(+)-naloxone and (+)-naltrexone limits nuclear factor K β translocation in LPS stimulated H9C2s

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Myocardial ischemic reperfusion injury remains the highest cause of mortality throughout the developed world. Previous experiments conducted in our lab showed that (+)-naloxone and (+)-naltrexone, toll-like receptor 4 (TLR4) antagonists, are able alter contractile recovery in isolated ischemic-reperfused mouse hearts. This study examines both compounds within the in-vitro setting using the H9C2 cell line. Immunocytochemistry measuring NF-kβ (an inflammatory transcription factor) nuclear translocation was used to determine the number of cells which responded to the TLR4 agonist LPS. H9C2s were pretreated with either compound for 1 hour at either 50, 100 or 200 μmol/l followed by LPS stimulation at 100 nanograms for 30 minutes. Cells were stained with NF-Kβ p65 followed by DAPI and Alexa Fluor 488 staining for visualization. Using Chi-square tests with Bonferonni post-hoc analyses both compounds reduced NF-Kβ translocation regardless of the dosage (P<0.05). Current experiments are aimed at optimizing an ischemic-reperfusion experiment within the in-vitro setting. H9C2s seeded at 1.0×10^5 cells / ml in 24 well plates were given a hypoxic buffer solution before placed into a hypoxic chamber for two hours. After this the supernatant was aspirated and the cells given a normoxic buffer and placed into an incubator. Preliminary studies using the MTT and trypan blue assay showed that this protocol significantly reduced cell viability. Future studies will examine whether (+)-naloxone or (+)-naltrexone is able to limit the loss in cell viability using this protocol.