Effect of S-nitrosoglutathione (GSNO) on excitation-contraction coupling in mechanically-skinned muscle fibres of the rat

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S-nitrosoglutathione (GSNO) is a naturally-occurring compound found in fast-twitch muscle fibres, which is able to S-glutathionate and/or S-nitrosylate various target proteins involved in excitation-contraction (EC) coupling (Aracena *et al.*, 2003). S-glutathionation or S-nitrosylation-induced changes to EC coupling proteins such as the Na^+/K^+ and SR Ca^{2+}-ATPases might alter their efficacy and contribute to muscle fatigue under certain conditions. We sought to identify which EC coupling steps are more susceptible than others to GSNO-modulated changes and whether these changes might be important in understanding of muscle fatigue.

Male Long-Evans hooded rats were killed under deep anaesthesia (2% v:v isoflurane) and the extensor digitorum longus and soleus muscles swiftly excised and immersed in paraffin oil at resting length. Single fibres were mechanically-skinned with forceps and a segment connected to a force transducer (stretched to 120% resting length) and immersed in either a standard K-HDTA solution with weak Ca^{2+} buffering (50 μM EGTA, pCa 7) to examine transverse tubular (T-) system depolarization-induced force responses or into heavily Ca^{2+}-buffered (50 mM EGTA) solutions to examine the contractile apparatus properties. All solutions contained as follows: 1 mM free Mg^{2+}, 8 mM ATP; 10 mM creatine phosphate, at pH 7.1, and were equilibrated to room temperature (~23°C) before use. GSNO was dissolved directly into solution immediately (~1 min) before use.

Single fibres were electrically stimulated (75 V cm^{-1}, 1 ms pulse) to produce twitch or tetanic (50 and 100 Hz) force responses before and after GSNO exposure (2 mM for 2 min in all cases). Additionally, paired pulses with differing intervals (0-20 ms) were applied to determine the repriming period of sodium channels in the T-system membrane (an indirect but sensitive measure of T-system polarization, see Dutka & Lamb, 2007). The Ca^{2+}-sensitivity of the contractile apparatus was determined by transferring the single skinned fibre segment from the 50 mM EGTA-based solution to solutions with progressively higher free [Ca^{2+}] (made by mixing 50 mM EGTA solution with 50 mM Ca-EGTA solution as appropriate, pCa range ~10-4.6) before and after GSNO treatment (2 mM, 2 min in 50 mM EGTA solution). In some other cases, 10 mM DTT (added from a 1M stock) was added to the solution. Maximum Ca^{2+}-activated force produced by the contractile apparatus was virtually unchanged by exposure to GSNO. The Ca^{2+}-sensitivity of the contractile apparatus was substantially potentiated (~0.12 pCa) after exposure to fresh GSNO (n = 7), and this effect was fully reversed by 10 mM DTT. In two slow-twitch SOL fibres GSNO exposure virtually had no effect. Interesting in electrically-stimulated fast-twitch fibres, the equivalent exposure to GSNO initially caused a moderate decrease of 35% and 20% in peak twitch and tetanic force responses respectively (compared to the pre-exposure level, n = 11). Furthermore, subsequent twitch and tetanic force responses became progressively smaller and this was not reversed fully by 10 mM DTT but was substantially ameliorated by a small additional Ca^{2+} load (10 s at pCa 6.7 or equivalent to ≤50% of the endogenous SR Ca^{2+} level), suggesting that the cause of the decline in force involved loss of SR Ca^{2+}. The progressive decrease in depolarization-induced force was not attributable to changes in T-system excitability because the repriming period for T-system Na^+ channels was not significantly altered (4.3 ± 0.2 ms and 4.0 ± 0.3 ms before and after GSNO). These data indicate that the GSNO exposure glutathionated the contractile apparatus, causing a substantial increase in Ca^{2+}-sensitivity (~0.12 pCa). GSNO treatment had little effect on T-system excitability, thus implying Na^+ channels, voltage-sensors, and Na^+/K^+ pumps remained functional unaltered. The progressive loss of depolarization-induced force after GSNO treatment was seemingly due to a loss of Ca^{2+} from the SR either through the SR Ca^{2+} pump itself or through the RyR. Alternatively, the uptake by SR Ca^{2+} pumps might be reduced at the resting Ca^{2+} level. Taken together, glutathionation in intact fibres might aid in maintaining force output when it might otherwise decline.

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