

# Novel aspects of the Renin-Angiotensin-Aldosterone System

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## Summary

An important hormonal regulatory system for both fluid balance and blood pressure regulation is the renin-angiotensin-aldosterone system (RAAS). Activation of the RAAS is therefore a vital mechanism to maintain homeostasis in the setting of decreased circulating volume (such as blood loss or dehydration). However, when the RAAS is chronically activated in the setting of cardiometabolic diseases, the two main effector hormones of this system, aldosterone and angiotensin II, have significant pathogenic actions on the cardiovascular and renal system, which include the stimulation of fibrosis, inflammation, cell proliferation, neovascularization and oxidative stress<sup>1</sup>.

Aldosterone levels are increased in 50% to 80% of all hypertensive disorders and are associated with obesity and metabolic disorders such as impaired glucose and lipid metabolism and insulin resistance<sup>2</sup>. Moreover, patients with primary aldosteronism (PA), have increased cardiovascular risk, and more renal and metabolic complications than essential hypertension<sup>3</sup>. Despite several decades of investigation, the mechanism of excess aldosterone secretion in PA remains poorly understood.

In addition to activation of RAAS, accumulation of the reactive glucose-metabolite methylglyoxal (MGO) is recognised as key a driver of cardiovascular disease and vascular complications in diabetes. There are some indications that the RAAS and the MGO pathway interact, but this is relatively underexplored. MGO is formed as a glycolytic intermediate and a byproduct of glycolysis<sup>4</sup>. MGO is detoxified to D-lactate by the glyoxalase system with the enzyme glyoxalase 1 (Glo1) as the rate limiting step<sup>5</sup>. It has been demonstrated that Glo1 is impaired in diabetes, and that this impairment can be restored by the angiotensin receptor blocker (ARB) candesartan. In mice, has been demonstrated that candesartan prevents experimental diabetic retinopathy by restoring Glo1 function<sup>6</sup>. In type 1 (T1DM) and type 2 diabetes mellitus (T2DM), higher plasma MGO levels were associated with chronic kidney disease (CKD) and cardiovascular disease (CVD)<sup>7,8</sup>. Blockade of the RAAS is one of the most successful interventions to combat diabetic complications<sup>9-11</sup>, but whether the beneficial effects of the blockade of the RAAS is due to a reduction of MGO has not be fully explored.

The aims of this thesis were to explore: 1. the mechanisms underlying the aldosterone production in primary aldosteronism; 2. the implication of the RAAS in primary aldosteronism; 3. the interaction of RAAS with glycation.

The topics covered in this thesis are introduced in the general introduction, described in Chapter 1. The main outcomes of this thesis were:

- In **Chapter 2**, we reviewed the concept of epithelial-to-mesenchymal transition (EMT) and its role in renal diseases, with particular focus on hypertensive kidney disease, the second leading cause of end-stage renal disease after diabetes mellitus.
- In **Chapter 3**, we described the role of angiotensin II type 2 receptor (AT2R) and the angiotensin-(1-7) receptor (MasR) on aldosterone and cortisol synthesis using a pharmacological approach. We did not find a significant effect of nanomolar concentrations of Compound 21 (C21), an AT2R agonist, on CYP11B1 (cortisol synthase) or CYP11B2 (aldosterone synthase) gene expression in the NCI-H295R and HAC15 adrenocortical cells lines. Furthermore, no effect of C21 was observed in aldosterone-producing adenoma (APA), a condition featuring hyperaldosteronism, and in APA-adjacent tissue. However, micromolar concentration of C21 markedly increased CYP11B1 and CYP11B2 gene expression through angiotensin II type 1 receptor (AT1R), as this effect was blunted by the angiotensin II type 1 receptor blocker irbesartan.
- In **Chapter 4**, we have investigated the effect of the macrolide antibiotic clarithromycin on aldosterone synthesis in cells isolated from APA with/without somatic mutations in the potassium channel Kir3.4 (KCNJ5). Clarithromycin lowered, in a concentration-dependent manner, the expression of the CYP11B2 gene. However, whether and aldosterone secretion in aldosterone-producing cells (CD56<sup>+</sup> cells) from KCNJ5 mutated APAs. However, when exposed to increasing concentrations of clarithromycin, the CD56<sup>+</sup> cells obtained from wild-type APAs showed no change of CYP11B2 gene expression and aldosterone secretion in response to the macrolide.
- In **Chapter 5**, we described that in adrenocortical cells HAC15 and in tissue strips obtained ex vivo from patients with APA, the G protein-coupled estrogen receptor (GPER), is the main mediator of CYP11B2 expression in response to aldosterone exposure as this effect was abolished by the selective antagonist G36 or molecular silencing of GPER. Furthermore, angiotensin II potentiated the GPER-mediated effect of aldosterone on CYP11B2 through a crosstalk between GPER-1 and AT1R receptors.
- We have studied in **Chapter 6** the titer of angiotensin II type-1 receptor autoantibodies (AT1AA) in APA patients. I described that the titer of AT1AA is increased in APA patients, which persisted after surgical cure of hyperaldosteronism. Moreover, I found in HAC15 cells, that the stimulation with IgG purified from sera of APA increased both CYP11B2 expression and aldosterone release.

In **part 2** of this thesis, I have studied the link between RAAS and glycation.

- In **Chapter 7**, we showed that in APA patients, serum levels of AT1AA are not associated with the serum dicarbonyls methylglyoxal, glyoxal and 3-deoxyglucosone, or protein-bound and free advanced glycation endproducts (AGEs). Moreover, free AGEs N<sup>ε</sup>-(1-carboxymethyl)lysine (CML), N<sup>ε</sup>-(1-carboxyethyl)lysine (CEL) and N<sup>δ</sup>-(5-hydro-5-methyl-4-imidazol-2-yl)-ornithine (MG-H1) serum levels were increased after adrenalectomy.
- We reported in **Chapter 8** that Irbesartan treatment did not change plasma levels of the dicarbonyls methylglyoxal, glyoxal and 3-deoxyglucosone, free AGEs or D-lactate in individuals with type 2 diabetes and albuminuria.
- Despite the null finding in chapter 8, we have studied the effect of angiotensin II in mice and reported in **Chapter 9** that angiotensin II infusion in mice downregulated Glo1 gene expression in the kidney and the liver in vivo with a concomitant increase in dicarbonyl stress.

The main findings are further discussed in the general discussion.