Pretreatment with Naltrindole Exhibits Robust Cardioprotection in an Isolated Rat Heart Model of Ischemia-Reperfusion Injury

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INTRODUCTION

Myocardial infarction (MI) is one of the deadliest forms of ischemic heart disease, resulting in millions of annual deaths globally. MI is characterized by cardiomyocyte cell death leading to compromised heart function due to insufficient supply of oxygenated blood to the heart.[1] In the clinical setting, percutaneous coronary intervention (PCI) is the gold standard to return blood flow as soon as possible. However, studies have shown that the restoration of blood to ischemic tissue (reperfusion) causes a paradoxical exacerbation of cardiomyocyte dysfunction and death in a phenomenon known as ischemia-reperfusion (I/R) injury (Fig.1) that threatens the long-term viability of the heart.[1] There are currently no therapeutic interventions that have been shown to clinically improve cardiac function and reduce infarct size in patients who have suffered from I/R-induced MI injury.

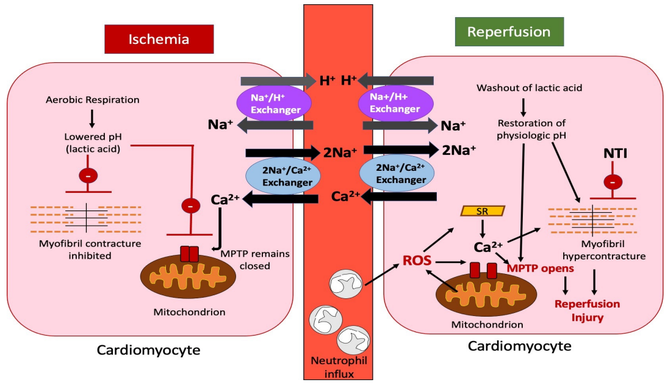


Figure 1. Proposed Mechanism of Ischemia/Reperfusion Injury in Cardiomyocytes. Acute myocardial ischemia results in a decrease in pH due to the build-up of lactic acid from anaerobic conditions. This results in closed mitochondrial permeability transition pore (MPTP). Reperfusion results in washout of lactic acid, resulting in the rapid restoration of physiological pH, which stops the inhibitory effect on the MPTP pore, induces Ca^{2+} overload, and cardiomyocyte hyper-contracture. The restoration of the mitochondrial membrane potential drives Ca^{2+} into the mitochondria, which can also induce MPTP opening (i.e. mitochondrial dysfunction) and cardiac contractile dysfunction. Neutrophils accumulate in the infarcted myocardial tissue in response to the release of chemo-attractants and generate reactive oxygen species (ROS) (adapted).[1] Naltrindole (NTI) exerted direct negative ionotropic effects on the heart during preconditioning (Figure 6) that correlated with attenuation of cardiac hypercontracture during reperfusion as evidenced by the dramatic restoration of left ventricular end diastolic pressure at 15 and 45min reperfusion (see Table 1).

In previous small and large animal studies of myocardial I/R injury (*ex vivo* and *in vivo*), activation of opioid receptors is known to mediate cardioprotective effects during preconditioning (prior to ischemia) that result in the resolution of cardiac arrhythmia or the reduction of infarct size.[2,3,4,5] The heart predominantly expresses delta (δ) and kappa (κ) opioid receptors throughout the atria and ventricles, making these opioid receptors attractive therapeutic targets.[4,5]

In our previous studies, a novel three-amino acid peptide (tripeptide), that is structurally similar to other amino acid-based delta and kappa-opioid receptor agonists, demonstrated a significant reduction in infarct size and improved cardiac function when administered during preconditioning in isolated rat hearts using the Langendorf model (*ex vivo*).[6]

To identify the specific opioid receptor subtype responsible, the cardioprotective effects of tripeptide combined with either Naloxone (NX; broad-spectrum opioid antagonist), Nor-binaltorphine (BNI; selective kappa-opioid receptor antagonist), or with Naltrindole (NTI; selective delta-opioid receptor antagonist) were studied. Co-treatment with either NX or BNI were found to inhibit the cardioprotective effects of tripeptide, indicating that tripeptide was a kappa-opioid agonist. Surprisingly, the co-treatment of tripeptide with NTI augmented the cardioprotective effects of tripeptide alone. The goal of this study was to determine the relative contribution of tripeptide and NTI in mediating the observed reduction of infarct size and restoration of cardiac function (e.g., left ventricular developed pressure [LVDP], the maximal rate of rise of LVDP [$+dP/dt_{max}$], and the maximal rate of decline of LVDP [$-dP/dt_{min}$]). To this end, the study evaluated the effects of the opioid antagonists: NTI, BNI, and NX independently in a Langendorf rat heart model.

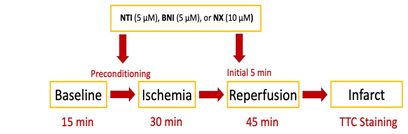
METHODOLOGY

Hearts from male Sprague-Dawley rats (275-325g, Charles River, Springfield MA) were isolated and subjected to 30 min global ischemia and 45 min reperfusion using a modified Langendorf heart preparation (Figure 2).

Naltrindole (NTI; 5 μM), Naloxone (NX; 10 μM), and Nor-binaltorphine (BNI; 5 μM) were given prior (last 5 min of baseline [15 min]) to the ischemic period (pre- treatment [i.e. preconditioning]) and during the initial 5 min of reperfusion (post-treatment).[2,6] Control I/R hearts did not receive any treatment.

Left ventricular (LV) cardiac function indices were measured using a pressure transducer (Fig.3). At the end of reperfusion (45min), hearts were frozen, cut into 2 mm sections, and stained with 1% triphenyl tetrazolium chloride (TTC). Weight of infarcted heart tissue (pale) was compared to total tissue at risk.

Statistical Analysis: All data in the figures are presented as means ± S.E.M. ANOVA analysis using Student-Neuman-Keuls test. Probability values of $p<0.05$ were considered statistically significant.



RESULTS

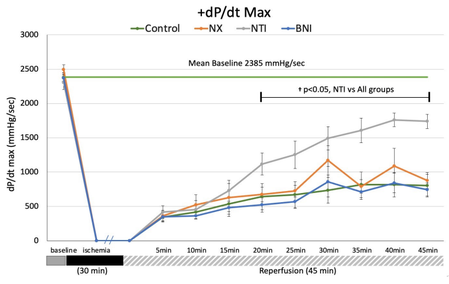


Figure 4. Time course of +dP/dt max for Control I/R, NX (10μM) + I/R, NTI (5μM) + I/R, and BNI (5μM) + I/R treated hearts. NTI + I/R significantly improved +dP/dt max during 20 min to 45 min of reperfusion. † $p<0.05$, NTI vs. all groups.

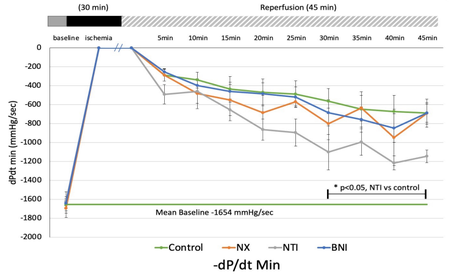


Figure 5. Time course of -dP/dt min for Control I/R, NX (10μM) + I/R, NTI (5μM) + I/R, and BNI (5μM) + I/R treated hearts. NTI + I/R significantly improved -dP/dt min during 30 min to 45 min of reperfusion. *$p<0.05$, NTI vs. Control.

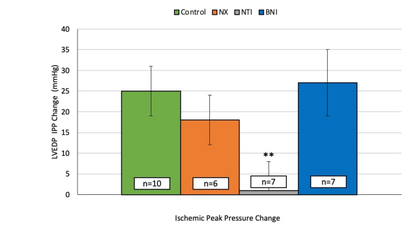


Figure 6. LVEDP ischemic peak pressure (IPP) change for Control, NX, NTI, and BNI. IPP change represents the peak pressure difference between preconditioning and IPP. NTI + I/R significantly attenuated the rise in IPP during ischemia. **$p<0.01$, NTI vs Control.

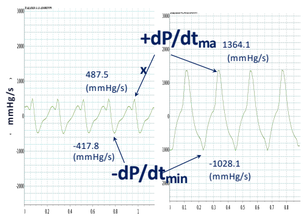


Figure 3. Representative tracings of the maximal rate of rise of left ventricular developed pressure (LVDP) [$+dP/dt_{max}$] and maximal rate of decline of LVDP [$-dP/dt_{min}$] for control I/R (left) and I/R + NTI (right) treated hearts at 45 min reperfusion following 30 min global ischemia.

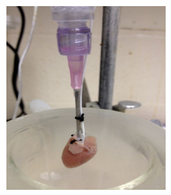


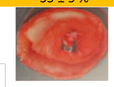
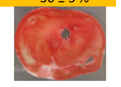
Figure 2. Flow diagram of the experimental protocol (left) and representative picture of isolated rat heart Langendorf preparation (top).

RESULTS (Cont.)

Table 1. Cardiac function baseline, reperfusion 15 mins, and final values (reperfusion 45 min) for control I/R; NX (10 μM) + I/R; NTI (5 μM) + I/R; and BNI (5 μM) + I/R treated hearts. NTI + I/R significantly improved post-reperfused cardiac function compared to control I/R (*$p<0.05$; **$p<0.01$). NTI exerted a negative ionotropic effect on cardiac function during preconditioning (#$p<0.05$; ##$p<0.01$, baseline NTI vs preconditioning NTI) which was associated with significant attenuation of ischemic peak pressure change compared to controls. **Abbreviations**: LVDP, left ventricular developed pressure (LVESP-LVEDP); +dP/dt max, maximal rate of rise in LVDP; -dP/dt min, maximal rate of decline in LVDP; LVESP, left ventricular end systolic pressure; LVEDP, left ventricular end diastolic pressure. Naloxone (NX), naltrindole (NTI), nor-binaltorphine (BNI) and ischemia-reperfusion (I/R).

	dP/dt max (mmHg/s)	dP/dt min (mmHg/s)	LVESP (mmHg)	LVEDP (mmHg)	LVDP (mmHg)	Heart Rate (bpm)	Coronary Flow (mL/min)
Control (n=10)							
Baseline	2379±65	-1660±87	105±4	8±1	97±3	265±7	20±2
Preconditioning (PC)	2455±72	-1736±90	105±3	9±1	97±3	266±8	19±2
Ischemia Peak Pressure (IPP)	11±5	-13±7	39±4	38±4	0.39±0.04	699±121	0
IPP Change	---	---	---	29±6	---	---	---
Reperfusion 15 min	532±181	-437±137	98±5	72±6	27±9	225±24	9±0.8
Reperfusion 45 min	800±168	-687±139	101±6	61±5	41±9	281±26	9±1
NTI (n=7)							
Baseline	2312±106	-1622±102	97±2	6±1	92±2	297±8	19±1
Preconditioning (PC)	1581±379**##	-991±305##	76±12**	17±5##	60±10**##	278±10	19±1
Ischemia Peak Pressure (IPP)	215±185	-221±192	21±3	18±3	2.5±1.6	287±133*	0
IPP Change	---	---	---	1±7	---	---	---
Reperfusion 15 min	729±151	-655±114	78±10*	39±7**	39±9	219±14	12±1
Reperfusion 45 min	1830±90**	-1185±82**	100±6	18±4**	82±4**	261±5	11±1
NX (n=6)							
Baseline	2496±69	-1696±96	100±3	8±1	92±3	256±5	16±2
Preconditioning (PC)	2433±102	-1624±134*	97±6	8±1	89±6	244±7	15±2
Ischemia Peak Pressure (IPP)	146±70	-140±68	26±6	26±6	0.85±0.24	676±137	0
IPP Change	---	---	---	18±6	---	---	---
Reperfusion 15 min	628±206	-551±166	91±9	67±9	24±5	208±34	6±1
Reperfusion 45 min	878±113	-692±112	101±10	65±8	36±7	240±25	6±1
BNI (n=7)							
Baseline	2371±80	-1642±51	94±3	6±1	88±2	288±11	20±2
Preconditioning (PC)	2319±83	-1566±85	92±5	6±1	86±4	279±10	19±2
Ischemia Peak Pressure (IPP)	24±19	-22±17	34±8	33±8	0.87±0.34	415±168	0
IPP Change	---	---	---	27±8	---	---	---
Reperfusion 15 min	483±96	-461±96	89±3	70±3	18±5	301±43	9±1
Reperfusion 45 min	729±93	-682±110	94±3	62±2	32±4	250±17	8±1

Table 2. Representative TTC stained mid-wall sections from isolated perfused rat hearts. Weight ratios of infarcted heart tissue vs at-risk (to heart tissue) left ventricular heart tissue in I/R as determined by TTC staining. Healthy red heart tissue and infarcted tissue stained white. NTI + I/R significantly decreased infarct size compared to control I/R. (†† $p<0.01$, NTI vs. all groups). **Abbreviations:** Triphenyl tetrazolium chloride (TTC), naltrindole (NTI) and ischemia-reperfusion (I/R), naloxone (NX) and nor-binaltorphine (BNI).

	Control	NTI	NX	BNI
Infarct Size (Infarct Area/Area At-Risk)	35 ± 3 %	7 ± 2 % ††	35 ± 5 %	36 ± 5 %
Representative Mid-Wall Heart Sections				

CONCLUSION

Results suggest that:

- Pretreatment with the delta-opioid receptor antagonist NTI leads to dramatic restoration of post-reperfused heart function (LVEDP, $-dP/dT_{min}$, and $+dP/dT_{max}$) to near pre-ischemic baseline levels.
- NTI may elicit direct cardioprotective effects on the heart during I/R injury that is independent of opioid receptor inhibition.
- There is a direct correlation between the attenuation of final LVEDP and infarct size which suggests that NTI mitigates myocardial I/R injury by preventing cardiac hypercontracture.

In future studies, we will:

- Determine whether the observed cardioprotective effects of NTI alone are reproducible in an *in vivo* rat myocardial I/R model.
- Examine potential mechanisms of NTI pretreatment that are responsible for the significant increase in resistance to I/R damage, and whether these effects are exerted independently of the delta opioid receptor.

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