Maastricht University

The role of Aldosterone and PTH in Human Primary Aldosteronism and Vascular Calcification

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Addendum

Summary

The present thesis focuses on mechanisms describing the bidirectional interaction between aldosterone and PTH and the impact on the cardiovascular system.
Although only a small proportion of cases of primary aldosteronism (PA) are familial, investigation of these cases has been elemental for understanding the molecular basis of the disease. One such example is the mechanism of apparent autonomy in hyperaldosteronism by the renin-angiotensin system. **Chapter 1** summarizes current knowledge on familial forms of PA and the association with main gene mutations. These familial genetic abnormalities are present as germline mutations in PA and account for up to 5% of cases and are inherited in an autosomal dominant manner. To date, four different forms of familial hyperaldosteronism (FH) have been described. FH-1 is caused by an unequal crossing-over of the promoter sequence of the CYP11B1 gene with the coding region of CYP11B2. As a consequence, the regulatory region of the resulting chimeric gene is altered such that the CYP11B2 gene becomes responsive to the adrenocorticotropic hormone (ACTH). FH-2 is caused by gain of function mutations in the gene CLCN2 coding for ClC-2 chloride channels. These mutations result in an enhanced chloride efflux and membrane depolarization causing increased CYP11B2 expression and aldosterone overproduction. In FH-3, Kir3.4 (KCNJ5) mutations cause a loss of selectivity for K^+, Na^+ influx, depolarization of the cell, with subsequent increase in intracellular Ca^{2+} via voltage-gated T-type Ca^{2+} channels and autonomous production of aldosterone. Finally, in FH-4, the gene encoding for T-type Cav3.2 (CACNA1H) activates channels at less depolarized potentials with ensuing increased Ca^{2+} influx.

Chapter 2 describes new horizons for diagnosis and treatment of aldosterone-producing adenoma caused by a somatic mutation in the potassium channel Kir 3.4 (KCNJ5), which explains for some 30% to 70% of PA cases. We tested effects of clarithromycin-macrolide on aldosterone synthesis and secretion in a population of aldosterone-secreting cells, obtained by immuno-separation of $CD56^+$ cells from APA tissue, with or without the 2 most common KCNJ5 mutations (G151R and L168R). When exposed to increasing concentrations of clarithromycin, $CD56^+$ cells obtained from wild-type APAs showed no change of CYP11B2 gene expression and aldosterone secretion in response to the antibiotic macrolide. Conversely, both G151R and L168R APA cells exposed to clarithromycin showed a concentration-dependent consistent blunted expression of the aldosterone synthase gene. Our results provide compelling evidence of the feasibility of blunting aldosterone synthesis specifically in aldosterone-

producing cells from tumors carrying the 2 most common KCNJ5 mutations, paving the way towards personalized treatment.

Chapter 3 highlights the role of estrogens in the context of autonomous over-secretion of aldosterone in PA. GPER, a G protein coupled estrogen receptor, is highly expressed in the normal human adrenocortical zona glomerulosa. Our previous results suggest GPER to have a potent secretagogue effect of 17b-estradiol on aldosterone when the estrogen β receptor is blocked. Multiple observations subsequently showed GPER to promiscuously bind other steroids, and mediate MR-independent aldosterone effects in various cell types. Based on these findings, we set up a study to investigate whether aldosterone actives GPER and acts as its own secretagogue. This was studied both in vitro using a human adrenocortical cell line (HAC15) and ex vivo using APA tissues (cut into 2-3mm) obtained at adrenalectomy from a cohort of primary aldosteronism patients with aldosterone-producing adenoma (APA). We found that aldosterone stimulated expression of CYP11B2 mRNA, mitochondrial Ca^{2+} and NADH-dependent step responsible for its biosynthesis. This effect occurred both in vitro and ex vivo in APA. Our findings provide important information on the regulation of aldosterone secretion under pathological and physiological conditions. Furthermore, using a pharmacological approach to block the MR and the GPER, we found that only GPER is the main mediator of CYP11B2 activation in response to aldosterone. Based on our data we propose an autocrine-paracrine mechanism whereby aldosterone, acting via GPER, increases its own biosynthesis and release.

In **Chapter 4** we present mechanisms underlying APA associated with a blunted expression of the twik-related acid-sensitive K^+ channel 2(TASK-2). For that, we investigated functional mutations in the promoter sequence of the KCNK5 gene known to lead to low expression of TASK-2. C999T, the most prevalent mutation detected in APA, significantly decreases transcriptional activity of the TASK-2 gene by some 35%, being a significant decrease compared to the percentage observed in WT. It is worth mentioning that the blunted expression of TASK-2 channel was also inversely related to the expression of 13 microRNAs. From these, only miR-23 and miR-34 significantly blunted expression of TASK-2, explaining only 25% of APAs. Thus, the low expression of TASK2 is associated with other genetic variations

acquired by the adrenal gland and/or with environmental factors, coinciding with development of PA.

In **Chapter 5** we report important limitations of a published double-blind placebo-controlled trial investigating whether PTH levels are lowered by short-term treatment with an Ang-II receptor blocker (valsartan) in postmenopausal women with low 25(OH)D levels. The hypothesis comes from evidence supporting the relevant interaction between parathyroid hormone (PTH) and the renin-angiotensin-aldosterone-system (RAAS). Interestingly, they found no effects of RAAS blockade with ARB treatment on PTH levels. Our objection to this statement was that Ang-II has a role in regulating PTH release, yet cannot be conclusively ruled out based on this trial. Firstly, the authors decided to recruit normotensive woman with no hyperparathyroidism, and thus cannot use a full BP lowering dose of valsartan. Secondly, the assessment of PTH changes can be influenced by lack of standardization of food, calcium and sodium intake during the study. Finally, considering that RAAS is progressively blunted by aging, it is conceivable that the effect of valsartan was low in 60-80 year old women that were selected for this study.

Chapter 6 describes the interaction between the adrenocortical zona glomerulosa and the parathyroid gland in both in vivo and ex vivo experimental data. In vivo, we examined PTH secretion when blocking angiotensin II in hypertensive patients with PA and in patients with EH. Interestingly, captopril, an inhibitor of angiotensin-converting enzyme, lowered PTH levels both in patients with EH and in patients with APA after adrenalectomy. Next, we used primary human parathyroid cells isolated from patients receiving surgery for primary hyperparathyroidism. We showed that both angiotensin II and aldosterone stimulated PTH secretion by acting via the AT-1R and the MR, respectively.

In **Chapter 7** we present the first in vivo study that investigates the combination of phosphate binders (PBs) with high vitamin K2 intake in a rat model of chronic kidney disease (CKD). It is known that patients with ESRD receive phosphate binders to lower circulating levels of phosphate, a known inducer of vascular calcification and vascular disease. However, although phosphate binders lower circulating phosphate, they do not decrease vascular calcification. One explanation is that phosphate binders also bind vitamin K, thereby further

aggravating the vitamin K deficiency of ESRD patients. Animals were given high phosphate combined with high or low levels vitamin K2 in their diet. Intriguingly, we found less VC in animals on a phosphate binder plus high vitamin K2 diet compared to that of phosphate binder with low vitamin K2. Our findings suggest a novel treatment of phosphate binder therapy combined with high vitamin K2 for patients with CKD that can prevent the progression of vascular calcification.

Chapter 8 discusses the findings of this thesis and gives an overview of the conclusions. The results were analyzed in light of published literature.