INTRODUCTION. Nitric oxide (NO) and nitric oxide synthase (NOS) are known to participate in the development of skeletal muscle ischemia/reperfusion (IR) injury (1). It is known that neuronal NOS (nNOS) is the most abundant NOS isoform in both rat and human skeletal muscle; however, its precise role in the development of skeletal muscle IR injury has not been clearly established. In the central nervous system (CNS), nNOS activity has been well studied and shown to contribute to the development of IR injury. However, in myocardial IR injury, genetically nNOS-deficient mice show no difference in infarct size compared to controls. This study utilized the highly selective nNOS inhibitor, N'-propyl-L-arginine (NPA) (149-fold selective for nNOS vs. eNOS; >3000-fold selective for nNOS vs. iNOS) (2), to demonstrate that nNOS inhibition is protective against skeletal muscle contractile function during early reperfusion.

METHODS: Fifty-seven rats weighing 175-225g were used. 1) Thirty rats underwent 3 h warm innerverated ischemia and 3 h reperfusion of the right extensor digitorum longus (EDL) muscle. Intravenous infusion of NPA (10 nmol/kg/min or 100 nmol/kg/min) or PBS (0.2ml/h) was started 15 min prior to reperfusion and continued throughout the experiment. After 3h reperfusion, the EDL was harvested and underwent in vitro contractile testing. The left EDL was harvested prior to ischemia or treatment and tested as an internal control. 2) Fifteen rats received a 3.25 h infusion of either NPA or PBS to examine the effects of NPA on non-ischemic skeletal muscle. The left EDL was harvested prior to treatment and underwent in vitro contractile testing as an internal control. The right EDL underwent in vitro contractile testing following agent administration. 3) Twelve rats were used to determine systemic effects of NPA or PBS administration on mean arterial pressure (MAP), heart rate (HR), and respiratory rate (RR).

For data analysis, the maximal twitch force and average isometric tetanic force of the treated EDL were compared with those of the contralateral nontreated EDL, and the results were expressed as a percentage of the contractile force achieved by the contralateral nontreated EDL. One-way analysis of variance (ANOVA) and then two-way repeated-measures ANOVA were performed to compare PBS to NPA-treated groups. A p-value <0.05 was considered significant.

RESULTS: 1) Following 3 h ischemia and 3 h reperfusion, rats treated with NPA produced maximal twitch contractile forces of 53±6% (mean±SEM) and 51±7% of normal in low and high dose NPA-treated rats, respectively, compared to 47±7% of normal in PBS-treated rats. This trend was not statistically significant. NPA-treated rats produced greater tetanic contractile force than PBS-treated controls at all tested stimulation frequencies (Figure 1). At 70 Hz stimulation, force produced was 30±5% of normal in PBS-treated rats and 37±6% and 40±7% of normal in low and high dose NPA-treated rats, respectively. At a stimulation frequency of 100Hz, force increased from 36±5% of normal in controls to 48±6% and 48±7% of contralateral normal in low and high dose NPA groups, respectively. At 120Hz stimulation, force increased from 39±6% of normal in controls to 53±7% and 53±6% of normal in low and high dose NPA-treated rats, respectively. The force produced across all frequencies was significantly greater for both high and low dose NPA compared to control by repeated measures ANOVA (p<0.05). There were no significant differences between muscles treated with either high or low dose NPA.

2) In non-ischemic skeletal muscle, there were no statistically significant differences in twitch or tetanic contractile force in rats treated with either high or low dose NPA compared to control.

3) During a 3.25 h infusion, mean arterial pressure decreased to a minimum of 91±7% of baseline at 10 minutes in the 10 nmol/kg/min group and 88±5% at 50 minutes in the 100 nmol/kg/min group compared to 99-103% of baseline in control rats during the first 50 minutes. These differences were not significantly different from control at any time point. There were no differences in heart rate or respiratory rate between NPA and PBS treated rats.

DISCUSSION: These results show that selective nNOS inhibition with NPA improves contractile function of innervated skeletal muscle following 3 h ischemia and 3 h reperfusion. There is no additional benefit of increasing the dosage of NPA from 10 nmol/kg/min to 100 nmol/kg/min. This finding suggests that NO synthesized by nNOS may be detrimental to contractile function of reperfused skeletal muscle. This result is similar to previous findings in the CNS, but different from studies in myocardium, where no benefit was demonstrated in nNOS deficient mice. The lack of increase in normal skeletal muscle function with treatment suggests that the beneficial effect of NPA is related to its ability to attenuate an increase in nNOS activity during reperfusion. In conclusion, this study shows that inhibition of nNOS is protective to skeletal muscle contractile function during early reperfusion and may represent a clinically useful mechanism for reducing the effects of IR injury.

REFERENCES

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