Angiotensin II via AT_1 receptors may mediate apoptosis in the cardiac conduction system of rats

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Apoptosis has been suggested as a possible cause of gradual development of complete heart block and fatal arrhythmias associated with absence of the AV node, sinus, and internodal pathways (James *et al*, 1996). Studies about apoptosis in the heart by means of cardiomyocyte cell culture have demonstrated that angiotensin II (Ang II) mediates cardiomyocyte apoptosis via angiotensin II type I receptors (AT_1) (Cigola *et al*, 1997). The transgenic *m(Ren-2)27* (TG) rat carries the additional *Ren-2* gene, the expression of which results in an increase of heart Ang II (Campbell *et al*, 1995), thus potentially affecting the cell growth/death equilibrium. This study addresses the question of role of Ang II/AT_1 receptors mediated apoptosis in the sinoatrial (SA) and atrioventricular nodes (AV).

Six, male 2 week TG and Hannover Sprague Dawley (SD) rats were anaesthetised by pentobarbitone sodium i.p. injection (100 mg/kg). The hearts were removed and fixed in 10% formaldehyde. Following dehydration and embedding in paraffin, 5 μm serial sections were cut then stained with Masson Trichrome to localize SA and AV nodes. The sections containing SA or AV node were processed for either: (a) calculation of apoptotic nuclei following terminal deoxnucleotidyl transferase nick end labelling of 3'-OH ends using Fluorescein-FragEL™; or (b) immunohistochemical labelling with antibodies to the AT_1 receptors prior to confocal scanning laser microscopical analysis. Quantification of AT_1 receptors was performed by using Microimage analysis software (Olympus).

Group	Apoptotic cells/mm^2		AT_1 receptors ($\times 10^3$)/mm^2	
	SA	AV	SA	AV
SD	0.040±0.07	0.164±0.12	1.14±0.17	7.63±1.91
TG	**0.140±0.37***	**0.433±0.11***	**1.67±0.26***	**12.50±3.97***

Data expressed as mean ± SD (n=6)

* = significant compared with control ($P<0.05$) (Independent-Sample T-test)

The table shows that the number of apoptotic cell in both the SA and AV node is significantly greater in the TG compared with the SD ($p<0.05$). Quantification of AT_1 receptors within SA and AV node shows that there were significantly more AT_1 receptors in the TG compared with the SD ($p<0.05$). These data suggest that an elevated level of apoptosis in the TG rat heart compared with the controls could be accounted for by *Ren-2* derived Ang II active via AT_1 receptors.

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