofibrillar Ca$^{2+}$ regulation in this preparation, the fourfold increase (pCa 9 to pCa 4) in actomyosin ATPase activity is in agreement with previous reports (19). Because the ATPase activity measured under relaxing conditions is that of the myosin head while either not bound to or weakly associated with actin, this result implies that NEM treatment affects the intrinsic ATPase activity of the myosin head. It is, therefore, likely that the effects of NEM on actomyosin ATPase activity and force production observed at pCa 4 are also due to modifications of the myosin head rather than due to an effect on other proteins involved in the activation process, such as troponin C or tropomyosin. To our knowledge, this is the first demonstration that contractile protein thiol modification causes a reduction in actomyosin ATPase activity in single permeabilized fibers. However, these observations are consistent with previous results obtained by using purified actin and myosin (26).

Whereas NEM irreversibly modifies protein thiols, the NO-donor compound SNP may reversibly modify protein thiols by transnitrosylation (34). Transnitrosylation involves transfer of NO$^+$ from a NO donor to a protein thiol and results in the formation of a stable S-nitrosoprotein. Functionally significant S-nitrosylation of proteins has been demonstrated both in purified proteins (1, 33) and in vivo (17). In the present study, it was observed that SNP decreased isometric force of permeabilized psoas fibers in a concentration-dependent manner, with no effects observed at SNP concentrations <0.5 mM. Thus permeabilized fibers were sensitive to SNP but to a much lesser extent than to NEM, which significantly decreased force and actomyosin ATPase activity at concentrations more than an order of magnitude lower. SNP also significantly decreased actomyosin ATPase in parallel with the reduction in maximal force produced at pCa 4. While the effective

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**Fig. 5.** Force-Ca$^{2+}$ relationship in control, timed-control (20 min), and skinned single muscle fibers treated with 5 mM SNP and 25 µM N-ethylmaleimide (NEM) for 20 min; n = 6 experiments for each data point. A: there is a measurable reduction in Ca$^{2+}$ sensitivity in timed-control single fibers. Treatment with either 5 mM SNP or 25 µM NEM results in further reduction in both maximal force and Ca$^{2+}$ sensitivity. B: 50% effective concentration (EC$_{50}$) pCa (pCa$_{50}$) is significantly decreased by SNP and NEM treatment (P < 0.01). Results are presented as means ± SE. *Significantly different from initial value (P < 0.01); †significantly different from initial value (P < 0.01).

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**Fig. 6.** Comparison of NEM and SNP-mediated changes in maximal force, actomyosin ATPase activity (ATPase) and unloaded shortening velocity ($V_o$, measured by slack test) with control fibers. All results are presented relative to initial control; n = 6 experiments for each data point. NEM- and SNP-related results are compared with timed-control (20 min) samples. Note that both force and actomyosin ATPase activity are reduced proportionately after 20-min treatment with SNP. $V_o$, however, was decreased to a lesser extent by SNP treatment. Results are presented as means ± SE. *Significantly different from control (P < 0.05); †significantly different from timed control (P < 0.01).
concentrations of SNP were very high, the SNP-mediated reduction in force and actomyosin ATPase activity at pH 4 and V₀ were reversed by treatment with the thiol-reducing agent DTT. This indicates that the SNP-mediated changes in force and actomyosin ATPase activity were related to modification of functionally significant contractile protein thiols. These results do not permit determination of which contractile proteins were affected by SNP-mediated thiol modification or whether more than one protein was modified. However, the most reactive thiols in this system are the myosin head SH-1 and SH-2 thiols (6, 36), although other potential target proteins include tropomyosin, troponin T (9), troponin C (13, 28), and F-actin (24).

The relationship between force, actomyosin ATPase activity, and V₀ may be analyzed by using Brenner’s analytic model (4), which is based on the Huxley two-state model of cross bridge cycling (15). The steady-state fraction of cycling cross-bridges (αₖ₆) in the force-generating state is given by Eq. 4

\[
\alpha_{k6} = \frac{f_{app}}{f_{app} + g_{app}} \tag{4}
\]

where \(f_{app}\) is the apparent rate constant for cross-bridge attachment and \(g_{app}\) is the apparent rate constant for cross-bridge detachment. Assuming the hydrolysis of one ATP molecule per cross-bridge cycle, isometric actomyosin ATPase activity is given by equation 5

\[
\text{ATPase} = n b_{app} \alpha_{k6} \tag{5}
\]

where \(n\) is the number of cross-bridges per half sarcomere and \(b\) is the number of half sarcomeres within the fiber. The maximum force developed by a fiber is largely a function of cross-bridge recruitment, and the ATPase depends on both cross-bridge recruitment \(n\) and cross-bridge cycling. \(V₀\) is correlated with both the actomyosin ATPase activity and cross-bridge cycling. In the present study, a nearly equal reduction in both the maximal force and the actomyosin ATPase was observed after treatment of the rabbit psoas skinned single fiber with SNP. Exposure to SNP caused only a small reduction in \(V₀\), while both maximal force and actomyosin ATPase activity were reduced to a greater extent. This suggests that the reduction in maximal force and actomyosin ATPase activity was mostly due to a reduction in cross-bridge recruitment \(n\). The smaller reduction in \(V₀\) suggests that SNP treatment also decreases the cross-bridge cycling rate.

These results are consistent with earlier results in which force and stiffness were decreased after treatment with maleimide adducts (6, 31) or DTNB (36). When DTNB was used as the thiol-modifying reagent in permeabilized fast- and slow-twitch single fibers, the effects on force and stiffness were reversible (36). The relationship between degree of thiol modification on the myosin head, particularly of SH-1, force and stiffness reduction, and actomyosin ATPase activity is unclear in permeabilized single fibers (31). Actomyosin ATPase activity may be either increased or decreased depending on the degree of SH-1 and SH-2 modification. Force and stiffness, however, are consistently reduced by modification of the myosin head critical thiols. Further studies are required to clarify these relationships.

The force-pCa relationship of permeabilized fibers is also altered by treatment with a thiol-modifying reagent such as DTNB (36). The slope of the force-pCa relationship and the pCa₅₀ were decreased in both fast-twitch (extensor digitorum longus) and slow-twitch (soleus) rat skeletal muscle single fibers (36). This work suggests that thiol modification of the myosin head may modulate the Ca²⁺-activation mechanism in permeabilized fibers, independent of any modification of other contractile proteins involved in Ca²⁺ regulation. In the present study, exposing permeabilized fibers to SNP also decreased both force at maximum [Ca²⁺] and the pCa₅₀. The possibility that this result is due to local changes in fiber core Ca²⁺ buffering and pH reductions that are proportional to the actomyosin ATPase cannot be ruled out in this study (25).

Although the effective concentration of SNP required to appreciably decrease maximal force and actomyosin ATPase activity in permeabilized fibers was in the millimolar rather than the micromolar range, this does not necessarily exclude the possibility that S-nitrosylation reactions with contractile proteins occur in vivo and may be functionally significant. Hemoglobin, which is reportedly S-nitrosylated in vivo, requires millimolar concentrations of a NO donor over a 30-min period to achieve appreciable S-nitrosylation in vitro (17). Similarly, the ras oncogene product p21 is modified at Cys113 and regulated by micromolar concentrations of NO in vivo, but it requires 0.3 mM and 1 mM concentrations of the NO donors S-nitroso-N-penicillamine and SNP, respectively, to achieve a similar extent of modification (23). This underscores the fact that not all NO donors or sources are equal but that effects observed at high concentrations with one, such as SNP, may mimic modifications that occur physiologically at much lower concentrations of NO. It was not feasible to examine the effects of NO itself in this system, since doing so would require exclusion of oxygen from the perfusion system. NO is unstable in O₂-containing aqueous solutions, giving rise, ultimately, to nitrous acid, which would result in an uncontrolled pH effect on the perfusion buffers. In addition, NO is unable to participate in S-nitrosylation reactions in the absence of O₂ (20). The effects of SNP on the contractile properties of permeabilized rabbit psoas fibers were, however, reversible with DTT, indicating that the reaction is, in part, thiol selective and that reversible thiol oxidation and possibly S-nitrosylation of contractile proteins had taken place.

In conclusion, the present results indicate that exposing permeabilized fibers to the NO donor SNP inhibits force, Ca²⁺ sensitivity, actomyosin ATPase activity and, to a lesser extent, decreases \(V₀\). These inhibitory effects of SNP are reversed by DTT, indicating that the SNP-mediated reduction in force, Ca²⁺ sensitivity, actomyosin ATPase activity, and \(V₀\) are due to reversible oxidation of thiols on the contractile proteins. The
results suggest the possibility of a cGMP-independent mechanism by which NO or NO donors may modulate skeletal muscle function under either normal or pathophysiological conditions, particularly those, such as sepsis, in which NO production is markedly increased.

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