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-agonists could be beneficial while κ-agonists could be detrimental. Even less is known about μ-agonists (2 - 5).

Previous *in vitro* models that have addressed ischemia/reperfusion injury utilize buffering systems which maintain a constant pH of 7.4 (2,4). 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 CO_2 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. 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.

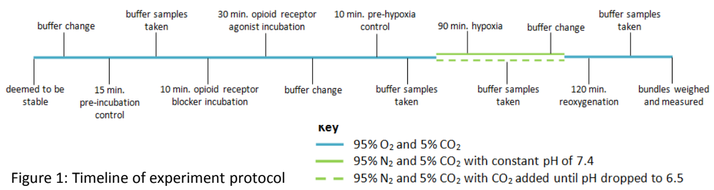

Figure 1: Timeline of experiment protocol

Figure 2: Dissecting swine biopsy

Figure 3: Bundle in muscle bath

Results

Drugs in this study	
[D-Ala2,D-Leu5]-Enkephalin (DADLE)	δ_1- δ_2-agonist
[D-Pen^2,D-Pen^5]-Enkephalin (DPDPE)	δ_1-agonist
Naloxone	δ- κ- μ-blocker/antagonist
Naltriben	δ_2-blocker/antagonist

Figure 4: Explanation of drugs used as preconditioners in this study.

Pr<0.05	
*	compared to Control no hypoxia
Δ	compared to Control pH 7.4
•	compared to Naloxone 10 uM / DPDPE 10 uM
θ	compared to DPDPE 10 uM
+	compared to Naloxone 10 uM

Figure 5: Explanation of symbols used. Differences between the means were tested at each time point using ANOVA and Tukey's Post Hoc Test. The probability Pr refers to the probability that the group corresponding to the column is the same as the group corresponding to the symbol. If the probability is <0.05, it is stastically probable that the two groups in question are different. All forces are presented as a percentage of their pre-incubation force ± StDev.

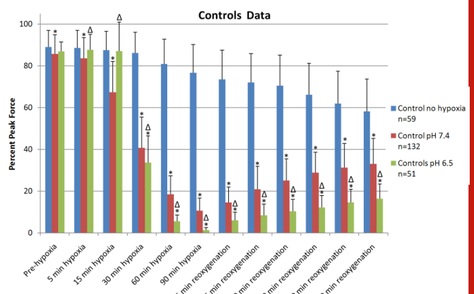

Figure 6: Graph of the controls throughout hypoxia and reoxygenation. Control no hypoxia columns reveal natural decay over time of muscle bundles while Control pH 7.4 and Control pH 6.5 show decay over time of bundles experiencing hypoxia and reoxygenation without any drug preconditioning at the corresponding 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.

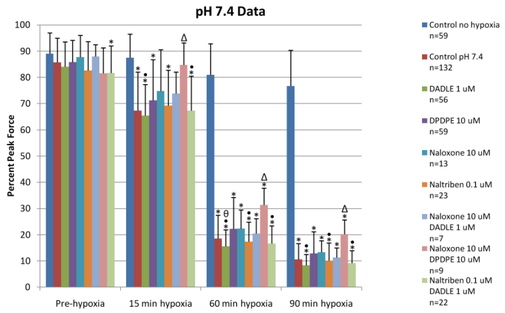

Figure 7: Graph of bundles at pH 7.4 during significant points of time of hypoxia. Throughout hypoxia, all groups were significantly lower than Control no hypoxia except the groups containing Naloxone at the 15 minute time point. Interestingly, the Naloxone/DPDPE group did not seem to decay to the same extent as the other Naloxone groups as it was considered significantly different from Control pH 7.4 throughout hypoxia.

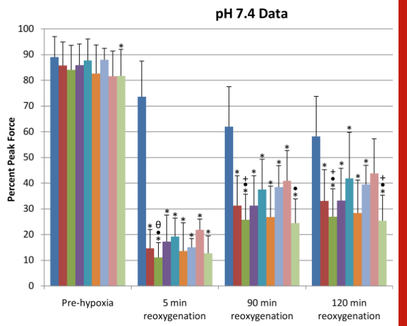

Figure 8: Graph of bundles at pH 7.4 during significant points of time of reoxygenation. By the end of reoxygenation the Naloxone/DPDPE group was no longer considered significantly different from Control no hypoxia.

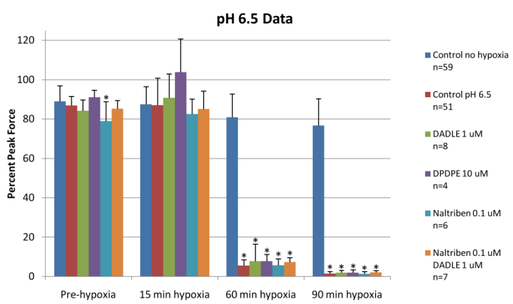

Figure 9: Graph of bundles at pH 6.5 during significant points of time of hypoxia Throughout hypoxia, none of the preconditioned groups were considered to be different than Control pH 6.5.

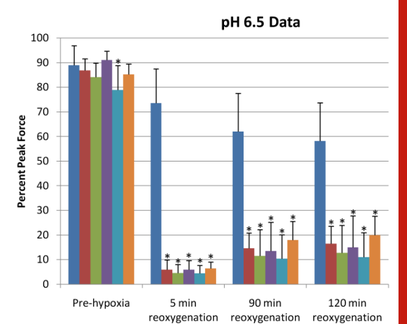

Figure 10: Graph of bundles at pH 6.5 during significant points of time of reoxygenation. Again, the recovery of the preconditioned groups was not considered different than the Control pH 6.5 group.

Conclusion

We hypothesized that δ-opioid receptor agonists, as preconditioners, would aid in the reduction of damage caused by periods of high CO_2 (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-agonist 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.

Discussion

The combination of Naloxone with DPDPE (a non-specific blocker in combination with a δ-agonist) 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-agonist, 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.

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 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

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