Nitric oxide alters the rate and sensitivity of sarcoplasmic reticulum calcium uptake in ovine skeletal muscle

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Calcium leakage from the sarcoplasmic reticulum (SR) to the cytosol can occur via reduced sarcoplasmic/ endoplasmic reticulum ATPase (SERCA) activity, increased efflux via the Ryanodine receptor (RyR) Ca^{++} channel or SR membrane leakage. The aim of this experiment was to investigate if nitric oxide (NO) influences SR Ca^{++} uptake and release from lamb carcasses after control (none), medium (300V, 14Hz) or high (700V, 14Hz) voltage electrical stimulation (ES) applied for 1 min approximately 5 min post-mortem. From 9 lambs, the SR was isolated from the *Longissimus thoracis et lumborum* (LTL) at the 13^{th} thoracic vertebra approximately 10 min post-mortem. Isolated SR membranes were incubated for 30 min with the 100mM final concentration of the NO donors Diethylamine NONOate (NONO) or Sodium nitroprusside (SNP) at 25^{o}C before assay of SR Ca^{++} uptake, release and ATPase activity. Incubation with NONO increased the linear and maximal rates V_{max} of SR Ca^{++} uptake ($P<.05$ and $P<.01$ respectively), without affecting the ATPase activity ($P>.05$). This resulted in an increased coupling ratio ($P<.05$) between V_{max} and ATPase for NONO, indicating greater efficiency of the SERCA pump. The calcium concentration for half maximal uptake ($[Ca^{++}]_{0.5}$) was also increased by NONO, indicating reduced sensitivity of Ca^{++} induced Ca^{++} uptake. Collectively, these data indicate that while NONO increases the rate of Ca^{++} uptake, NO desensitised the SERCA to initiate Ca^{++} uptake. No effect of SNP or ES was observed on SR Ca^{++} uptake ($P>0.05$). Neither NONO nor SNP affected SR Ca^{++} release via the RyR. However, ES resulted in increased SR Ca^{++} efflux following thapsigargin-induced inhibition of the SR ATPase. Due to the low rates of release observed, this was most likely due to membrane damage or increased SR permeability, not opening of RyR. In conclusion, the NO donor NONO influenced the SERCA, reducing its Ca^{++} sensitivity, but increasing its rate of uptake. Reduced sensitivity of Ca^{++} induced Ca^{++} uptake ($[Ca^{++}]_{0.5}$) may increase cytosolic Ca^{++} concentrations due higher Ca^{++} required to induce uptake, likely increasing cytosolic Ca^{++} concentrations and activating Ca^{++} dependent proteases.