Using δ-opioid receptor agonists to protect skeletal muscle against low pH hypoxic damage

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Background
During many surgical procedures, tissue experiences a period of ischemia which can cause stunning, apoptosis, and necrosis of the tissue. By minimizing this damage, procedural outcomes and patient recovery could be improved (1).

It is unclear as to whether opioid receptor agonists demonstrate protective properties against ischemic injury or not. It is thought that δ-opioid receptors could be beneficial while κ-opioids could be detrimental. Even less is known about µ-opioids (2).

Previous in vitro models that have addressed ischemia/reperfusion injury utilize buffering systems which maintain a constant pH of 7.4 (3, 4). This is not the most accurate representation of ischemia as it does not account for the drop in tissue pH caused by the buildup of metabolic wastes. By creating a high CO2 hypoxic event, thus lowering the pH to 6.5, our model was better able to mimic real-life ischemic conditions.

Methods
These studies were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Minnesota. A biopsy of rectus abdominis muscle was dissected from healthy, castrated male Yorkshire swine weighing approximately 80kg anesthetized using telazol and thiopental. The biopsy was placed in an oxygenated modified Krebs buffer solution and dissected into 16 bundles approximately 2-4mm wide by 30mm long. These were suspended in two banks of 8 muscle baths each and stretched to their optimal length. Baths were randomized to one of the pre-treatment groups: Naloxone, DPDPE, Naltriben, or DADLE. Platinum electrodes stimulated the muscles with 15V, 1msec pulses every 10 seconds. Transducers were calibrated at 0 and 10 grams. Protocol began once the bundles were stabilized. The peak-twitch force was used to indicate the viability of the muscle samples.

Results

during hypoxia and reoxygenation. By the end of reoxygenation the 7.4 group, the Control pH 6.5 group had significantly lower force preconditioning at the corresponding pH. Compared to the Control pH 7.4 group, the Control pH 6.5 group had significantly lower force production throughout hypoxia and reoxygenation suggesting additional tissue injury.

Discussion
The combination of Naloxone with DPDPE (a non-specific blocker in combination with a δ-opioid) trended higher than either of the two independently, suggesting that any beneficial effect may be derived from the need to block some receptors while activating others. More specifically, the data suggests that the beneficial effect might be due to Naloxone’s ability to block the κ- and µ-receptors and DPDPE’s ability to activate the δ-receptor. This is suggested by the fact that neither DPDPE or Naloxone on its own appeared either helpful or detrimental, essentially matching Control pH 7.4 in figures 7 and 8, yet the combination of the two was not considered significantly different than the Control with no hypoxia group by the end of reoxygenation.

No clear conclusions can be drawn about Naltriben and DADLE. Naltriben, a δ-blocker, did slightly worse than Control as a trend, and DADLE, a δ-opioid, performed even worse. The combination of the two was no improvement, possibly because Naltriben does not block either the κ- or µ-receptors.

In figures 9 and 10 it can be observed that each group had a specific relationship to the Control pH 6.5 group established from the initial decay occurring in the incubation period. This relationship was maintained throughout hypoxia and reoxygenation, suggesting that the individual outcome of any of these bundles may be more related to the initial decay of the bundle rather than any trend related to the treatment.

Conclusion
We hypothesized that δ-opioid receptor agonists, as preconditioners, would aid in the reduction of damage caused by periods of high CO2 (low pH) hypoxia. Our results were not entirely concurrent with our hypothesis. From the analysis of these data, it can be concluded that the most protective group was the combination of the non-specific opioid receptor blocker and δ-opioid DPDPE (Naloxone/DPDPE). Additionally, the overall lower recovery of the low pH group suggests that using a model without pH modification might be an inadequate model of ischemia.

Future Work
Much more information is needed. With a larger sample size, the standard deviation for some groups could be lower which could be beneficial in more accurately calculating whether two groups are statistically different. In particular, more experiments are needed in the pH 6.5 range, especially focusing on Naloxone and its combinations with DPDPE as these performed the best in the pH 7.4 range. Different dosages need to be looked into, particularly to determine why the combination of Naltriben and DADLE wasn’t an improvement over Control.

References