How Little Aldosterone is Able to Raise Blood Pressure?

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Clin J Am Soc Nephrol 4: 703–710, 2009. doi: 10.2215/CJN.01220209

Salt sensitive blood pressure in mice with increased expression of aldosterone synthase. *Hypertension* 51: 134–140, 2008

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After its first description, primary aldosteronism was long felt to be a rare condition (1). More recently, a debate has been ongoing about the true prevalence of primary hyperaldosteronism (2). Primary aldosteronism is caused by bilateral adrenal hyperplasia, by aldosterone-producing adenoma, more rarely by primary adrenal hyperplasia, aldosterone-producing malignancies, or infrequent genetic syndromes (3). Since the early 1980s, ever higher proportions of hypertensive patients were diagnosed as having primary aldosteronism after the plasma aldosterone:renin ratio was introduced as a diagnostic tool and as an index of aldosterone synthesis out of proportion to the stimulation by the renin-angiotensin system (RAS) (4). This epidemiologic tsunami prompted the sarcastic question "Is there an unrecognized epidemic of primary aldosteronism?" (2). Because of the limited sensitivity and specificity of the aldosterone/renin ratio, because of problems collecting truly representative cohorts of hypertensive patients and further issues, past estimates of up to 40% of hypertensive patients having primary hyperaldosteronism are certainly exaggerated, although it remains certainly wise to screen patients with a higher *a priori* probability of primary hyperaldosteronism (*i.e.*, patients with hypertension plus hypokalemia, young patients with hypertension, hypertension in a patient with an incidental adrenal mass, and patients with severe or resistant hypertension (3)).

Nevertheless, there is increasing evidence that even in the absence of overt primary hyperaldosteronism more subtle faults in the regulation of aldosterone synthesis play a role in the genesis of so-called essential or primary hypertension.

First, there is impressive evidence that for patients with resistant hypertension spironolactone is very effective for the control of blood pressure (BP) (5). One recent example is the efficacy of spironolactone as a fourth-line treatment in the ASCOT trial (6).

Even more compelling is the Framingham offspring study in which Vasan found in nonhypertensive individuals a significant relationship between the serum aldosterone concentration and the increase in BP as well as the incidence of hypertension; this group also found that in nonhypertensive individuals ambulatory BP and incidence of hypertension during follow-up correlated with the aldosterone:renin ratio obtained with meticulous methodology (7). The high ratio of plasma aldosterone concentration/plasma renin activity suggests relative autonomy of aldosterone synthesis and inappropriately high production of aldosterone in relation to the stimulation by the RAS (8). Remarkably, the authors also found significant heritability, suggesting linkage to 7p22, although confounding by medication could not be excluded. There had already been reports of abnormal regulation of aldosterone synthase (CYP 11B2) and abnormal CYP11B2 gene polymorphism in idiopathic hyperaldosteronism. Even more relevant to the issue of primary or essential hypertension, the BRIGHT study (British Genetics of Hypertension) found that hypertensive homozygotes for the 344 T-allele of CYP11B2 had altered 11-beta hydroxylase efficiency (9–11). This finding has been confirmed in a preliminary report (12).

Despite these provocative findings it is generally accepted that to prove causality in clinical studies is problematic. This is particularly true for epidemiologic studies in essential hypertension: its cause is not monogenetic and BP is presumably the outcome of small changes in many genes. Recently, the elegant technique of Mendelian randomization has opened one methodological window; that is, comparing BP of heterozygotic carriers of known mutations of a renal sodium transporter with individuals without such mutations (13). The recent study of Makhanova opens another window (14). It shows (to the extent that animal data can be extrapolated to humans) that even minor dysregulation of one candidate gene (in this case a minor increase in aldosterone synthesis by increased stability of aldosterone synthase mRNA) causes hypertension. It might also play a role in the genesis of human hypertension. The implications go beyond the current discussion on the frequency of primary hyperaldosteronism and suggest a potential role of aldosterone, at least in a proportion of individuals with essential hypertension, particularly salt-sensitive hypertension.

The experimental approach adopted in the laboratory of Oliver Smithies (who received the Nobel Prize for his work on the development of techniques) was the genetic manipulation of aldosterone synthase as one candidate gene of essential hypertension. The relatively unstable 3′ untranslated region of the mRNA of aldosterone synthase was replaced by the more stable 3′ untranslated region of bovine growth hormone, thus

Published online ahead of print. Publication date available at www.cjasn.org.

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ISSN: 1555-9041/404–0703

selectively stabilizing the aldosterone synthase mRNA in the adrenal gland. The strategy was to expose the mice with high adrenal expression of aldosterone synthase ($AS^{high/high}$) *versus* controls to two maneuvers: (1) diets with different sodium content (low salt, 0.1%; medium salt, 4%; high salt, 8% sodium chloride), and (2) infusion of angiotensin II (AngII) (2 mg/kg per d for 14 d on a 4% sodium chloride diet).

$AS^{high/high}$ mice had the following characteristics:

- The mRNA for aldosterone synthase in the adrenals was higher by 50% on the normal salt diet, although plasma aldosterone did not differ significantly. (Such a relatively modest increase is relevant if one tries to extrapolate to human essential hypertension as contrasted with primary hyperaldosteronism).
- Increased expression of aldosterone synthase did not affect survival, growth, or fertility, whereas changes in the dietary salt intake did.

What was the reaction of blood pressure to changes in dietary salt? On a low- and normal-salt diet, the $AS^{high/high}$ mice with high aldosterone synthase activity remained normotensive, but renin expression in the kidney increased; however, the increase was less than in wild-type mice. In other words the stimulation by low salt was attenuated, illustrating adaptation to salt restriction with less activation of the RAS. On a 4% salt diet, BP did not differ from that of wild-type mice, but the BP increase in response to AngII infusion was increased (see below).

One major finding is that on a high-salt diet, the $AS^{high/high}$ mice had a significantly greater increase of BP than wild-type mice (117 ± 3 *versus* 107 ± 3 mmHg, respectively). On high salt, there was no significant difference of renin mRNA between wild-type and $AS^{high/high}$ mice, excluding one potential confounder. In the $AS^{high/high}$ mice, a selective significantly higher expression of the epithelial sodium channel (ENaC) α subunit mRNA was found (*i.e.*, of the epithelial aldosterone-sensitive epithelial sodium transporter—its excessive activity causes Liddle's syndrome). This finding suggests that sodium reabsorption in the distal tubule was increased. Other potentially interesting transporters (*e.g.*, sodium/chloride cotransporter; sodium/hydrogen exchanger 3) were not affected. This constellation of high BP in the presence of upregulation of an aldosterone-sensitive renal sodium transporter suggests that aldosterone secretion was not sufficiently suppressed, implicating a certain degree of autonomy of aldosterone secretion.

What was the reaction of BP and target organs to AngII infusion in the $AS^{high/high}$ mice on 4% salt diet? The BP response to the chronic infusion of AngII was significantly greater in the $AS^{high/high}$ mice than in the wild-type mice ($\Delta 57$ *versus* 41 mmHg).

The authors also looked for evidence of target organ damage. In $AS^{high/high}$ mice on a 4% salt diet and chronically infused with AngII, the authors found a higher heart weight/body weight ratio compared with wild-type mice; this might potentially be attributed to higher BP. But the authors also found significantly higher collagen III mRNA and perivascular fibrosis in the heart, a known hallmark of aldosterone-induced cardiac damage even in the absence of BP elevation (15). The higher urinary excretion of 8-isoprostane as a marker of systemic oxidative stress suggests that this may be at least one of the causes of increased target organ damage.

The finding that even minor changes of aldosterone synthesis amplify salt sensitivity is of great interest given the long known high frequency of salt sensitivity in individuals with essential hypertension, particularly in blacks (16–18). Particularly interesting is the fact that in human observations as in the above animal model, high dietary salt intake is necessary for, and an amplifier of, target organ damage. The role of salt in the genesis of target organ damage is illustrated by primitive societies that live on a virtually sodium-free diet and have low BP as well as no evidence of target organ damage despite rocket-high activation of the renin-angiotensin-aldosterone system with high aldosterone concentrations (19).

There are many pathways through which high salt may amplify target organ damage (heart, vessels, atherogenesis); although high salt intake suppresses circulating AngII, it increases the AngII precursor angiotensinogen in the vessel wall and increases AngII signaling molecules. High salt upregulates the AT1 receptor; it alters the vessel structure and composition, reduces nitric oxide (NO) bioavailability, and increases superoxide generation, *etc.* (20–23). In human endothelial cell cultures, aldosterone in the presence of even minute elevations in sodium concentration reduces NO generation and increases endothelial cell stiffness (24).

What are the ramifications of this breakthrough observation? To the extent that one can extrapolate from mice to humans (which is plausible), one conclusion is that an effective strategy to reduce the burden of hypertension in Western societies is to reduce salt intake, which has led to initiatives to do this despite opposition from the food industry (25–27).

Second, if the above experimental observations can be confirmed in humans, the above evidence that only minor elevations of aldosterone concentrations sensitize target organs to aldosterone-induced organ damage would justify the more widespread use of aldosterone receptor blockade in the treatment of hypertension. Target organ damage includes renal damage. From a renal perspective it is of note that patients with primary aldosteronism have more frequent microalbuminuria for any given level of GFR and also more frequent loss of renal function (28,29). Animal studies have shown that aldosterone promotes progression of kidney damage and of proteinuria (30). Recent studies show that addition of an aldosterone receptor blocker to RAS blockade causes BP-independent reduction of proteinuria; this would be consistent with the hypothesis that aldosterone contributes to renal injury (31,32).

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Aldosterone receptor sites on plasma membrane of human vascular endothelium detected by a mechanical sensor. *Eur J Physiol* Epub November 19, 2008

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Aldosterone is arguably one of the most underestimated and neglected aspects in renal disease. This is explained not to the least by the fact that excessive concentrations of aldosterone are not necessary to cause serious target organ damage, as illustrated by the fact that—given the right circumstances—even plasma aldosterone concentrations within the normal range cause cardiovascular and renal target organ damage, which is prevented by mineralocorticoid receptor blockade. This is illustrated, for instance, by experimental observations in salt-sensitive Dahl rats and clinical observations in patients with congestive heart failure (1–3). This is explained by the fact that positive sodium balance or elevated sodium concentration, inflammation causing oxidative stress, shifts in redox potential, and activation of the mineralocorticoid receptor by cortisol in states of malfunction of the cortisol degrading enzyme 11βHSD2 (11-β-hydroxysteroid dehydrogenase 2) are permissive factors for the adverse effects of normal aldosterone concentrations, somewhat misleadingly called "relative aldosterone excess" (4–12). The importance of such ancillary conditions is illustrated by the fact that, for instance, the adverse effects of high salt are prevented by a low-salt diet (6,9,10). If maintained on a low-salt diet, rats do not develop high BP or vascular damage; the same is seen in Yanomama Indians with plasma aldosterone concentrations of 85.6 ng/dl, low BP, and no evidence of vascular damage—their daily consumption of sodium is 1 mmol and of potassium is 203 mmol (13,14).

After identification of the mineralocorticoid receptor, it was widely believed that the effects of aldosterone were mediated via this receptor only by genomic mechanisms; although earlier, but confirmed by later observations in experimental animals and humans, effects with a very fast time course incompatible with transcriptional mechanisms were observed (15–18).

Very recently the group of Oberleithner had used the impressive nanotechnique of atomic force microscopy to study fast interactions between aldosterone and endothelial cells with the resulting endothelial cell stiffening—an issue of obvious great clinical interest because vascular pathology is a hallmark of absolute or relative aldosterone excess: patients with hyperaldosteronism have impaired endothelium-dependent flow-mediated vasodilatation and the same is seen even in patients with relative aldosterone excess (19–21). Here it is presumably the result of downregulated NO-synthase as documented, for instance, in the renal vasculature of hypertensive patients after salt loading and of increased concentrations of the NO-synthase inhibitor asymmetric dimethylarginine (22,23).

The rationale for testing the effect of sodium was the fact that endothelial cells express the ENaC, causing endothelial cells and vessels to swell and stiffen, which is prevented by the sodium channel blocker, amiloride (21,24–26). What Oberleithner had found was that in the presence of aldosterone, but not in its absence, the stiffness of endothelial cells increased within minutes when the sodium concentration was increased once a threshold of 135 mmol/L was transgressed. Increased stiffness was associated with increased cell volume and reduced deformability (19). The increase was abrogated by eplerenone. Varying the sodium concentration in human plasma could reproduce this effect. Stiffening was prevented when the sodium transporter ENaC was blocked using amiloride. In agreement with *in vivo* observations, NO production was salt-dependently reduced (22).

To further provide evidence that a classical mineralocorticoid receptor is involved, the authors used atomic force microscopy in the study discussed here: one single molecule of aldosterone was covalently bound to one linker molecule of polyethylene glycol, which in turn was tethered to the tip of a cantilever. When this aldosterone-coated tip was approached the surface of human endothelial cells, the cell membrane mineralocorticoid receptor interacted with the one aldosterone molecule. The so-called unbinding force in the incredibly low range of pico-Newtons could be measured when withdrawing the tip. The binding was not affected by spironolactone.

Why should the binding of aldosterone by the mineralocorticoid receptor of human endothelial cells and the variability of the effect on cell stiffness and NO production be of interest to the clinical nephrologist? Past studies had shown that in hypertensive patients plasma sodium concentration higher by a minor degree (1 to 3 mmol/L) are found in hypertensive patients (27). So it is not only sodium balance, but, as documented by the *in vitro* observations of Oberleithner, also sodium concentration that is important—not only for BP but also, at least in part, NO-dependently—for vascular functions spanning the spectrum from vascular stiffness to atherogenesis. Given the above effect of sodium concentration on properties of human endothelial cells (and presumably vessels), it would be wise to reassess the effect of dialysate sodium concentration in hemodialyzed patients.

Direct effects of aldosterone on BP independent of sodium balance are suggested by the surprising finding, so far not followed up or confirmed, that in anuric hemodialyzed patients predialysis systolic BP is lowered substantially by 11 mmHg after administration of 50 mg of spironolactone—obviously suggestive of direct effects on vascular resistance. There were no effects on plasma potassium concentration (28). In anuric patients it is unlikely that this effect is mediated by sodium balance; it is more likely that the above-mentioned factors responsible for "relative aldosterone excess" are involved (11,12).

Finally what is the role of aldosterone and sodium in chronic kidney disease? In renal damage models adrenal hyperplasia was documented years ago, and Greene and Hostetter documented elevated aldosterone concentrations(29,30). In their study, administration of aldosterone was able to overcome the benefit from RAS blockade and aggravated renal injury. Blocking the mineralocorticoid receptor with spironolactone was not only able to protect but even to reverse glomerulosclerosis in experimental studies (31). Although spironolactone has been

successfully administered in proteinuric patients on top of RAS blockade, there remains a hazard (32). Experimental data point to the importance of high salt intake in the genesis of aldosterone-induced glomerular and vascular injury in the kidney (33,34). So when treating patients with chronic kidney disease, it would be plausible to go back to what was done in such patients at the beginning of the last century (usually grossly neglected today): to reduce sodium intake, the rationale for which has become much clearer today with increased insight into the underlying pathomechanisms.

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Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. *J Clin Invest* 118: 2526–2534, 2008

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It is well known that in early stages of diabetes mellitus high plasma prorenin concentrations may be found; as renal target organ damage progresses increased renin activity is also seen (1,2). In general the role of the RAS has recently become more complex with the recognition of angiotensin converting enzyme 2, receptors binding (pro)renin, local RAS systems in different renal structures including podocytes and proximal tubules, the difference between AngII concentration in plasma and renal interstitial fluid, the increased response of renal vascular tone to AngII, and of many other aspects of the RAS (3–12).

But over many years, it was not quite clear why hyperglycemia upregulates the activity of the RAS in the first place. This issue is all the more important because the activation of the RAS, presumably as a result of hyperglycemia, contributes to diabetic complications such as hypertension, proteinuria, and renal injury (13,14). These complications have become the target of therapeutic interventions by blockade of the RAS. It was therefore of obvious interest to identify the signal through which hyperglycemia causes release of renin from the juxtaglomerular apparatus (recent studies show that renin is even expressed in nonclassical sites of the nephron, particularly the collecting duct (15)). There was no evidence that the classical pathways by which hyperglycemia causes target organ injury are involved. It was therefore logical to examine whether hyperglycemia itself was the culprit (16).

A G-protein coupled receptor, GPR91, was previously identified as being involved in the monitoring and detection of citric acid cycle intermediates (17). This finding resolved one of the long-standing mysteries of how tissues monitor changes in the rate of intermediary metabolism (18). In a long series of brilliant experiments, Peti-Peterdi recently identified the known succinate receptor GPR91 as the missing link between high glucose levels and renin release from the juxtaglomerular apparatus. It is known that ischemia causes accumulation of the Krebs cycle intermediate succinate, which is normally present within mitochondria but may be released under conditions of ischemia. Ischemia has long been suspected to be involved in renin release. Interestingly, the succinate-sensing receptor GPR91 is most strongly expressed in the kidney, including the juxtaglomerular apparatus (17). It is also known that the release of renin is triggered by prostaglandins 1 and 2, NO, and prostanoids (19,20). Furthermore, modulation of ionized calcium was known to be involved in the control of renin release, and calcium-sensing receptors as well as vitamin D receptors are present on juxtaglomerular cells and modulate renin release (21–23). Long ago, Baumbach *et al.* showed that succinate caused renin release from the kidney, but the whole story had never been tied together (24).

In an elegant series of experiments, Peti-Peterdi *et al.* studied the effect of high glucose in a model of microperfusion of the juxtaglomerular apparatus using innovative fluorescence confocal imaging techniques (25). When the glucose concentration in the perfusate of the afferent arteriole was increased, they observed a prompt degradation of prorenin in the juxtaglomerular cells and renin release into the perfusate. Unexpectedly, it was not the juxtaglomerular cell that was the target of high glucose. Further experiments unraveled a complex interplay between endothelial cells of the afferent arteriole on the one hand and juxtaglomerular cells on the other hand. If high glucose was presented to endothelial cells of the afferent arteriole, they produced succinate in the Krebs cycle and this triggered renin release from juxtaglomerular cells; the same was seen when succinate was added to the perfusate. The effects of high glucose and succinate were not additive. When the downstream Krebs cycle inhibitor malonate (which causes cumulation of succinate) was added to the perfusate, the same sequence was seen as with high glucose. If the Krebs cycle was blocked upstream by fluorocitrate to prevent generation of succinate, the effect of high glucose on renin release was abolished. The major proportion of the effect of high glucose was mediated directly via succinate; however, a certain proportion of the effect was also mediated by the change in osmolality.

It was puzzling that the metabolite receptor GPR91 is expressed by endothelial cells but not by juxtaglomerular cells, raising the issue: How do the two types of cells communicate? Upon exposure to high glucose, the intracellular calcium concentration increased in endothelial cells. The authors provided evidence of a paracrine signal transduction cascade from endothelial cells to juxtaglomerular cells. In endothelial cells, the increase in intracellular calcium triggered the production of NO and prostaglandin 2, which triggered the renin release from juxtaglomerular cells—cell-to-cell cross talk. Such paracrine signaling via GPR91 is not unique to the kidney; for instance, it has also been shown in the liver (26).

The hypothesis that succinate triggers the release of renin in response to hyperglycemia via the GPR91 receptor required

that high glucose increased succinate concentrations to the levels that were able to activate the GPR91 receptor in the experiments of He (17). Indeed, in animals with streptozotocin diabetes the succinate concentration was dramatically increased. The involvement of the succinate-specific receptor GPR91 was further addressed by comparing GPR91 wild-type with GPR91 knockout mice. The release of renin in response to high glucose was significantly diminished in GPR91 −/− mice. In these animals, the release of fluorescence-stained renin granules from the juxtaglomerular apparatus, as seen in the wild-type mice, was largely abrogated. Hyperglycemia apparently does not only cause release of renin by degranulation of juxtaglomerular cells, but it also increases the synthesis of renin as documented in GPR91wild-type and knockout mice.

It is also of note that in the study of He succinate caused an increase in BP, presumably also mediated via renin (17). This novel mechanism may explain the findings of Deboer that in type 1 diabetes hyperglycemia is a risk factor for incident hypertension and that intensive insulin therapy reduces the long-term risk of developing hypertension (27).

These experiments prove that the effect of hyperglycemia is direct and not mediated by systemic or alternative intrarenal factors. In the past, one candidate to explain the link was a macula-densa-mediated feedback: it was assumed that a primary increase of glucose reabsorption via the sodium glucose cotransporter in the proximal tubule activates the macula-densa-mediated feedback and thus causes glomerular hyperfiltration and renin activation. This hypothesis has become less plausible, however, because in A1 adenosin receptor knockout mice with absent tubuloglomerular feedback, hyperglycemia still induces hyperfiltration and the experiments discussed here may be the nail in the coffin of this hypothesis (28).

The succinate-triggered and GPR91-mediated renin release may be relevant not only for hyperglycemia, but also for states in which succinate cumulates in the kidney, such as hypoperfusion. The complex arrangement between endothelial cells and the juxtaglomerular apparatus may indeed be ideal to monitor renal oxygen tension and the ensuing local accumulation of succinate.

Interestingly, Kang identified high concentrations of renin not only in the classical juxtaglomerular apparatus, but also in the collecting duct of diabetic animals (29). The release mechanisms and the role of renin at this site await clarification. It has been speculated that upregulation of (pro)renin production is caused by high AngII, which promotes buildup and release of (pro)renin (29,30). Because AngII directly stimulates the collecting duct EnaC, it is plausible to assume that this is a local mechanism augmenting salt reabsorption.

The above data are not only interesting to explain the physiology of renin release in response to hyperglycemia, but also have important clinical implications. The link between hyperglycemia and renin release adds further weight to the importance of aiming for near normoglycemia in diabetic patients (27). The link between hyperglycemia and renal damage has identified novel culprits, and in the future these might even provide potential targets for intervention.

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