INDUCTION OF APOPTOSIS IN A7R5 RAT AORTIC VASCULAR SMOOTH MUSCLE CELLS BY THE CALCIUM CHANNEL BLOCKERS SKF96365 AND NIFEDIPINE

KE Kerry¹, RD Jones¹, KS Channer² and TH Jones¹³¹. Hormone and Vascular Biology Group, Academic Unit of Endocrinology, University of Sheffield, UK. ²Department of Cardiology, Royal Hallamshire Hospital, Sheffield, UK. ³Centre for Diabetes and Endocrinology, Barnsley Hospital NHS Foundation Trust, Barnsley, UK.

Alterations in intracellular calcium homeostasis and signalling are proposed to play a key role in vascular smooth muscle cell (VSMC) apoptosis, an event important in vascular remodelling. Calcium channel blockers have been demonstrated to inhibit vascular lesion formation in animal models and are widely used in the treatment of hypertensive heart disease. We have utilised the A7r5 rat aortic VSMC cell line to investigate the effects of the L-type calcium channel blocker nifedipine and the store-operated calcium channel blocker SKF96365 on VSMC apoptosis.

A7r5 cells were plated at 150000 cells/well in 6-well cell culture plates in DMEM supplemented with 10% foetal bovine serum (FBS), and left to adhere over night at 37°C. Media was then replaced with phenol red-free DMEM supplemented with 2% FBS containing either H₂O (0.1%) vehicle or SKF96365 (1, 5, 50 or 100µM), or DMSO (0.1%) vehicle or nifedipine (1, 10 or 100µM) and incubated for 24 hours at 37°C. On the day of analysis, adherent cells were removed with 0.25% trypsin-EDTA and combined with floating cells via centrifugation at 1000rpm for 5 minutes, washed in ice-cold PBS, and resuspended in ice-cold annexin-binding buffer. Cells were then incubated with 5µl of FITC-conjugated annexin V for 15 minutes before the addition of 10µl of propidium iodide (100µg/ml). Cells were then analysed and apoptotic cells identified by fluoresce-activated cell sorting. 10000 events were counted per test.

Compared to vehicle control, 24 hour nifedipine 1, 10 and 100µM treatment induced dose-dependent apoptosis in early; 4.84±0.41%, 5.56±0.34%* and 7.59±0.60%* respectively, mid; 3.90±0.42%, 4.66±0.43% and 10.8±0.74%* respectively, and late; 4.31±0.29%, 4.51±0.24% and 11.86%* respectively, stages of apoptosis. Compared to vehicle, 24 hour treatment with SKF96365 1, 5, 50 and 100µM also induced apoptosis at early; 6.74±0.88%*, 12.5±0.75%*, 4.31±0.32% and 40.1±2.98%* respectively, mid; 1.34±0.20%, 2.30±0.19%*, 6.71±1.06%* and 4.14±0.32%* respectively, and late; 6.16±0.46%, 11.26±0.77%*, 11.68±2.4% and 41.57±3.52%* respectively, stages of apoptosis. Results expressed as % events counted ± S.E.M. * = p<0.05 by unpaired Students T-Test. N = 8 in all experiments.

In conclusion, the store-operated calcium channel blocker SKF96365 and the L-type calcium channel blocker nifedipine induce a dose-dependent increase in apoptosis of A7r5 VSMCs. These results demonstrate an important role of calcium in VSMC apoptosis and indicate a mechanism by which calcium channel blockers may act to contribute towards vascular remodelling in the prevention of lesion formation and treatment of heart disease.