Signalling through endothelial connexin40 modulates the myogenic constriction of arteries and regulates blood pressure

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Introduction. Cardiovascular functions such as the heart beat and regulation of artery diameter require the unified action of millions of individual cells. This logistical feat is in part dependent on gap junctions that allow the rapid spread of electrical and chemical signals between adjacent cells. Connexin40 (Cx40) is one of four gap junction subunit proteins expressed in the cardiovascular system and its extensive expression in the vascular endothelium implies an important role in the coordination of vascular responses (Hill *et al.*, 2001). Indeed, global deletion in mice (Cx40KO) has been shown to cause impaired propagation of vasodilation in the microcirculation and extreme hypertension (de Wit *et al.*, 2000). Furthermore, Cx40 polymorphisms in humans have been associated with atrial fibrillation and hypertension (Firouzi *et al.*, 2006; Juang *et al.*, 2007). However, in addition to endothelial cells, Cx40 is expressed in the heart and the renin secreting cells of the kidney and its loss causes dysregulation of renin secretion as well as down-regulation of another major cardiovascular connexin, Cx37, at the sites of Cx40 deletion (Wagner *et al.*, 2010). We therefore aimed to determine the role of endothelial expression of Cx40 on the regulation of blood pressure and vascular function through the use of new transgenic mouse models.

Methods. To analyse the function of endothelial Cx40, we generated transgenic mice expressing a dominant negative form of Cx40 (ΔCx40), under control of the endothelial specific promoter, Tie2. The cardiovascular effects of ΔCx40 expression were determined by measurement of blood pressure, renin secretion and the reflexive vasoconstriction of small mesenteric resistance arteries to increased intraluminal pressure *in vitro* (myogenic response). Serum samples for analysis of renin secretion were obtained from the retroorbital sinus of mice using heparinized capillary tubes after anaesthesia was induced by isoflurane inhalation. Tissues for *in vitro* experiments were obtained from mice euthanized after isoflurane anaesthesia.

Results. Isolated small mesenteric arteries from ΔCx40 transgenic mice exhibited increased sensitivity of the myogenic response to pressure increases (EC_{50}: wildtype, 45mmHg; ΔCx40, 37mmHg; $P<0.001$). Impairment of nitric oxide was not responsible for these effects as inhibition by L-NAME (100μM) and hydroxocobalamin (100μM) increased the sensitivity of both wild type and ΔCx40 arteries to pressure increase. However, subsequent inhibition of the endothelium dependent hyperpolarisation (EDH) by blockade of small and intermediate calcium activated potassium channels (1μM TRAM-34 and 1μM UCL1684, respectively) revealed that this pathway was impaired in ΔCx40 arteries as no effect was seen, while wild type arteries showed a further increase in sensitivity to pressure. A modest but significant increase in the blood pressure of ΔCx40 mice was observed without alteration in renin secretion or loss of Cx37 observed in Cx40KO mice.

Conclusion. Endothelial Cx40 is necessary for modulation of myogenic constriction by endothelium dependent hyperpolarisation and its impairment causes elevated blood pressure.

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