Angiotensin-independent Mechanism for Aldosterone Synthesis during Chronic Extracellular Fluid Volume Depletion

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Abstract

Wild-type (*Agt*+/+) and homozygous angiotensinogen deletion mutant (*Agt*−/−) littermates were placed on normal (NS) or low Na diet (LS) for 2 weeks. Plasma aldosterone levels (P_{aldo}) were comparable during NS, and similarly elevated during LS in *Agt*+/+ and *Agt*−/−. Moreover, in both, the elevation in P_{aldo} was accompanied by marked increase in adrenal zona glomerulosa cells and adrenal P450aldo mRNA. *Agt*−/− mice were distinguished from *Agt*+/+ mice by their higher plasma K level, by ~ 1.5 and ~ 3.8 mEq/liter during NS and LS, respectively. Within the *Agt*−/− group, P_{aldo} was directly proportional to plasma K. The importance of K for the hyperaldosteronism during dietary Na restriction was verified by the observation that superimposition of K restriction led to hypotension in *Agt*+/+ and uniform death in *Agt*−/− mice along with a reduction in P_{aldo} by 75 and 90%, respectively. Thus, suppression of potassium, but not angiotensin, led to a marked attenuation of hyperaldosteronism during dietary Na restriction. Therefore, (*a*) a powerful angiotensin-independent mechanism exists for the hyperaldosteronism during LS; (*b*) high K is a central component of this mechanism; (*c*) contrary to current belief, the tonic effect of high K on aldosterone synthesis and release does not require an intact renin-angiotensin system; and (*d*) normally, intermediary feedback signals for hyperaldosteronism, i.e., both hypotension and high K, are effectively masked by aldosterone actions. (*J. Clin. Invest.* 1997. 99:855–860.) Key words: potassium • renin • sodium • aldosterone synthase

Introduction

Aldosterone plays a central role for the extracellular fluid (ECF) volume homeostasis in mammals. Dietary sodium restriction is a potent stimulus for aldosterone secretion. Since experimental blockade of angiotensin II (Ang II)[1] attenuates aldosterone secretion, Ang II has been thought to mediate the aldosterone secretion during sodium restriction (1–3). Indeed, the term "the renin-angiotensin aldosterone system" has been coined to describe this connection (4). Some studies (5) have suggested, however, that the increased aldosterone in this condition may also be under the influence of non-Ang II mechanism(s). For example, plasma Ang II level was shown in some settings to dissociate from aldosterone secretion (6–8). Nephrectomized rats respond to sodium restriction with a significant rise in aldosterone secretion (9). However, these early, apparently important, findings have been viewed only as anomalies (10) and the "anomalous" observations are attributed today to an activation of the *adrenal* renin-angiotensin system (11) and/or alteration of the adrenal *sensitivity* to Ang II (12, 13). In support of the latter, dietary salt restriction was shown to lead to an upregulation of *adrenal* Ang II receptors (14, 15).

Extracellular potassium concentration is another major modulator of aldosterone synthesis. Indeed, plasma potassium is a potent regulator of aldosterone secretion (16), and Young et al. (17) demonstrated in the dog a synergistic effect of rising plasma potassium levels and angiotensin II in stimulating aldosterone. In this regard, treatment of rats with captopril inhibits the potassium-induced increase in aldosterone and aldosterone synthase (2, 18). Moreover, potassium supplement, although it decreases plasma renin activity, increases adrenal renin (18). Therefore, it has been proposed that locally generated Ang II in the adrenal glomerulosa cells may mediate the aldosterone secretion induced by potassium.

Even more unclear is the role of potassium during dietary salt restriction. 40 years ago, studies in dogs (19) and humans (20) documented that potassium depletion attenuates the increase in urinary mineralcorticoid activity or aldosterone during sodium restriction. 15 years later, Boyd et al. (21) documented some 0.5 mEq/liter increase in plasma potassium level during dietary salt restriction and speculated on an intermediary role of potassium in the secondary hyperaldosteronism. However, studies (1–3, 22) have since demonstrated that the attempt to inhibit Ang II by a pharmacological measure is commonly effective in reversing the hyperaldosteronism, leading to the current prevailing notion that the role of potassium in this condition, if any, is channelled through the action of the renin-angiotensin system (23).

A new opportunity has recently arisen to seek a definitive answer to the role of Ang II and potassium in the enhanced aldosterone synthesis and release during sodium restriction when mutant mice with targeted deletion of the angiotensinogen gene were produced by genetic engineering (24–26). Using these mice developed in our laboratory, we examined (*a*) whether Ang II is essential for the induction of aldosterone during volume depletion; if not, (*b*) whether potassium plays a role in aldosterone secretion in the absence of angiotensin, and if potassium is important; (*c*) why the importance has heretofore not been widely appreciated. Our studies have revealed

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1. *Abbreviations used in this paper: Agt*, angiotensinogen gene; Ang II, angiotensin II; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; LS, low sodium; NS, normal sodium; P450aldo, aldosterone synthase; P_{aldo}, plasma aldosterone concentration.

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that potassium functions as a regulatory signal for aldosterone synthesis and release even in the absence of intact renin-angiotensin system, thereby being capable of effectively maintaining extracellular fluid volume homeostasis during dietary sodium restriction.

Methods

Mice. Wild-type mice and angiotensinogen deletion homozygous littermates were used in this study. These are the offspring of the angiotensinogen deletion mutants generated earlier by gene targeting in our laboratory (26).

The mice used in the present study were genotyped by Southern blot analysis of tail DNA. 3 μg of tail DNA were digested with both XbaI and XhoI, and hybridized with a ^{32}P-labeled 0.7 kb BglII–PstI fragment of the angiotensinogen genomic DNA (5′ flanking probe), which was external to the 5′ end of the targeting vector. F_4 mice homozygous for angiotensinogen gene null mutation (*Agt*−/−) and wild-type (*Agt*+/+) littermates were subjected to the analyses described below.

Dietary sodium and potassium contents. Mice were fed either high sodium purified diet (3.15% Na, Purina Mills, MO), low sodium purified diet (0.02% Na), low sodium–low potassium purified diet (0.03% Na, 0.02% K) or normal mouse chow (0.46% Na, 0.72% K). All animals had free access to food and tap water.

Measurement of plasma aldosterone and potassium concentrations. Blood was collected by one of two methods. Some (results shown in Fig. 1) were decapitated, and truncal blood collected promptly. Others (results shown in Fig. 5, *A* and *B*) were anesthetized with an intraperitoneal injection of pentobarbital (Nembutal, 50 mg/Kg body wt ip; Abbott Laboratories, North Chicago, IL) and placed on a temperature-controlled warm table. PE10 tubing (Becton Dickinson, Sparks, MD), heated and tapered at one end, was then inserted into the left carotid artery, and blood was collected through this catheter to avoid hemolysis. In both of these methods, blood was collected into tubes kept on ice in the presence of EDTA (10 mM) for the measurement of plasma aldosterone concentration and into heparinized micro-hematocrit capillary tubes for the measurement of potassium. The plasma was rapidly separated and kept frozen at −70°C until measurement. Plasma aldosterone was measured by the Coat-A-Count RIA procedure (Diagnostic Products, Los Angeles, CA).

Northern blot analysis for aldosterone synthase, 11β-hydroxylase and side chain cleavage mRNAs. RNA was isolated from the adrenal gland immediately after death using RNAzolB™ (Tel Test Inc., Friendswood, TX). 3.5 μg of RNA was electrophoresed in 1.0% agarose gel, transferred to a nylon membrane (ZETABIND™; Cuno Inc., Meriden, CT) and hybridized with ^{32}P-labeled aldosterone synthase, 11β-hydroxylase (gifts from Dr. Keith L. Parker, Duke University) and side chain cleavage probe (a gift from Dr. Jeffrey Milbrandt, Washington University). The membrane was rehybridized with a human glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA probe (Clontech, Palo Alto, CA) as a control for RNA loading.

Histological study. Adrenal glands were fixed in 4% buffered paraformaldehyde for 24 h, embedded in paraffin, sectioned in a thickness of 3 μm, and stained with hematoxylin eosin.

Blood pressure measurement. Mice (5 wk old) were anesthetized with an intraperitoneal injection of pentobarbital as above and placed on a temperature-controlled warm table. PE10 tubing, heated and tapered at one end, filled with heparin (100 U/ml)-saline solution, was inserted into the left carotid artery. The remaining portion was threaded under the skin and exited at the nape, where the end of the cannula was sealed by heating. 24 h after surgery, a piece of PE50 tubing was connected to the carotid artery cannula. The other end of the tubing was connected to a swivel to allow free mobility of the mouse. Blood pressure was measured in these conscious mice with a Cobe CDX III transducer which was connected to a Blood Pressure Analyzer (Micro-Med, Inc. Louisville, KY). Blood pressure and heart rate were continuously monitored for 30–60 min until they became stable in a quiet and unrestrained environment.

Statistical analysis. Data are presented as the means±SEM. Statistical significance was assessed by using analysis of variance and unpaired *t* test.

Results

Effect of dietary manipulation on plasma aldosterone and potassium concentrations. In the first set of studies, wild-type (*Agt*+/+) and angiotensinogen targeted-deletion homozygous (*Agt*−/−) littermates were divided into normal, low and high sodium (Na) diet groups. Plasma aldosterone concentration was determined after 14 d of the dietary regimen, and the results are shown in Fig. 1. In both *Agt*+/+ and *Agt*−/− mice, plasma aldosterone concentration was low during high Na diet, averaging 7±4 ng/dl (n = 7) and 19±6 (n = 3), respectively, and remained so during normal Na diet, averaging 36±10 ng/dl (n = 9) and 17±3 (n = 6), respectively. Moreover, during low Na diet, plasma aldosterone concentration was comparably elevated in *Agt*+/+ and *Agt*−/− mice, averaging 757±236 ng/dl (n = 8) and 774±229 (n = 8), respectively. Thus, in *Agt*−/− mice, plasma aldosterone increased just as markedly as in *Agt*+/+ mice after 2 wk of dietary sodium restriction. Since *Agt*−/− mice are completely devoid of Ang, Ang is clearly not essential for achieving the degree of hyperaldosteronism that is compatible with survival during dietary sodium restriction.

In both *Agt*+/+ and *Agt*−/− mice, the transcripts of enzymes involved in steroidogenesis were quantitated in the adrenal gland during dietary manipulation (Fig. 2). The amount of mRNA for P450aldo, but not P450scc or P450c11 (relative

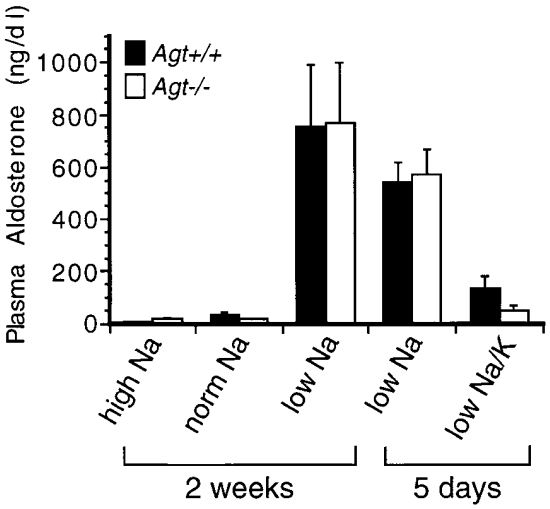


Figure 1. Plasma aldosterone concentrations measured in wild-type (*Agt*+/+) and homozygous angiotensinogen deletion mutant (*Agt*−/−) mice. No statistical difference was noted between *Agt*+/+ vs. *Agt*−/− mice on high, normal (*norm*), or low Na diet (for 2 wk or 5 d), or low Na/K diet (5 d). However, plasma aldosterone concentration was significantly and comparably elevated in both *Agt*+/+ and *Agt*−/− mice on 2-wk ($P < 0.01$ and $P < 0.05$) and 5-d ($P < 0.0001$ and $P < 0.01$) low Na diet when compared with those on normal Na diet; and was significantly and comparably depressed in both *Agt*+/+ and *Agt*−/− mice on low Na/K diet ($P < 0.01$ and $P < 0.005$) when compared with those on low Na diet. Values are given as mean±1SE. [Number of animals studied (*Agt*+/+, *Agt*−/−): 2 wk of high Na (7, 3), normal Na (9, 6), low Na (8, 8); 5 d of low Na (7, 5), low Na/K (7, 8).]

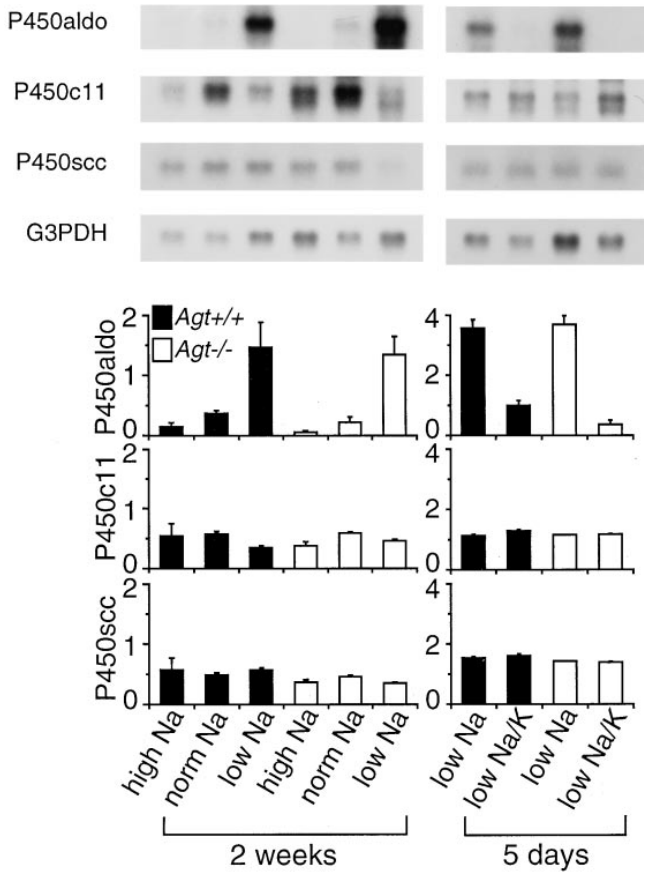


Figure 2. Northern blot analysis for the steroidogenic P450 enzymes in whole adrenal gland obtained from *Agt*+/+ and *Agt*−/− mice. Animals were placed on either high, normal (*norm*) or low Na-containing diet for 2 wk; or low Na or low Na/K-containing diet for 5 d. Typical hybridization patterns are shown on the top; densities obtained with specific P450 enzyme probes are standardized by those with the G3PDH probe and given at the bottom. Relative densities are comparable between *Agt*+/+ and *Agt*−/− for all three P450 enzymes during all diets tested. In both *Agt*+/+ and *Agt*−/− mice, relative densities were comparable during high and normal Na diet, again, for all three P450 enzymes, whereas low Na diet led to a uniform and selective increase in relative density for P450aldo over the level measured during normal Na diet in both *Agt*+/+ (4.0-fold) and *Agt*−/− mice (6.1-fold). The increase in relative density was completely nullified when K restriction was superimposed upon Na restriction, again in both *Agt*+/+ and *Agt*−/− mice. [Number of animals studied (*Agt*+/+, *Agt*−/−): 2 wk of high Na (4, 6), normal Na (4, 5), low Na (5, 5); 5 d of low Na (7, 6), low Na/K (7, 6).]

to that of G3PDH mRNA), was substantially elevated in the adrenal of *Agt*+/+ mice after 2 wk of dietary Na restriction ($n = 5$), when compared with that of *Agt*+/+ mice on normal Na diet ($n = 4$). Of note, P450aldo mRNA was also selectively and markedly increased in the adrenal of *Agt*−/− mice after 2 wk of Na restriction ($n = 5$) when compared with that without Na restriction ($n = 5$).

Our morphological analysis (Fig. 3) further revealed that these changes in P450aldo mRNA were accompanied by parallel changes in the density of zona glomerulosa cells, again, in both *Agt*+/+ and *Agt*−/− mice. Thus, in both *Agt*+/+ and *Agt*−/− mice, dietary Na restriction caused an increase in zona glomerulosa cell population relative to the entire adrenal cortical cell population (Fig. 4). Therefore, both qualitatively and quantitatively, *Agt*+/+ and *Agt*−/− mice displayed identical patterns when they were challenged with dietary Na restriction, in terms not only of plasma aldosterone level but also of the response of specific steroidogenic enzyme and adrenocortical cell type (27, 28).

Given that plasma potassium (K) level has a significant tonic influence on aldosterone synthesis and release, we determined in separate mice plasma K and aldosterone levels simultaneously (Fig. 5 *A*). In *Agt*+/+ mice, plasma potassium level remained relatively low regardless of high ($n = 8$), normal ($n = 8$) or low Na diet ($n = 7$). In contrast, in *Agt*−/− mice, plasma potassium level was uniformly higher than in *Agt*+/+ mice, particularly when they were fed low Na diet (Fig. 5 *A*). Thus, plasma potassium level of *Agt*−/− mice was above *Agt*+/+ level by 1.2–1.5 mEq/liter during high ($n = 3$) and normal Na diet ($n = 7$), and this difference increased to ~ 3.8 mEq/liter when they were placed on low Na diet ($n = 6$). Of note, within the *Agt*−/− mouse group, we found a significant positive correlation between the two indices when data from different dietary regimens were pooled (Fig. 5 *B*). It therefore appeared likely that the high aldosterone level found in *Agt*−/− mutants during sodium restriction was dependent on the high plasma potassium level prevailing uniformly in these animals.[2]

This notion was examined in our additional experiments in which the effect of concurrent dietary Na and K restriction on aldosterone was tested in *Agt*−/− mice. Thus, we assessed the effect of 5-day dietary restriction of both Na and K in both *Agt*+/+ and *Agt*−/− mice. During this low Na/K diet regimen, plasma aldosterone concentration averaged 47±20 ng/dl ($n = 8$) in *Agt*−/− mice and 136±45 ng/dl in *Agt*+/+ mice ($n = 7$, $P < 0.1$). Of note, measurements made in separate groups of *Agt*+/+ ($n = 7$) and *Agt*−/− mice ($n = 5$) which were placed on low Na diet alone for the same duration of 5 days revealed that aldosterone levels in these control mice were comparably elevated (Fig. 1). The above relatively low aldosterone levels of *Agt*+/+ and *Agt*−/− mice on low Na/K diet, therefore, reflect the effect of superimposition of K restriction, which reduces aldosterone level by ~ 75% and ~ 90% in *Agt*+/+ and *Agt*−/− mice, respectively. Since *Agt*−/− mice are completely devoid of angiotensin, the observed K-dependency of hyperaldosteronism during Na restriction does not involve angiotensin. Moreover, by comparing the mRNA levels and adrenal morphology of mice placed on low Na vs. those on low Na/K diet (Figs. 2, 3, and 4), it is apparent that K restriction of only 5 d duration is highly efficient in dampening the markedly upregulated P450aldo mRNA and zona glomerulosa cell proliferation that are caused by Na restriction alone in both *Agt*+/+ and *Agt*−/− mice.

The vital importance of the above potassium-dependent aldosterone secretion and release for body fluid homeostasis was attested in additional studies, in which concurrent Na and

2. Plasma ACTH level assessed by the ACTH ^{125}I RIA Kit (INCSTAR Corp., Stillwater, MN) averaged 111±30 pg/ml ($n = 9$) and 130±47 ($n = 7$) after 2-wk normal and low Na diet regimen, respectively, in $Atg^{+/+}$ mice ($P > 0.1$). Similarly, plasma ACTH level averaged 150±25 pg/ml ($n = 6$) and 207±42 ($n = 7$) after 2-wk normal and low Na diet regimen, respectively, in $Atg^{-/-}$ mice ($P > 0.1$). No significant correlation was found between plasma ACTH and aldosterone levels in *Agt*+/+ ($r = 0.08$, $P > 0.2$) or *Agt*−/− mice ($r = 0.05$, $P > 0.4$).

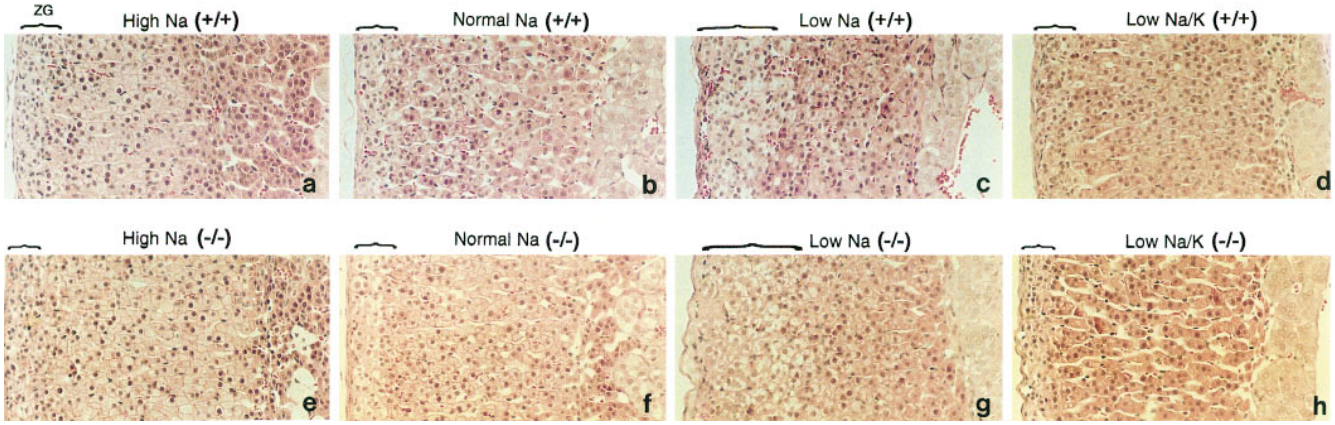


Figure 3. Adrenal gland morphology in *Agt*+/+ (*top*) and *Agt*−/− mice (*bottom*). Animals were placed on high (*a* and *e*), normal (*b* and *f*) or low Na-containing (*c* and *g*) diet for 2 wk or low Na/K-containing diet for 5 d (*d* and *h*). Zona glomerulosa (*ZG*) cells are appreciably and comparably increased in density in *Agt*+/+ and *Agt*−/− mice during low Na diet, but not low Na/K diet. Adrenal glands from *Agt*+/+ and *Agt*−/− mice on low Na diet for 5 d showed patterns essentially identical to those of *c* and *g*, respectively (not shown). (H & E stain, ×200)

K restriction for > 5 days led to significant hypotension in *Agt*+/+ mice by 5 d ($n = 7$) (Fig. 6), and uniform (6 out of 6) death in *Agt*−/− mice within 7 d. Indeed, it was based on this experience of losing all *Agt*−/− mice that the 5-day regimen described above was designed. While blood pressure of *Agt*+/+ mice on low Na/K diet was low but measurable, blood pressure of *Agt*−/− mice was already too low to measure after 5 d of Na and K restriction. Clearly, in both *Agt*+/+ and *Agt*−/− mice, K-dependent aldosterone secretion is critically important for the circulating volume when their sodium intake is restricted.

Discussion

In the present study, we found a vital role of potassium in achieving hyperaldosteronism and maintaining volume homeostasis of angiotensin deficient mice that are subjected to chronic dietary sodium restriction. This role of potassium was once predicted by Boyd et al. (21) a quarter century ago, but has otherwise been unappreciated (10). The marked hyperkalemia present in Na-restricted *Agt*−/− mice led us to uncover the existence of a direct relationship between potassium and aldosterone. Although it is beyond the scope of the current investigation how hyperkalemia could increase plasma aldosterone level, it is assumed that an activation of aldosterone synthesis and release occurred in response to increased K concentration within the zona glomerulosa cells (29), where plasma K is a reflection of intracellular K. In this regard, plasma K was unaffected by dietary manipulation of Na in *Agt*+/+ mice. Moreover, a marked dampening of plasma aldosterone level in *Agt*+/+ mice by K restriction was accompanied by only a subtle reduction in plasma K level, echoing the familiar notion that plasma K in rodents is notoriously insensitive to alterations in total body K content. One may speculate, then, that the mechanism of chronic activation of aldosterone by angiotensin during dietary salt restriction involves increased partition of K within zona glomerulosa cells (vs. extracellular compartment) through an induction of potassium transporter(s), such as Na/K ATPase (20). Obviously, assessment of K concentration within the zona glomerulosa cells is required to verify this possibility. In any case, we observed, during dietary Na and K manipulations, an impressive parallelism between the effects of high endogenous Ang II vs. potassium on three independent parameters, namely plasma aldosterone level, aldosterone synthase mRNA (vs. mRNAs of two other steroidogenic enzymes) and the morphology of zona glomerulosa cells (vs. other adrenal cells). This parallelism warrants a search for a common intermediary pathway for the effect of Ang II and potassium in inducing aldosterone synthesis and release.

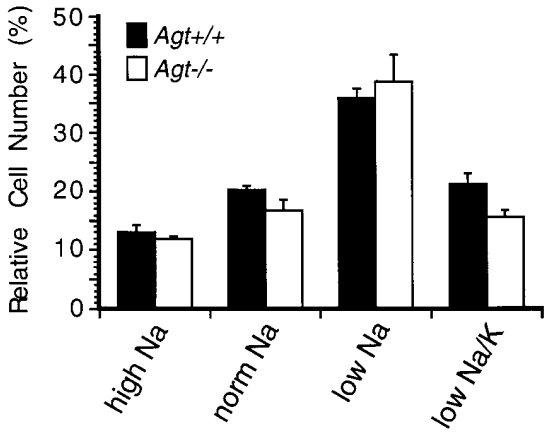
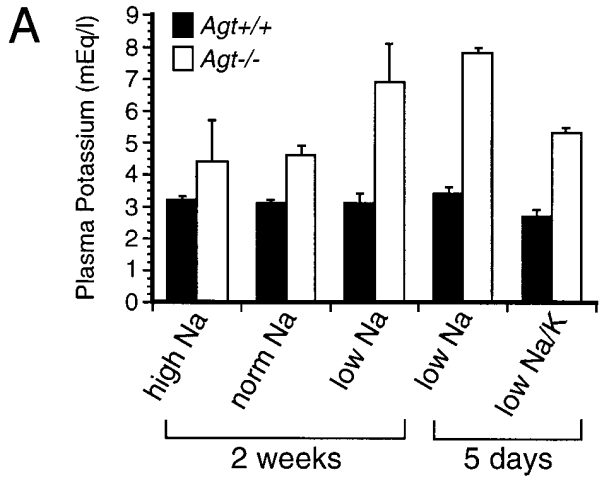


Figure 4. Semiquantitative analysis of the relative proportion of zona glomerulosa cells. The number of nuclei within the zona glomerulosa was assessed as a percentage of the number of all nuclei in the entire cortex. Specimens were obtained from animals placed on high, normal (norm) or low Na-containing diet for 2 wk or low Na/K-containing diet for 5 d. In both *Agt*+/+ and *Agt*−/− mice, a significant increase in this parameter was observed during low Na diet when compared with the level during normal Na diet ($P < 0.01$ and $P < 0.02$); which was dampened significantly during low Na/K diet ($P < 0.01$ and $P < 0.01$). [Number of animals studied (*Agt*+/+, *Agt*−/−): 2 wk of high Na (3, 3), normal Na (3, 3), low Na (3, 3); 5 d of low Na/K (3, 3).]

Our studies with low Na/K diet further helped us to gain insight into the identity of the stimulatory signal for the hyperaldosteronism during dietary sodium restriction. In *Agt*+/+ mice on Na restriction, superimposition of K restriction led to a reduction in aldosterone level and systemic blood pressure. The observation, therefore, is consistent with the long-standing presumption that, hypotension, besides the macula densa mechanism (31), triggers the angiotensin upregulation during



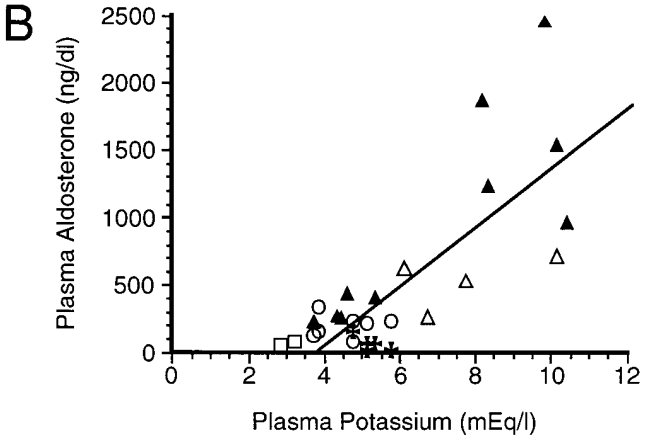


Figure 5. Plasma potassium levels in *Agt*+/+ and *Agt*−/− mice (*A*). Whereas potassium level remained uniformly low in *Agt*+/+ mice regardless of dietary regimen, that of *Agt*−/− mice was uniformly high in response to all diets [$P > 0.1$, $P < 0.0001$, $P < 0.01$, $P < 0.005$, $P < 0.0001$ vs. *Agt*+/+ mice on 2-week high Na, normal (*norm*) Na, low Na and 5-d low Na and low Na/K diet, respectively], and was particularly high with low Na diet. Plasma potassium level decreased significantly in *Agt*−/− mice during low Na/K diet from the level of *Agt*−/− mice on low Na diet ($P < 0.05$). Values are expressed as mean±1SE. [Number of animals studied (*Agt*+/+, *Agt*−/−): 2 wk of high Na (8, 3), normal Na (8, 7), low Na (7, 6); 5 d of low Na (5, 4), low Na/K (8, 5).] Correlation between plasma potassium concentration and aldosterone concentration simultaneously determined in *Agt*−/− mice (*B*). Data collected from *Agt*−/− mice placed on 4 different Na- and K-content diets were pooled (□, high Na 2W; ○, normal Na 2W; ▲, low Na 2W; △, low Na 5D; +, low Na/K 5D). Each point represents data from a single mouse. A significant correlation was noted between these two parameters ($P < 0.001$, $r = 0.613$). The correlation between K and aldosterone remained still statistically significant for data points that are K < 8 mEq/liter and aldosterone < 1000 ng/dl ($P < 0.05$, $r = 0.209$). For plasma potassium measurement, blood was collected through an indwelling catheter from anesthetized mice to avoid hemolysis (11). Plasma aldosterone levels were slightly elevated in all samples (plasma aldosterone levels were 36±10 ng/dl ($n = 2$), 170±31 ($n = 7$), 944±249 ($n = 10$), 507±99 ($n = 4$), 40±24 ($n = 5$) in high, normal, low Na diet for 2 wk and low Na, low Na/K diet for 5 d, respectively) when the values were compared with those determined in blood collected from conscious animals by decapitation shown in Fig. 1.

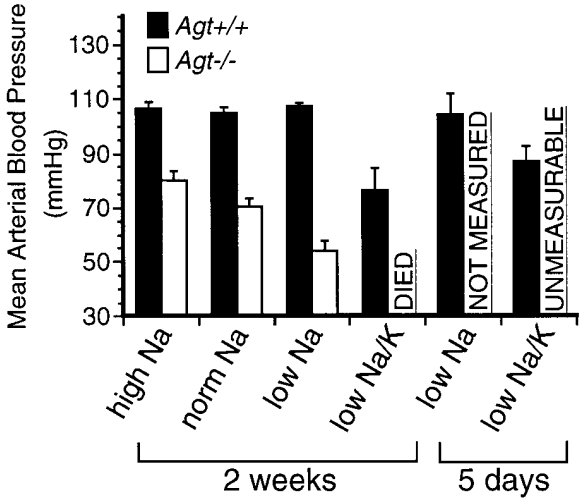


Figure 6. Mean systemic arterial blood pressure measured in *Agt*+/+ and *Agt*−/− mice. Animals were placed on either high, normal (*norm*) or low Na-containing diet for 2 wk; or low Na or low Na/K-containing diet for 5 d. Blood pressure was unaffected by dietary Na content in *Agt*+/+ mice whereas all *Agt*−/− mice were significantly hypotensive, and blood pressure had a significant direct relationship with dietary Na content. In *Agt*+/+ mice, however, superimposition of K restriction with low Na/K diet led to a modest to marked reduction in blood pressure at 5 d ($P < 0.1$) and at 2 wk ($P < 0.05$), when compared with those on low Na alone. *Agt*−/− mice died within 7 d of starting low Na/K diet in the first set of study ($n = 6$). In the second set of study, blood pressure was too low to measure after 5 d of low Na/K diet. Values are expressed as mean±1SE. [Number of animals studied (*Agt*+/+, *Agt*−/−): 2 wk of high Na (3, 3), normal Na (4, 3), low Na (3, 3), low Na/K (6, 0); 5 d of low Na (4, 0), low Na/K (4, 0).]

dietary Na restriction. In this context, the K-induced aldosterone upregulatation effectively masks this otherwise-occurring hypotension (32, 33) (Fig. 7). It should be noted that dietary K restriction led to hypotension (Fig. 6) and normalization of plasma aldosterone level (Fig. 1) not only in *Agt*−/− mice but also *Agt*+/+ mice, indicating that the hyperaldosteronism of wild-type mice during salt restriction is also highly dependent on potassium.

Our observations in *Agt*−/− mice point to the importance not only of K but also of Ang in inducing aldosterone during dietary Na restriction. Thus, in the complete absence of Ang, dietary Na restriction led to marked hyperkalemia in *Agt*−/− mice. The lack of such a severe hyperkalemia in wild-type animals during Na restriction must, therefore, be attributed to the renin-angiotensin system, the effect of which is, through aldosterone induction, to minimize the potent K retaining influence of dietary Na restriction (Fig. 7). This explains why the importance of potassium in aldosterone secretion has heretofore not been widely appreciated (10). Overall, therefore, the secondary hyperaldosteronism during dietary Na restriction depends on the potent stimulatory effect of both hypotension (through renin-angiotensin) and increased potassium. Together with the stimulus from a reduction in distal delivery, which leads to renin release (31) and potassium retention (34), these stimuli appear to be capable of inducing aldosterone sufficiently to raise blood pressure and potassium to near baseline levels (Fig. 7).

In conclusion, studies with angiotensinogen deletion mu-

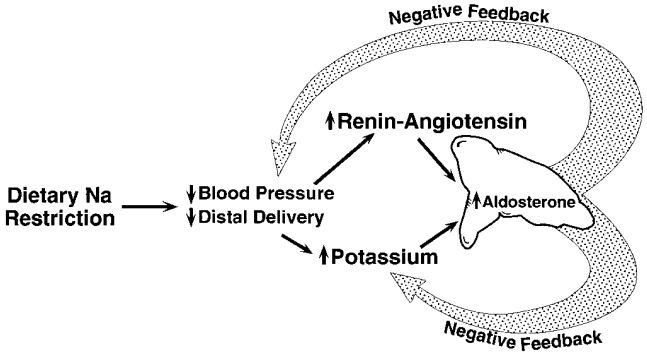


Figure 7. Hypotension and increased potassium as stimuli for the secondary hyperaldosteronism during dietary sodium restriction. It has been speculated that the secondary hyperaldosteronism during dietary Na restriction depends on the activation of renin-angiotensin system, which, in turn, is mediated by the baroreceptor mechanism (i.e., hypotension) and the macula densa mechanism (i.e., decreased distal delivery). The present study proposes a presence of a similarly important mechanism, i.e., increased potassium (intra- or extracellular). All these mechanisms can collectively upregulate aldosterone synthesis and release that is sufficient to bring the level of blood pressure and potassium levels to near baselines.

tant mice have provided unequivocal evidence for the existence of a mechanism independent of angiotensin for the activation of aldosterone secretion during ECF volume depletion. The studies have also indicated that high K content in the cell or plasma can stimulate aldosterone independently of *adrenal* angiotensin or its receptors.

Acknowledgments

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