091P ANANDAMIDE REDUCES INFARCT SIZE IN RAT ISOLATED HEARTS BY A MECHANISM INDEPENDENT OF CB_1 OR CB_2 RECEPTOR ACTIVATION

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Cannabinoids have recently been identified as cardioprotective agents (Lépicier *et. al.,* 2003). However, the endocannabinoid, anandamide (AEA), was reported to be ineffective at reducing infarct size in rat isolated hearts subjected to ischaemia-reperfusion. In that study, AEA was dissolved in propylene glycol. Therefore, we tested whether anandamide, delivered in Tocrisolve100^{TM} (Tocris, UK), could reduce infarct size in a similar experimental model to the previous study.

Methods: Hearts taken from Male Wistar rats (250-350g) were perfused at a constant pressure (80 mmHg) and immersed in perfusate (Krebs bicarbonate solution + insulin, 100 mU.ml^{-1}) maintained at 37˚C. Electrical pacing was applied at 5 Hz during periods of perfusion. Left ventricular developed pressure (LVDP) was measured by means of a pressurised (5-10 mmHg) balloon inserted into the left ventricle and connected to a pressure transducer. Coronary flow (CF) was measured with a Transonic probe, placed between the perfusate reservoir and heart, connected to a T206 flow meter. Baseline mechanical function was recorded during an initial 15 min aerobic perfusion. Hearts were then subjected to 30 min of global, no-flow ischaemia followed by 2 h reperfusion and were electrically paced at 5 Hz whilst perfused. Infarct size (% of the left ventricle) was determined from triphenyltetrazolium chloride staining. Where used, SR141716A (SR6 CB_1 receptor antagonist, 1 μM) and SR144528 (SR8 CB_2 receptor antagonist, 1 μM) were present in the perfusate throughout the protocol. Vehicle (Tocrisolve100^{TM} + DMSO 0.1% vol.vol^{-1}, n = 9), AEA (1 μM, n = 7) or ACPA + JWH133 (1 μM each, n = 5) were infused 5 min prior to the onset of ischaemia and then throughout reperfusion. SR6 and SR8 were tested alone (n = 5 and 6, respectively) or in the presence of AEA (n = 6 and 6, respectively). Values (mean ± s.e.m) obtained after 2 h reperfusion were compared among the different experimental groups by analysis of variance supported by Dunnett's *post hoc* test.

In vehicle-treated hearts, by the end of reperfusion, LVDP had recovered to 26±5% and coronary flow to 17±4% of baseline. Infarct size, measured at the end of reperfusion, was 21±3% of the left ventricle. AEA had no significant effect on recovery of LVDP or CF compared to control but significantly reduced infarction to 10±1% of the left ventricle. Alone, neither SR6 nor SR8 had any significant effect on infarct size (22±4% and 20±2% of the left ventricle respectively) or recovery of LVDP (24±4% and 28±5% of baseline, respectively) or CF (32±6% and 26±6% of baseline, respectively). However, both SR6 and SR8 blocked the reduction in infarct size induced by AEA (26±4% and 21±2% of the left ventricle, respectively), recoveries of LVDP and CF were not significantly affected. The combination of ACPA and JWH133 had no significant effect on the recovery of LVDP (10±1% of baseline) or CF (26±17% of baseline) or extent of infarction (19±5% of the left ventricle).

AEA delivered in Tocrisolve100^{TM} reduces infarct size when administered prior to ischaemia in rat isolated, Langendorff-perfused hearts. Activation of either CB_1 or CB_2 alone, or together, does not appear to account for cardioprotection induced by AEA.

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