Cellular localisation of relaxin receptors in arteries and veins and region-dependent responses to *in vivo* relaxin administration in male rats

M. Jelinic,[1] J.H. Gooi,[1] M.E. Wlodek,[2] H.C. Parkington,[3] M. Tare[3] and L.J. Parry,[1] [1]Department of Zoology, The University of Melbourne, Parkville, VIC 3010, Australia, [2]Department of Physiology, The University of Melbourne, Parkville, VIC 3010, Australia and [3]Department of Physiology, Monash University, Clayton, VIC 3193, Australia.

Cardiovascular diseases contribute to nearly one third of all deaths worldwide, with vascular dysfunction and increased vascular stiffness as high risk factors. Recent Phase I/II clinical trials have identified the peptide hormone relaxin (RLX) as an effective treatment of acute heart failure (Teerlink *et al.*, 2009), although the mechanisms of action in this clinical application are unknown. RLX is a potent vasodilator of small renal and mesenteric arteries in rodents, and human subcutaneous arteries (Conrad, 2010). It acts directly on endothelial cells (ECs) to stimulate nitric oxide production. This involves activation of vascular matrix metalloproteinases (MMPs) and ET_B receptors (Conrad, 2010). *In vivo* administration of RLX in rodents also increases passive compliance in some arteries through outward hypertrophic remodelling (Chan & Cipolla, 2011; Debrah *et al.*, 2011). Less is known about the vascular effects of RLX on veins. In addition, RLX-family peptide receptors 1 and 2 (RXFP1 and RXFP2) have not been localized to specific cell types in blood vessels. Therefore, the two objectives of this study in male Wistar rats were to: i) localize RXFP1 and RXFP2 in arteries and veins; and ii) test the hypothesis that RLX reduces vascular stiffness and increases vasodilation but that these effects may vary between different arteries and veins.

Study 1: *Rxfp1* and *Rxfp2* expression was compared in the aorta, mesenteric and femoral artery and vein by quantitative PCR. *Rxfp1* was more highly expressed in the aorta and femoral vein whereas *Rxfp2* was predominantly expressed in the mesenteric and femoral arteries. Immunohistochemistry using rat arteries demonstrated that RXFP1 was largely expressed in the vascular smooth muscle cells. These novel findings show that *Rxfp1* and *Rxfp2* are differentially expressed in arteries and veins, and that RLX may act directly on the vascular smooth muscle to alter the extracellular matrix or cause relaxation.

Study 2: Male rats (n=8) were chronically infused *in vivo* with 4 μg/h human recombinant RLX for 5 days. Rats were anaesthetized using isofluorane inhalation (2% in oxygen). Passive mechanical wall stiffness and *Mmp2* and *Mmp9* expression in the femoral and mesenteric artery were compared with vehicle-controls (n=6). Vascular reactivity in response to *in vitro* RLX treatment was also investigated in the aorta, femoral artery and femoral vein. *In vivo* RLX treatment significantly reduced stiffness in the mesenteric artery but had no effect on the femoral artery. This was associated with outward hypertrophic remodelling. RLX treatment had no effect on *Mmp2* or *Mmp9* suggesting no involvement of vascular gelatinases. Incubation of vessels with RLX for 45 min *in vitro* had no effect on PE-induced constriction. In summary, these data demonstrate that RLX has region-specific functions in the vascular system. The ability of relaxin to reduce arterial wall stiffness may be beneficial in the treatment of some cardiovascular diseases.

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