Calcium paradox of aldosteronism and the role of the parathyroid glands

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Vidal, Alex, Yao Sun, Syamal K. Bhattacharya, Robert A. Ahokas, Ivan C. Gerling, and Karl T. Weber. Calcium paradox of aldosteronism and the role of the parathyroid glands. Am J Physiol Heart Circ Physiol 290: H286–H294, 2006; doi:10.1152/ajpheart.00535.2005—The hypercalcuria and hypermagnesuria that accompany aldosteronism contribute to a fall in plasma ionized extracellular Ca2+ and Mg2+ concentrations ([Ca2+]i and [Mg2+]i). Despite these losses and the decline in extracellular levels of these cations, total intracellular and cytosolic free Ca2+ concentration ([Ca2+]C) is increased and oxidative stress is induced. This involves diverse tissues, including peripheral blood mononuclear cells (PBMC) and plasma. The accompanying elevation in plasma parathyroid hormone (PTH) and reduction in bone mineral density caused by aldosterone (Aldo)-1% NaCl treatment (AldoST) led us to hypothesize that Ca2+ loading and altered redox state are due to secondary hyperparathyroidism (SHPT). Therefore, we studied the effects of total parathyroidectomy (PTx). In rats receiving AldoST, without or with a Ca2+-supplemented diet and/or PTx, we monitored urinary Ca2+ and Mg2+ excretion; plasma [Ca2+]i and [Mg2+]i, and PTH; PBMC [Ca2+]i, and H2O2 production; plasma α1-antiprotease activity; total Ca2+ and Mg2+ in bone, myocardium, and rectus femoris; and gp91phox labeling in the heart. We found that 1) the hypercalcuria and hypermagnesuria and decline (P < 0.05) in plasma [Ca2+]i and [Mg2+]i, that occur with AldoST were not altered by the Ca2+-supplemented diet alone or with PTx; 2) the rise (P < 0.05) in plasma PTH with AldoST, with or without the Ca2+-supplemented diet, was prevented by PTx; 3) increased (P < 0.05) PBMC [Ca2+]i and H2O2 production, increased total Ca2+ in heart and skeletal muscle, and fall in bone Ca2+ and Mg2+ and plasma α1-antiprotease activity with AldoST were abrogated (P < 0.05) by PTx; and 4) gp91phox activation in right and left ventricles at 4 wk of AldoST was attenuated by PTx. AldoST is accompanied by SHPT, with parathyroid gland-derived calcitropic hormones being responsible for Ca2+ overload in diverse tissues and induction of oxidative stress. SHPT plays a permissive role in the proinflammatory vascular phenotype.

aldoosterone; magnesium; oxidative and nitrosative stress; parathyroid hormone

ALDOSTERONISM, defined as chronic, inappropriate (relative to dietary Na+) elevations in plasma aldosterone (Aldo), is accompanied by a proinflammatory phenotype characterized by oxidative and nitrosative stress, immune cell activation, vascular remodeling by invading inflammatory cells, and bone loss (3, 18, 31, 66, 87, 90). Mechanisms responsible for the vascular phenotype have been under investigation for some time. Aldo-salt treatment (AldoST)-induced elevations in arterial pressure have been considered and found to be noncontributory. This conclusion is based on multiple lines of evidence. 1) Systemic hypertension poses a hemodynamic over-load on the left ventricle and aorta over the short term while sparing the atria, right ventricle, and pulmonary artery. On the other hand and given the in-parallel arrangement of the right and left heart created by a common coronary circulation, a circulating factor gains access to the entire heart as well as to the great vessels via their vasovasorum. Vascular lesions are found throughout the right and left atria and ventricles and the adventitia of the aorta and pulmonary artery (13, 57, 74, 86, 93). This is not the case for elevations in arterial pressure caused by an occlusive band around the abdominal aorta below the renal arteries, where renal ischemia does not occur with activation of the renin-angiotensin-Aldo system (13). 2) Vascular lesions do not appear when spironolactone, an Aldo receptor antagonist, is coadministered in a small (nonpressor) dose, which does not prevent the AldoST-induced elevation in arterial pressure, or in a larger (depressor) dose (12, 25, 54, 56, 57, 61, 66, 75). 3) Although they prevent a rise in arterial pressure, nonspecific vasoactive agents do not prevent vascular lesions (53, 57). Hypertension has also been considered to cause oxidative stress in the left ventricle and aorta (9, 33, 43, 83). However, such evidence has been found in the normotensive right ventricle (87) and in postcapillary venules (63), where elevations in arterial pressure are not expected. Additionally, an altered redox state is not seen with comparable elevations in arterial pressure induced by exogenous norpinephrine (43). Thus multiple lines of evidence have linked AldoST-induced vascular remodeling and oxidative stress to a circulating factor, rather than hemodynamic effects. Activated immune cells appear to represent this circulating factor (see below).

Aldosteronism in rats is accompanied by increased urinary and fecal excretion of Ca2+ and Mg2+, which contributes to a fall in plasma ionized concentrations of Ca2+ and Mg2+ (17, 18). Despite these losses and the fall in extracellular levels of these divalent cations, total intracellular and cytosolic free Ca2+ concentrations ([Ca2+]i) each rise, and oxidative stress is induced (2, 3, 17). This involves diverse tissues, including peripheral blood mononuclear cells (PBMC). A Ca2+ overload of PBMC with an accompanying induction of oxidative stress has been considered to be responsible for the activation of these cells (2, 3, 17, 31), which can be prevented by a Ca2+ channel blocker (2). Furthermore, an antioxidant abrogates vascular invasion by inflammatory cells (2, 87).

In rats and humans with chronic mineralocorticoid excess (plus dietary Na+), the increase in urinary and fecal excretion of Ca2+ and Mg2+ (15, 17, 18, 30, 35, 50, 85) and fall in their plasma ionized concentrations lead, eventually, to a reduction

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in bone mineral density (17, 18). On the basis of this collective evidence, we have inferred that secondary hyperparathyroidism (SHPT) is responsible for the Ca\(^{2+}\) overload of PBMC (17). However, the evidence has been circumstantial. Plasma parathyroid hormone (PTH) levels are increased in rats receiving AldoST, and hyperparathyroidism (HPT) has been reported in patients with primary or secondary aldosteronism (24, 45, 70, 76, 80). More definitive proof of the pathophysiological role of the parathyroid glands is the aim of the present study. We therefore hypothesized that the parathyroid glands are responsible for the Ca\(^{2+}\) overload and oxidative and nitrosative stress that accompany aldosteronism. To this end, the effects of AldoST, with or without a Ca\(^{2+}\) stress that accompany aldosteronism. To this end, the effects of

**MATERIALS AND METHODS**

**Animal model.** Nine-week-old male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were anesthetized with an intramuscular injection of ketamine (87 mg/kg body wt) and xylazine (13 mg/kg body wt). A midline incision was made along the anterior neck, and subcutaneous fat and platysma muscles were carefully dissected. Submaxillary salivary glands were laterally displaced to expose the sternohyoid muscle, which was longitudinally dissected. The thyroid gland was isolated, and the parathyroid glands were located and excised under a dissecting microscope at ×10 magnification. PTX was considered successful only in rats with serum Ca\(^{2+}\) <6.0 mg/dl (1.5 mmol/l) 48 h postoperatively. Within 48 h of PTX, some rats exhibited signs of hypocalcemia: 1 in 5 animals showed frequent muscle fasciculations; 1 in 10 had fasciculations and tetany; and 1 in 20 had seizures. After a single 1-ml dose of a 2% CaCl\(_2\) solution by gavage, the rats were able to consume a normal chow diet (Harlan Teklad Rodent Diet) supplemented with 2.5% CaCO\(_3\) and 1% lactose (Ca\(^{2+}\)-supplemented diet) to enhance gastrointestinal Ca\(^{2+}\) absorption. Thereafter, they remained permanently free of these signs and symptoms associated with hypocalcemia. The standard laboratory chow contained 1.13% Ca\(^{2+}\), 0.94% phosphorus, 0.4% Na\(^{+}\), 1% K\(^{+}\), 0.24% Mg\(^{2+}\), and 2.99 IU/g vitamin D\(_3\).

Unoperated, untreated, age- and gender-matched rats served as controls (n = 32). On the same day, uninephrectomized rats received Aldo (0.75 μg/h) by implanted minipump (Alzet, Cupertino, CA) together with 1% NaCl-0.4% KCl in drinking water (AldoST) and standard laboratory chow. A separate group of rats was treated with AldoST and fed the Ca\(^{2+}\)-supplemented diet, and another group of PTX rats was treated with AldoST and fed the Ca\(^{2+}\)-supplemented diet. Experimental groups were studied at 1, 2, 4, and 6 wk on the basis of gaps in our knowledge for the specific time points not previously reported. Animals were anesthetized and killed, and blood, PBMC, hearts, and skeletal muscle were harvested. Each time point for treated animals consisted of five rats. The study was approved by the institution’s Animal Care and Use Committee.

**Urinary excretion of Ca\(^{2+}\) and Mg\(^{2+}\).** Individual animals were placed in a metabolic cage for collection of urine on the day before they were killed and tissues were sampled. Urinary concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) were determined with an atomic absorption spectrophotometer as previously reported (17, 18). Urinary excretion rates of Ca\(^{2+}\) and Mg\(^{2+}\) are expressed in micrograms per 24 h.

**Plasma ionized extracellular Ca\(^{2+}\) and Mg\(^{2+}\) concentrations.** Plasma ionized extracellular Ca\(^{2+}\) and Mg\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\), and [Mg\(^{2+}\)]\(_i\)) were determined by the direct ion-selective electrode technique with a Nova 8 Analyzer (Nova Biomedical, Waltham, MA) (2).

**Plasma PTH.** Plasma PTH was measured by the intact PTH immunoradiometric assay with a commercial kit (Nichols Institute Diagnostics, San Clemente, CA) as reported elsewhere (17).

**PBMC intracellular Ca\(^{2+}\) concentration, H\(_2\)O\(_2\) production, and plasma α\(_1\)-antiproteinase activity.** PBMC were isolated, and cytotoxic free [Ca\(^{2+}\)]\(_i\), was measured as described previously (3) by a modification of the ratiometric method and the fluorescent molecular probe fura-2 (Molecular Probes, Eugene, OR). As reported previously, H\(_2\)O\(_2\) production by PBMC was measured fluorometrically with 2′,7′-dichlorofluorescin diacetate with a flow cytometer (FACS Caliber, Becton, Dickinson, Franklin Lakes, NJ) (3). Plasma α\(_1\)-antiproteinase (α\(_1\)-AP) activity was assessed using the α\(_1\)-AP 410 assay system (Oxis Research, Portland, OR) (31).

**Bone Ca\(^{2+}\) and Mg\(^{2+}\) concentrations.** Total Ca\(^{2+}\) and Mg\(^{2+}\) concentrations in tibia were determined by atomic absorption spectrophotometry and expressed in micrograms per milligram of fat-free dry bone as reported previously (17, 18).

**Myocardial and skeletal muscle Ca\(^{2+}\) and Mg\(^{2+}\).** Microdeterminations for Ca\(^{2+}\) and Mg\(^{2+}\) were carried out in defatted ventricular tissue and rectus femoris muscle and are expressed as nanoequivalents per milligram of fat-free dry tissue as reported elsewhere (17).

**Activation of gp91phox.** Coronal cryostat sections (6 μm) of both ventricles were prepared for immunohistochemistry as previously reported (87). Sections were incubated with primary antibody to gp91phox at a dilution of 1:100. Negative controls were incubated with secondary antibody alone.

**Statistical analysis.** Values are means ± SE. Data were analyzed using analysis of variance. Significant differences between individual means were determined using Bonferroni’s post hoc multiple comparisons test. Significance was assigned to \(P < 0.05\).

**RESULTS**

**Urinary Ca\(^{2+}\) and Mg\(^{2+}\) excretion.** A marked increase in urinary Ca\(^{2+}\) and Mg\(^{2+}\) excretion above control levels was found at 6 wk of AldoST (Fig. 1). We previously demonstrated the presence of hypercalcuria and hypermagnesuria at 1, 2, and 4 wk of AldoST (17). AldoST with the Ca\(^{2+}\)-supplemented diet or AldoST with PTX and the Ca\(^{2+}\)-supplemented diet was not altered by Ca\(^{2+}\) overload of PBMC (Aldo) salt treatment (AldoST, n = 5), AldoST + Ca\(^{2+}\)-supplemented diet (n = 5), and AldoST + Ca\(^{2+}\)-supplemented diet + parathyroidectomy (PTX, n = 5). AldoST-induced hypercalciuria and hypermagnesuria was not altered by Ca\(^{2+}\)-supplemented diet or prior PTX. Values are means ± SE. [Ca\(^{2+}\)]\(_i\), and [Mg\(^{2+}\)]\(_i\), extracellular Ca\(^{2+}\) and Mg\(^{2+}\) concentrations. \(*P < 0.05\) vs. controls.
The presence of oxidative and nitrosative stress systemically is evidenced by the fall in plasma $\alpha_1$-AP activity at 1 and 6 wk of AldoST (Fig. 4) and extends our previous observations at 4 wk of AldoST. The Ca$^{2+}$-supplemented diet did not prevent this decline in plasma $\alpha_1$-AP activity. PTx prevented the decline in plasma $\alpha_1$-AP activity that accompanies AldoST, consistent with oxidative stress in diverse tissues.

**Bone Ca$^{2+}$ and Mg$^{2+}$ concentrations.** Reduction of Ca$^{2+}$ and Mg$^{2+}$ concentrations in tibia at 4 and 6 wk of AldoST is consistent with PTH-mediated bone resorption. The Ca$^{2+}$-
supplemented diet did not prevent the loss of bone Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. On the other hand, PTx with the Ca\textsuperscript{2+}-supplemented diet prevented the loss of these minerals from tibia (Fig. 5).

Myocardial and skeletal muscle Ca\textsuperscript{2+} and Mg\textsuperscript{2+} concentrations. Ca\textsuperscript{2+} loading of myocardial tissue is found at 4–6 wk of AldoST, with or without supplemental dietary Ca\textsuperscript{2+} (Fig. 6). Prior PTx prevented this rise in intra- and extracellular or total Ca\textsuperscript{2+} in myocardial tissue. Total Mg\textsuperscript{2+} concentration in myocardial tissue was not altered during 4–6 wk of AldoST with or without dietary Ca\textsuperscript{2+} supplements or with prior PTx and Ca\textsuperscript{2+}-supplemented diet.

As in myocardial tissue, Ca\textsuperscript{2+} concentration was increased in the rectus femoris at 4–6 wk of AldoST, with or without supplemental dietary Ca\textsuperscript{2+}, and was prevented by PTx (Fig. 6). Skeletal muscle Mg\textsuperscript{2+} concentration was not altered during AldoST with or without dietary Ca\textsuperscript{2+} supplements or PTx.

Activation of gp91phox. In coronal sections of right and left ventricles, gp91phox labeling was positive in a small number of cells in interstitial and perivascular spaces of control rats (Fig. 7). Inflammatory cells and myofibroblasts appear in the perivascular space of the intramural coronary vasculature of the right and left ventricles at 4–6 wk of AldoST (14, 87). At these sites, we found markedly increased gp91phox-positive cells (Fig. 7), which are primarily inflammatory cells and myofibroblasts. This remodeling was attenuated by PTx (Fig. 7).

**DISCUSSION**

Previously, we merely implicated SHPT in the Ca\textsuperscript{2+} paradox that accompanies aldosteronism. Use of total PTx in the present study offers direct evidence in support of this hypothesis and addresses the impact of the parathyroid gland and its calcitropic hormones on the Ca\textsuperscript{2+} loading of PBMC and cardiac and skeletal muscle, as well as the appearance of oxidative and nitrosative stress. Our study led to several major findings.

Hypercalciuria and hypermagnesuria were observed at 6 wk of AldoST. We previously found a comparable level of urinary Ca\textsuperscript{2+} and Mg\textsuperscript{2+} excretion at 1–4 wk of AldoST, along with even greater gastrointestinal wasting of these divalent cations (17, 18). Together, these sustained losses led to a fall in plasma ionized [Ca\textsuperscript{2+}]o and [Mg\textsuperscript{2+}]o, each of which is a potent stimulus to the parathyroid glands’ release of calcitropic hormones (51). The decline in plasma [Ca\textsuperscript{2+}]o and [Mg\textsuperscript{2+}]o with 1–6 wk of AldoST was not prevented by our Ca\textsuperscript{2+}-supplemented diet or the amount of vitamin D\textsubscript{3} available in standard chow and produced endogenously (albeit not measured). These findings suggest that supplemental calcitriol, together with a diet supplemented with Ca\textsuperscript{2+} and Mg\textsuperscript{2+}, may be required to regulate PTH secretion in SHPT (68, 81). Bone resorption alone, as reflected by the fall in bone Ca\textsuperscript{2+} and Mg\textsuperscript{2+}, was unable to preserve extracellular homeostasis of Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. We did not monitor other well-known effects of PTH, such as urinary phosphate excretion and acidification (1, 36).

In future studies, the importance of PTH and 1,25-dihydoxyvitamin D\textsubscript{3} in AldoST rats with PTx can be addressed by autotransplantation of cryopreserved parathyroid glands and calcitriol supplements, respectively. We previously reported that cotreatment with an aldosterone receptor antagonist, spironolactone, attenuates urinary and fecal losses of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} and, thereby, prevents the fall in plasma [Ca\textsuperscript{2+}]o, [Mg\textsuperscript{2+}]o, and loss of bone mineral density (17). A reduction in plasma ionized [Ca\textsuperscript{2+}]o, has also been observed in humans with primary aldosteronism and is corrected by spironolactone or adrenal surgery (70, 76). The fall in plasma [Ca\textsuperscript{2+}]o, and [Mg\textsuperscript{2+}]o, is accompanied by an elevation in plasma PTH, which declines over time, con-
sistent with the downregulation of the parathyroid glands’ Ca$^{2+}$-sensing receptor (21). Prior PTx eliminates the rise in plasma PTH that accompanies AldoST. Elevations in plasma PTH have been found in rats treated with deoxycorticosterone acetate (DOCA)-salt (94), and chemical evidence of SHPT is corrected by spironolactone or adrenal surgery in humans with primary aldosteronism (17, 76). As in SHPT, bone Ca$^{2+}$ and Mg$^{2+}$ each fall significantly at 4 and 6 wk of AldoST, consistent with our previous findings for bone mineral density and bone strength (17, 18). SHPT and bone loss have also been observed in patients with advanced congestive heart failure (CHF), where secondary aldosteronism is expected, together with the hypercalcemia and hypermagnesemia, which accompany chronic treatment with furosemide, a potent loop diuretic (6, 45, 80).

A significant increase in total Ca$^{2+}$ concentration of PBMC at 1–4 wk of AldoST is associated with the SHPT found in rats with aldosteronism (17). The persistent rise in intracellular Ca$^{2+}$ that accompanies downregulated PTH levels is related to increased membrane permeability to Ca$^{2+}$ and increased expression of Ca$^{2+}$ channels (26, 79, 88). Ca$^{2+}$ channel expression is also increased with aldosteronism (7, 77). The Ca$^{2+}$ loading of PBMC involves its initial distribution within organelles, such as mitochondria and endoplasmic reticulum (10, 60, 73). Mitochondria regulate cytosolic Ca$^{2+}$ (22, 82). Their ability to sequester cytosolic Ca$^{2+}$ at a 3-log concentration gradient provides mitochondria with the unique capacity to regulate intracellular Ca$^{2+}$ during transient or passive Ca$^{2+}$ ingress. Mitochondria and endoplasmic reticulum chaperone proteins regulate cytosolic Ca$^{2+}$. Cytosolic free [Ca$^{2+}$]$_i$ of lymphocytes and monocytes are increased after 1 wk. Total Ca$^{2+}$ concentrations in myocardium and skeletal muscle are also increased, as shown here and reported previously for the heart (17). Others have documented a rise in intracellular Ca$^{2+}$ in vascular tissue and platelets during chronic mineralocorticoid-salt treatment (32, 38, 40). This Ca$^{2+}$ loading of tissues, against a background of Ca$^{2+}$ loss and reduced extracellular Ca$^{2+}$, has been termed a Ca$^{2+}$ paradox (28). This rise in total intracellular Ca$^{2+}$ concentration does not mandate an increase in Ca$^{2+}$ availability given the presence of Ca$^{2+}$ binding proteins. Here we demonstrate that PTx prevents this Ca$^{2+}$ paradox in myocyte- and nonmyocyte-containing tissues. Ca$^{2+}$ channel blockade prevents the Ca$^{2+}$ loading of diverse tissues in humans and rats with SHPT (2, 41). PTx and diltiazem are likewise protective in dystrophic hamsters, where Ca$^{2+}$ loading occurs in skeletal muscle and heart (10, 60). Calcitropic hormones, which are released by the parathyroid glands and may contribute to the Ca$^{2+}$ paradox, include PTH and endothelin (ET)-1 (27). PTx does not allow us to distinguish whether either or both of these hormones contribute to Ca$^{2+}$ loading. Future studies to address the role of ET-1 will involve an ET-1 receptor antagonist in AldoST rats.

The Ca$^{2+}$ overload of PBMC is accompanied by an induction of oxidative stress in these cells and is reflected in their increased production of H$_2$O$_2$, as shown for 1 and 2 wk of AldoST and as previously reported at 4 wk of AldoST (2, 3). H$_2$O$_2$ participates in the signal transduction that leads to an activation of PBMC. As observed in the present study, prior PTx prevents the induction of oxidative stress in PBMC. Amlodipine, a Ca$^{2+}$ channel blocker, and N-acetylcysteine, an antioxidant, prevent the rise in H$_2$O$_2$ production in PBMC (2). Mitochondria take up and accumulate Ca$^{2+}$ and represent a rich source of reactive oxygen species. Mitochondrial Ca$^{2+}$ uptake stimulates net production of reactive oxygen species through activation of membrane permeability transition, release of cytochrome c, respiratory inhibition, and release of antioxidants (84). Oxidative stress occurs in response to Ca$^{2+}$ accumulation within these organelles and when antioxidant defenses are exhausted. Another example of oxidative stress induced by mitochondrial Ca$^{2+}$ overload is found in cardiomyocytes after ischemia-reperfusion (23). In lymphocytes, H$_2$O$_2$ acts as an intracellular messenger involved in signal transduction and amplification to activate these cells in a manner that simulates antigen-antigen receptor binding (for review see Ref. 72).

Oxidative and nitrosative stress at a systemic level is evidenced by the fall in plasma $(\alpha_1$-AP activity, an inverse correlate of oxidative and nitrosative stress, which we report here for 1 and 6 wk of AldoST. Together with our previous finding, these observations demonstrate the sustained nature of oxidative and nitrosative stress in AldoST rats (3). In the secondary aldosteronism that accompanies CHF, oxidative and nitrosative stress has been reported in such diverse tissues as skin, skeletal muscle, heart, PBMC, and plasma (16, 34, 55, 89). Plasma levels of $8$-isoprostane and thiobarbituric acid-reactive substances are increased in rats with chronic mineralocorticoidism and can be prevented by spironolactone or tempol, a superoxide dismutase mimetic (37, 66, 90). Here, we show that PTx prevents the decline in plasma $(\alpha_1$-AP activity that accompanies AldoST and is related to Ca$^{2+}$ loading of diverse tissues.

Another major finding of the present study is oxidative stress in inflammatory cells that invade the intramural coronary vasculature of the right and left ventricles at 4 wk of AldoST and is expressed as increased gp91phox labeling in these cells.
We previously reported labeling of these cells with 3-nitrotyrosine, a stable residue indicative of a reactive nitrogen intermediate, peroxynitrite (3, 87). As noted here, this evidence of oxidative and nitrosative stress within the heart was not prevented by a Ca\textsuperscript{2+}/H\textsuperscript{+}-supplemented diet but was attenuated by PTx with the Ca\textsuperscript{2+}-supplemented diet. Prior PTx has been reported to ameliorate the incidence and severity of vascular lesions in the heart, kidneys, and/or cerebral vasculature in response to DOCA-salt treatment (58) and a high-salt diet in Dahl salt-sensitive rats (39) and in stroke-prone spontaneously hypertensive rats (46). NADPH oxidase activity has been found in the aorta and mesenteric vasculature in rats with mineralocorticoidism and is attenuated by apocynin, an NADPH oxidase inhibitor, tempol, or an ET (ET\textsubscript{A}) receptor antagonist (5, 37, 47, 62, 66, 90).

Our findings draw attention to a permissive role of the parathyroid glands in the vascular remodeling that accompanies AldoST. PTx attenuated, but did not completely prevent, these lesions. Other factors may also be operative. ET\textsubscript{A} receptor antagonists attenuate the appearance of oxidative and nitrosative stress and expression of adhesion molecules in the affected vasculature in rats with a chronic excess of mineralocorticoid (47, 66, 90). Extraparathyroid sources of ET-1 include the adrenal glands and endothelium. Circulating vasopressin is elevated during AldoST and DOCA-salt treatment and provides a stimulus to ET-1 synthesis (48), as does an associated deficiency of Mg\textsuperscript{2+} (8). An increase in plasma norepinephrine in DOCA-salt-treated rats may result from centrally mediated increases in peripheral sympathetic neural activity (20, 69). Elevations in substance P, a neurotransmitter, may also contribute to chronic mineralocorticoid excess because of reductions in plasma ionized [Mg\textsuperscript{2+}]\textsubscript{o} (42, 92). This uncertainty notwithstanding, a direct and immediate effect of AldoST on the vasculature appears less likely. This is further evidenced in a rodent model with cardiac overexpression of Aldo synthase and increased tissue levels of Aldo, where vascular remodeling was not found (29). Future studies are needed to address these issues. Hemodynamic factors, on the other hand, have been discounted (for review see Ref. 91).

Several clinical correlations can be drawn from the present study. Increased urinary Ca\textsuperscript{2+} excretion, reduced plasma ionized Ca\textsuperscript{2+}, elevated plasma levels of PTH, and increased cytosolic free [Ca\textsuperscript{2+}]\textsubscript{i} are found in patients with low-renin essential hypertension (11, 59, 71). High dietary Na\textsuperscript{+}, which suppresses renin and aldosterone, and elevated circulating aldosterone inappropriate for 1% dietary NaCl are each accompanied by hypercalciuria, which can lead to SHPT. As in rats with aldosteronism and SHPT, the paradoxical loading of cells with Ca\textsuperscript{2+} may explain the efficacy of Ca\textsuperscript{2+} channel blockers in reducing blood pressure and preventing oxidative stress (2, 52). These agents also protect against the immune cell activation that accompanies the SHPT of chronic renal failure (4). Evidence of SHPT, including elevated circulating levels of IL-6 and TNF-\alpha, resembles the proinflammatory CHF phenotype (49). Loop diuretics, commonly used in these patients, will exaggerate the urinary Ca\textsuperscript{2+} and Mg\textsuperscript{2+} excretion found with aldosteronism, thus promoting further PTH release and greater bone loss (44). On the other hand, the combination of a thiazide diuretic and spironolactone

![Image](Fig. 7. Immunohistochemical study of activation of gp91\textsuperscript{phox} in coronal cryostat sections of ventricle. Top: control. Middle: gp91\textsuperscript{phox}, a subunit of NADPH oxidase, activity (arrows) during AldoST. AldoST-induced gp91\textsuperscript{phox} activity was not altered by Ca\textsuperscript{2+}-supplemented diet (not shown). Bottom: AldoST-induced gp91\textsuperscript{phox} activity was attenuated (arrow), but not prevented, by prior PTx.)
reverses these losses and preserves bone health (78). The negative impact of non-K+-sparking diuretics on morbidity and mortality in patients with CHF has been called into question (19). On the other hand, Aldo receptor antagonists reduce mortality and morbidity when added to an angiotensin-convert-
ing enzyme inhibitor and loop diuretic, with or without β-receptor blocker and digoxin (64, 65).

GRANTS

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REFERENCES


