

Introduction

I/R injury induces cell death and organ dysfunction in large part due to a burst of reactive oxygen species that occurs upon the reintroduction of oxygen into the ischemic tissue area, leading to vascular endothelial dysfunction. This dysfunction causes decreased blood NO bioavailability and increased hydrogen peroxide (H_2O_2) levels. Previously we have shown that gp91ds-tat, a selective NADPH oxidase inhibitor, attenuated cardiac contractile dysfunction and reduced infarct size compared to controls in isolated rat hearts subjected to I/R injury. We speculate this occurs due to inhibition of NADPH oxidase-induced superoxide release. (1) NADPH oxidase is activated during I/R injury via cytokine receptor stimulation and utilizes molecular oxygen to produce superoxide. Gp91ds-tat selectively inhibits NADPH oxidase assembly by blocking p47^{phox} interaction with gp91^{phox}, also known as NOX2, and is principally expressed in endothelial and myeloid cells. Superoxide (SO) overproduction from activated NADPH oxidase can quench NO via the formation of peroxynitrite and also be converted to H_2O_2 in blood via superoxide dismutase. Peroxynitrite can further cause endothelial NO synthase uncoupling which produces SO instead of NO. (2) To understand the potential cardioprotective mechanism of gp91ds-tat, we performed real-time analyses of blood NO bioavailability and H_2O_2 levels using our rat hind limb I/R model.

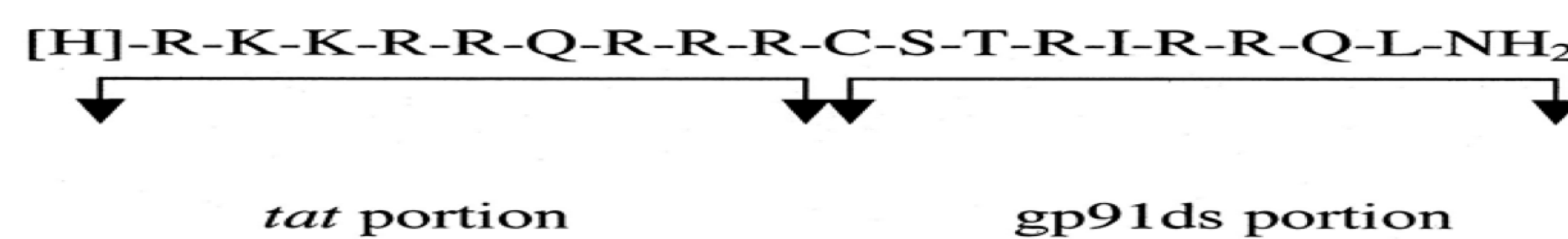


Figure 1. gp91ds-tat sequence (1.2 mg/kg, MW: 2452 g/mol) Adapted from Rey et al. (3)

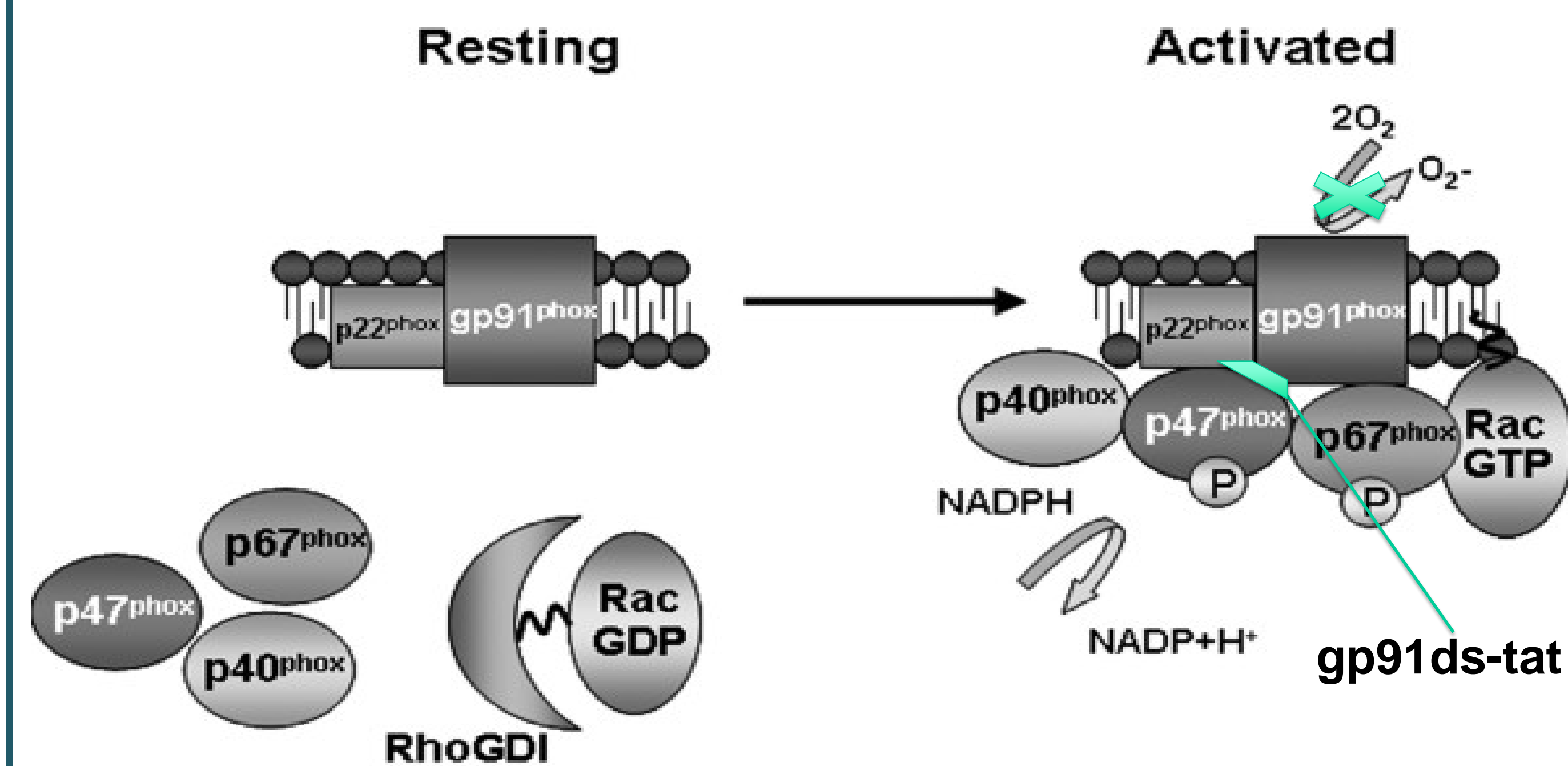


Figure 2. Schematic showing the inactive and active forms of NADPH oxidase. Green-lines denote areas of inhibition performed by gp91ds-tat. Adapted from Wilkinson et al. (4)

Hypothesis

We hypothesized that gp91ds-tat will attenuate I/R induced increase in H_2O_2 levels and decrease in NO levels in the blood when compared to the sham hind limb within the same rat. We expect that the gp91ds-tat treated rats will show a decrease in H_2O_2 blood levels and increased NO blood levels compared to the non-drug treated controls. As an outcome, there will be a decrease in ROS release and endothelial dysfunction.

Methods

Male Sprague-Dawley (SD) rats (275-325 grams, Charles River, Springfield, MA) were anesthetized with an induction dose of 60 mg/kg and maintenance dose of 30 mg/kg sodium pentobarbital intraperitoneally (i.p.). The rats also received an anticoagulant, sodium heparin (1000 USP units/mL) i.p. We measured blood H_2O_2 or NO levels from both femoral limbs in real-time: one limb was subjected to I/R while the other was used as a non-ischemic sham control. H_2O_2 or NO microsensors (100 μ m, WPI Inc., Sarasota, FL) were connected to a free radical analyzer (Apollo 4000, WPI Inc.) and were inserted into a catheter placed in each femoral vein (Figure 3). Ischemia was induced by clamping the femoral artery and vein of one limb for 30 min followed by 45 min of reperfusion. Gp91ds-tat (1.2 mg/kg) or saline (for control group) was given as a bolus injection via the jugular vein at the beginning of reperfusion. We continuously monitored the H_2O_2 or NO release and reported data collected at 5 min intervals during the 15 min baseline, 30 min ischemia and 45 min reperfusion periods. The changes in H_2O_2 or NO release during reperfusion are expressed as relative change to baseline after correction to the calibration curve of H_2O_2 (μ M) or NO (nM) microsensors. Experimental group data was compared by Student's t-test with $p < 0.05$ considered statistically significant.



Figure 3. The experimental preparation for measuring blood H_2O_2 or NO release from I/R and sham femoral veins in the male SD rats.

Results

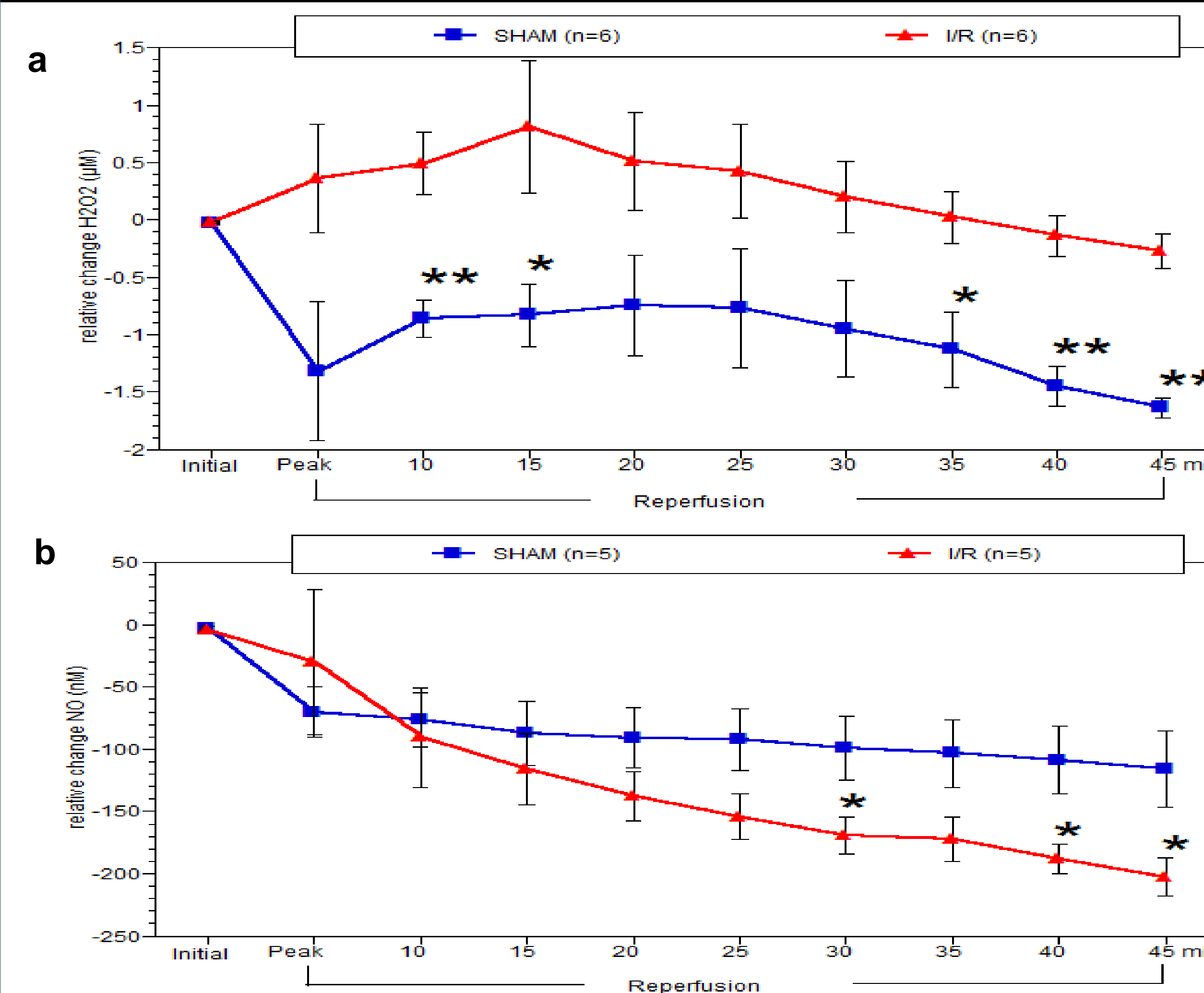


Figure 4. The time course of change relative to baseline in the saline control group of H_2O_2 (μ M) or NO (nM) from anesthetized male SD rat femoral limbs. (a) A significant increase in H_2O_2 release occurred from 10 min to 45 min reperfusion that ranged between 1.3 μ M and 1.4 μ M from I/R compared to sham limbs in saline controls (* $p < 0.05$, ** $p < 0.01$ from sham). (b) There was a significant decrease in NO release that ranged between -70 nM to -87 nM from I/R compared to sham limbs during the last 15 min (30-45 min) of reperfusion in saline controls. (* $p < 0.05$ from sham).

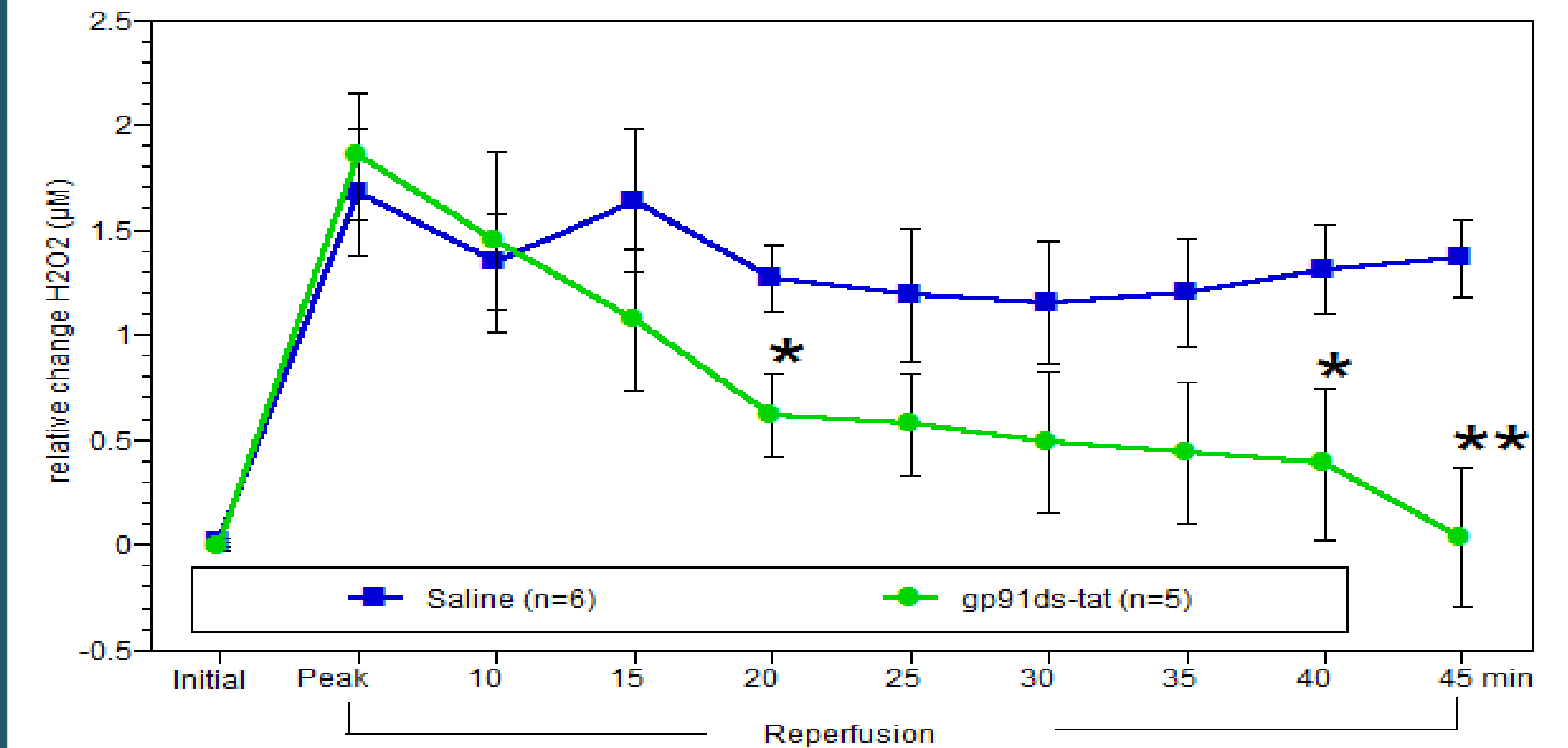


Figure 5. The comparison of H_2O_2 release in the blood between the I/R and sham femoral hind limbs (IR-Sham) during reperfusion between saline and gp91ds-tat treated SD rats. A significant decrease was observed in H_2O_2 release in the gp91ds-tat treated group at 20 min and 40-45 min of reperfusion that ranged between 0.65 μ M and 1.33 μ M. (gp91ds-tat * $p < 0.05$, ** $p < 0.01$ from saline).

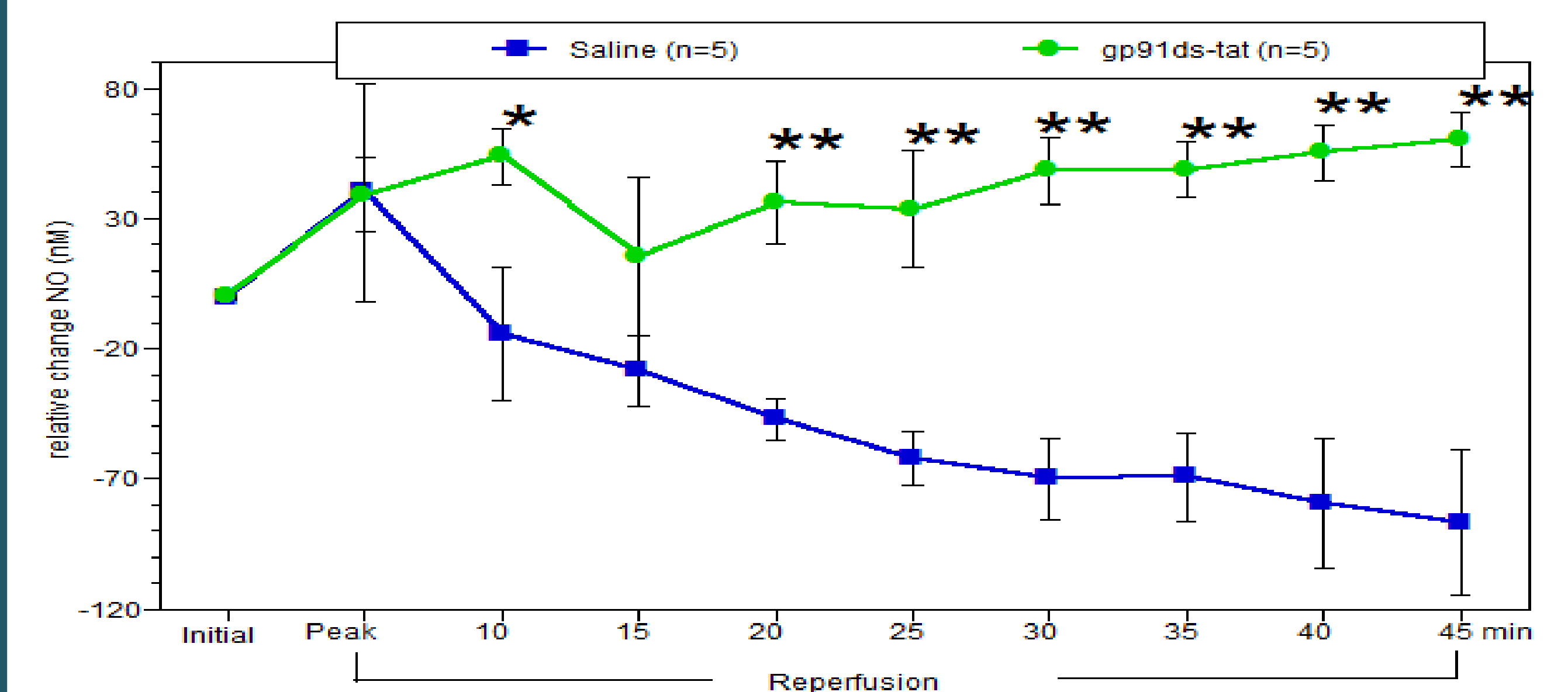


Figure 6. The relative change in NO release in the blood between the I/R and sham femoral hind limbs (IR-Sham) during reperfusion between saline and gp91ds-tat treated SD rats. A significant increase was observed in NO release in the gp91ds-tat treated group at 10 min and 20-45 min of reperfusion ranged between 68 nM and 146 nM. (gp91ds-tat * $p < 0.05$, ** $p < 0.01$ from saline).

Conclusions

I/R injury can cause an increase in blood H_2O_2 levels and a decrease in endothelial-derived NO bioavailability. A significant reduction of blood H_2O_2 levels and a significant increase in endothelial-derived NO bioavailability was observed in gp91ds-tat treated SD rats compared to the saline controls. The results of this study support our hypothesis that the NADPH oxidase peptide inhibitor, gp91ds-tat, significantly attenuates reperfusion-induced ROS release and leads to an increase in NO bioavailability. Overall, the data suggests that NADPH oxidase inhibitors like gp91ds-tat, may be a viable therapeutic option for future clinical use in I/R injury.

References

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