

## Role of gap junctions in renal vascular conducted response

The juxtaglomerular apparatus (JGA) is an essential structure in the regulation of renal function and embodies two major functions: tubuloglomerular feedback (TGF) and renin secretion. Both mechanisms function to regulate renal microcirculation, in addition to a plethora of systemic effects. The JGA is a unique structure to study intercellular communication. Intercellular connections, gap junctions (GJ), allow exchange of ions, nutrients, and small signalling molecules between neighbouring cells. GJs consist of two connexons, one from each cell, with connexins (Cx) as their building blocks. Several Cx isoforms are expressed in the JGA (1), making the JGA a functional syncytium. Disruption of the signalling pathways in the JGA is associated with reduced TGF response, dysregulation of renin secretion and hypertension (2, 3). The aim of this study was to determine if reduced intercellular communication affects the conduction of TGF-induced vasoconstriction in the afferent arteriole.

Experiments were performed using the isolated perfused juxtamedullary nephron preparation. Kidneys were isolated from wild type (WT); Cx40 knockout (Cx40 KO) and Cx45 KO mice and perfused with a Tyrodes buffer containing 5% BSA and an amino acid mixture, at pH 7.4. The inner renal surface contains a unique nephron population and allows access to the afferent arteriole. Local afferent vasoconstriction was induced by electrical pulse stimulation (300ms, 90V), administered via 2M NaCl filled microelectrode (0.5-0.8 M $\Omega$ ) at the glomerular entrance. These electrical stimulations emulate the initiation of vascular TGF responses. Renal perfusion pressure was kept constant at 95 mm Hg. Inner afferent arteriolar diameter was measured at the stimulation site (0  $\mu$ m) and every 50  $\mu$ m, at distances upstream (up to 400  $\mu$ m), following a protocol consisting of 5 min acclimation period, 30 s baseline, 30 s electrical stimulation, and 30 s recovery measurements. Response between WT, Cx40 and Cx45 KO were compared.

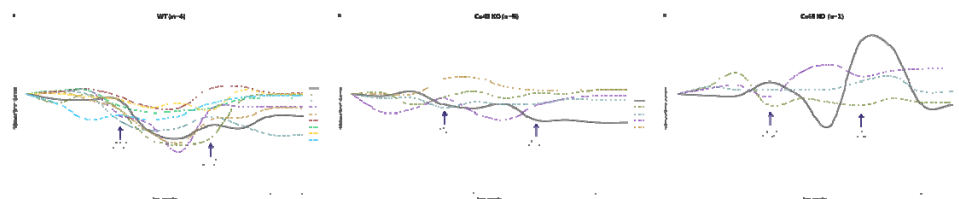


Fig 1. Relative afferent diameter in wild type (WT), Cx40 knockout (KO) and Cx45 KO groups in response to electrical stimulation both local and upstream sites. A: WT (n=6). B: Cx40 KO (n=6). C: Cx45 KO (n=2).

Preliminary data shows that electrical stimulation at the glomerular pole of the afferent arteriole reduces the diameter in the WT groups (Fig 1A) at both the local stimulation and upstream sites. In contrast, only the local stimulation site was affected in the KO groups, and no constriction was observed upstream (Fig 1B, C). Statistical analysis comparing the trend in Cx40 KO with the WT group indicates a significant difference ( $P=0.0016$ ). These results suggest that both Cx40 and Cx45 play a significant role in the propagation of the afferent vasoconstriction elicited by electrical stimulation, and thus in the TGF signalling in the JGA.

- (1) Hanner *et al.* (2010). *Am J Physiol Regul Integr Comp Physiol* **298**: 1143-1155.
- (2) Kurtz *et al.* (2007). *J Am Soc Nephrol* **18**: 1103-1111.
- (3) Sorensen *et al.* (2012). *Am J Physiol Renal Physiol* **303**: 855-863.