(+)-naloxone and (+)-naltrexone transiently block cardioprotection in ischemic-preconditioned mouse hearts

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Introduction: Cardiac ischemic-reperfusion injury remains problematic in the clinical setting. Acute bouts of ischemia & reperfusion, preceding ischemic-reperfusion injury, are cardioprotective. (+)-naloxone and (+)-naltrexone, both non-opioid receptor antagonists with anti-inflammatory properties, remain poorly defined within the cardiac setting. This study investigates if either drug affects the cardioprotective properties of ischemic-preconditioning.

Method: Isolated Balb/c (12 weeks) mice hearts were cannulated onto the constant pressure Langendorff apparatus (n = 8 each group). Upon removal of the left atrial appendage a 9 mm balloon was inserted into the left ventricle to record left ventricular developed pressure (LVDP). Hearts were exposed to 35 min of ischemia (LTI-35m) followed by 40 min of reperfusion. Non-preconditioned (NPC) hearts were perfused for 41 min before LTI-35m. Ischemic-preconditioned (IPC) hearts were given 3 bouts 2.5 min of ischemia and 2.5 min of reperfusion followed by LTI-35m. (+)-naloxone and (+)-naltrexone were administered directly into hearts at 20 μM. Drugs were infused for 15 min in NPC hearts followed by a 2.5 min washout with krebs buffer before LTI-35m. In IPC hearts, drug infusion started 10 min before IPC and during the 1st and 2nd bout of reperfusion. The left ventricle was dissected and flash frozen in liquid nitrogen upon completion of each experiment. Tissue homogenate will be quantified using western blots for cytosolic Il-1β, cardiac-FABP and HMGB1 levels. Post LTI-35m effluent collected from each heart was assayed for cell death using LDH assays. Data was analysed using two-way ANOVAs with repeated measures and post-hoc Tukey HSD.

Results: Measurement taken of LVDP post-ischemic recovery (LVDP%) revealed that NPC hearts (time: 80 min, LVDP%: 56.5 ± 3.5%; mean ± SEM) recovered poorly compared to IPCs (time: 80 min, LVDP%: 84.6 ± 2.9%) by the end of each experiment ($P<0.05$). (+)-naltrexone-IPC (time: 45 min, LVDP% 64.7 ± 5.1%) and (+)-naloxone-IPC (time: 45 min, LVDP% 43.7 ± 4.0%) both blocked LVPD recovery during early reperfusion (Tukey HSD; $P<0.05$). However, both drug treated group's LVDP returned to 70% of their original pre-ischemic values 20 minutes after ischemia [(+)-naltrexone-IPC (time: 80 min, LVDP% 71.1 ± 3.4%) and (+)-naloxone-IPC (time: 80 min, LVDP% 71.1 ± 4.0%). Neither drugs affected the LVDP recovery in NPC hearts ($P>0.05$).

Conclusion: (+)-naltrexone and (+)-naloxone at 20μM blocks early LVDP recovery after ischemia. LDH assays and western blot experiments are the next step in this project.