The angiotensin type 2 receptor prevents cell death in neonatal cardiomyocytes of the hypertrophic heart rat

E.R. Porrello[1,2], A. D'Amore[2], C.L. Curl[1], S.B. Harrap[1], W.G. Thomas[2] and L.M.D. Delbridge[1], [1]Department of Physiology, The University of Melbourne, VIC 3010, Australia and [2]Baker Heart Research Institute, Prahran, VIC 3004, Australia.

The Hypertrophic Heart Rat (HHR) displays cardiomyocyte hypertrophy in Association with an apparent reduction in myocyte number in adulthood (Harrap *et al.*). This suggests the possibility of reduced hyperplasia or increased apoptosis during very early cardiac development. The angiotensin AT_1 and AT_2 receptor subtypes have been implicated in both cellular growth and apoptosis, although the precise mechanisms are unclear. Cardiac AT_2 receptor expression is high during early development (Bastien *et al.*), and it has been suggested that AT_2 receptor-mediated actions counterbalance those of the AT_1 receptor. Specifically, it has been proposed that the AT_2 receptor inhibits growth and promotes apoptosis, but data from transgenic and knock-out experiments do not support this hypothesis. The aim of this study was to determine the relationship between cardiac AngII receptor expression levels and neonatal cardiomyocyte growth and apoptotic responses in the HHR compared with their Normal Heart Rat (NHR) control strain.

Cardiac ventricles were freshly harvested from HHR and NHR neonates at post-natal day 2. Tissue AT_{1A} and AT_2 mRNA expression levels were quantified by real-time RT-PCR. Relative to NHR, HHR neonatal hearts exhibited significantly higher AT_2 and lower AT_{1A} receptor expression levels (4.6-fold higher AT_2/AT_1 ratio in HHR compared with NHR).

Neonatal cardiomyocytes were isolated by enzymatic digestion and plated at high density (1250 cells/mm^2). Adenoviruses containing constructs for either the AT_{1A} or AT_2 receptors were created. After 48 hours, myocytes were infected with either AT_{1A} and/or AT_2 receptors to achieve a physiological level of receptor expression (150 fmol receptor protein/mg total cell protein). In addition, to mirror receptor expression in neonatal HHR hearts, cells were infected with AT_{1A} and AT_2 receptors in a 4:1 ratio. Adenoviruses also co-expressed green fluorescent protein (GFP), making possible to identify and morphologically assess infected cells. To assess myocyte apoptosis counts were performed (5 fields from each triplicate well in n = 4 experiments) of infected HHR and NHR cells that displayed vacuolisation. The incidence of apoptosis was studied after 72 hours exposure to 0.1 μM AngII.

When infected with the AT_{1A} receptor alone, HHR myocytes showed significantly higher proportions of apoptotic cells than NHR (22.7%, SE 4.1 vs 1.1%, SE 0.6, $P < 0.001$). With the addition of the AT_{1A} receptor antagonist candesartan (1 μM), the proportion of apoptotic cells in HHR with the AT_{1A} receptor alone fell to levels similar to those seen in NHR (1.8%, SE 0.8). A similar suppression of apoptosis was observed (2.0%, SE 0.9) when the PKC signal transduction pathways that mediate AT_{1A} receptor signalling were inhibited with BIM (1 μM). When cells were infected with both the AT_{1A} and AT_2 receptors, evidence of apoptosis in HHR cells virtually disappeared (0.4%, SE 0.1).

In HHR neonatal cardiomyocytes, intrinsic (presumably genetic) differences seem to predispose to significantly increased AngII-induced apoptosis when the AT_{1A} receptor is expressed in isolation. Co-expression of the AT_{1A} and AT_2 receptors rescues the cells from apoptosis. These findings suggest novel protective physiological mechanisms for the AT_2 receptor in early cardiac growth.

Bastien, N.R., Ciuffo, G.M., Saavedra, J.M. & Lambert, C. (1996) *Regulatory Peptides*, **63**, 9-16.

Harrap, S.B., Danes, V.R., Ellis, J.A., Griffiths, C.D., Jones, E.F. & Delbridge, L.M.D. (2002) *Physiological Genomics*, **9**, 43-48.