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Steroid Hormones and Receptors

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Aldosterone Production Is Regulated By Gap Junctions In Human Adrenal Cells

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Aldosterone plays a critical role in blood pressure regulation via the renin-angiotensin-aldosterone system. Autonomous production of aldosterone, primary aldosteronism (PA), is the commonest curable cause of hypertension worldwide. Two cell types in the adrenal cortex produce aldosterone: zona glomerulosa (ZG) cells and aldosterone producing nodules (APN), and in cells of aldosterone producing adenomas (APA). Adrenal cortex cells undergo phenotypic metamorphosis as they migrate from the subcapsular region, centripetally, towards the corticomedullary junction, changing from aldosterone- to cortisol- producing cells as they go. Gap junctions (GJs) are specialised channels of cytoplasmic communication between cells, permitting the regulated transfer of substrates. Loss-of-function mutations in the CADM1 gene, which affects GJ protein CX43, have been found in APAs, implicating CX43 in aldosterone regulation. This study aims to demonstrate the dysregulation of aldosterone production by CX43 inhibition in human adrenal cell line, H295R, and elucidate GJ distribution in human adrenal cortex.

Methodology: H295R cells were treated with 1×10-8M angiotensin II and connexin mimetic GJ inhibitor, GAP27, at 0μM, 25μM, 83μM and 250μM. Immunofluorescence (IF) was used to identify the abundance of CX43 in different cell types of the adrenal cortex, performed on ex-vivo para-APA adrenal tissue, using antibodies against CX43 (GJA1), colocalised with markers of aldosterone-producing cells (aldosterone synthase, CYP11B2), cells of the zona glomerulosa (ZG) (VSNL1, and DAB2) and zona fasciculata (ZF) (CYP7A1).

Results: Inhibition of CX43 with Gap27 results in a dose-dependent increase in CYP11B2 mRNA expression and aldosterone production. At the highest concentration of 250μM GAP27, CYP11B2 mRNA expression increased by 133-fold (p=<0. 00001) and aldosterone production by 29-fold (p=0. 0006). IF studies demonstrate a crescendo gradient of CX43 in a centripetal direction from cells of APN to ZF. The abundance was lowest in aldosterone-producing APN cells, moderate in non-aldosterone-producing ZG cells and highest in cortisol-producing ZF cells. GJ distribution was pan-membranous in ZF, but, where present, mostly cytoplasmic in APN. Evidence of recent active communication, in the form of annular gap junctions (AJG), was present in all three cell types.

Conclusion: The CX43 inhibition-induced upregulation of CYP11B2 expression and aldosterone synthesis indicates that it has an important regulatory role in physiological aldosterone production via CYP11B2 expression. The distribution of CX43 in adrenal tissue suggests its transport substrate not only regulates aldosterone synthesis, but also in adrenal cortex cell phenotypic metamorphosis/migration. Future work will investigate the substrate of CX43 in adrenal cells and its possible effect on adrenal cell phenotype.

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