Calcium paradox of aldosteronism and the role of the parathyroid glands

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The hypercalcuria and hypermagnesuria that accompany aldosteronism contribute to a fall in plasma ionized extracellular Ca\(^{2+}\) and Mg\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\), and [Mg\(^{2+}\)]\(_i\)). Despite these losses and the decline in extracellular levels of these cations, total intracellular and cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_t\)) 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 Ca\(^{2+}\) 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 Ca\(^{2+}\)-supplemented diet and/or PTX, we monitored urinary Ca\(^{2+}\) and Mg\(^{2+}\) excretion; plasma [Ca\(^{2+}\)]\(_o\), [Mg\(^{2+}\)]\(_o\), and PTH; PBMC [Ca\(^{2+}\)]\(_t\), and H\(_2\)O\(_2\); production; plasma \(\alpha_1\)-antiproteinase activity; total Ca\(^{2+}\) and Mg\(^{2+}\) in bone, myocardium, and rectus femoris; and gp91\(^{phox}\) labeling in the heart. We found that 1) the hypercalcuria and hypermagnesuria and decline \((P < 0.05)\) in plasma [Ca\(^{2+}\)]\(_o\), and [Mg\(^{2+}\)]\(_o\), that occur with AldoST were not altered by the Ca\(^{2+}\)-supplemented diet alone or with PTX; 2) the rise \((P < 0.05)\) in plasma PTH with AldoST, with or without the Ca\(^{2+}\)-supplemented diet, was prevented by PTX; 3) increased \((P < 0.05)\) PBMC [Ca\(^{2+}\)]\(_t\), and H\(_2\)O\(_2\); production, increased total Ca\(^{2+}\) in heart and skeletal muscle, and fall in bone Ca\(^{2+}\) and Mg\(^{2+}\) and plasma \(\alpha_1\)-antiproteinase activity with AldoST were abrogated \((P < 0.05)\) by PTX; and 4) gp91\(^{phox}\) 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 Ca\(^{2+}\) overload in diverse tissues and induction of oxidative stress. SHPT plays a permissive role in the proinflammatory vascular phenotype.

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 overload 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 oscillatory 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 noradrenaline (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 Ca\(^{2+}\) and Mg\(^{2+}\), which contributes to a fall in plasma ionized concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) (17, 18). Despite these losses and the fall in extracellular levels of these divalent cations, total intracellular and cytosolic free Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\)) each rise, and oxidative stress is induced (2, 3, 17). This involves diverse tissues, including peripheral blood mononuclear cells (PBMC). A Ca\(^{2+}\) 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 Ca\(^{2+}\) 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 Ca\(^{2+}\) and Mg\(^{2+}\) (15, 17, 18, 30, 35, 50, 85) and fall in their plasma ionized concentrations lead, eventually, to a reduction...
in bone mineral density (17, 18). On the basis of this collective evidence, we have inferred that secondary hyperparathyroidism (SHPT) is responsible for the \( \text{Ca}^{2+} \) 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 pathophysiologic role of the parathyroid glands is the aim of the present study. We therefore hypothesized that the parathyroid glands are responsible for the \( \text{Ca}^{2+} \) overload and oxidative and nitrosative stress that accompany aldosteronism. To this end, the effects of AldoST, with or without a \( \text{Ca}^{2+} \), stress that accompany aldosteronism. To this end, the effects of AldoST, with or without a \( \text{Ca}^{2+} \)

**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 \( \times 10 \) magnification. PTx was considered successful only in rats with serum \( \text{Ca}^{2+} \) \( <6.0 \) mmol/l (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 contractions for \( \text{Ca}^{2+} \) overloaded and oxidative and nitrosative stress that accompany aldosteronism. To this end, the effects of AldoST, with or without a \( \text{Ca}^{2+} \) overload and oxidative and nitrosative stress that accompany aldosteronism. To this end, the effects of AldoST, with or without a \( \text{Ca}^{2+} \)

**RESULTS**

**Urinary \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) excretion.** A marked increase in urinary \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) excretion above control levels was found at 6 wk of AldoST (Fig. 1). We previously demonstrated the presence of hypercalcemia and hypermagnesemia at 1, 2, and 4 wk of AldoST (17). AldoST with the \( \text{Ca}^{2+} \)-supplemented diet or PTx with the \( \text{Ca}^{2+} \)-supplemented
diet did not alter the augmented urinary losses of these divergent cations seen with AldoST.

Plasma ionized \([\text{Ca}^{2+}]_o\) and \([\text{Mg}^{2+}]_o\). Plasma ionized \([\text{Ca}^{2+}]_o\) and \([\text{Mg}^{2+}]_o\) levels were reduced \((P < 0.05)\) during AldoST compared with controls (Fig. 2); in controls, plasma \([\text{Ca}^{2+}]_o\) was 0.98 ± 0.01 mmol/l (range 0.89–1.16) and plasma \([\text{Mg}^{2+}]_o\) was 0.36 ± 0.01 mmol/l (range 0.32–0.40), and during 1–6 wk of AldoST, plasma \([\text{Ca}^{2+}]_o\) was 0.82 ± 0.02 mmol/l (range 0.71–0.94) and plasma \([\text{Mg}^{2+}]_o\) was 0.28 ± 0.01 mmol/l (range 0.24–0.33, \(P < 0.05\) vs. controls). In AldoST rats fed the \([\text{Ca}^{2+}]_o\)-supplemented diet, plasma \([\text{Ca}^{2+}]_o\) and \([\text{Mg}^{2+}]_o\) were also reduced: 0.81 ± 0.02 and 0.29 ± 0.01 mmol/l, respectively \((P < 0.05)\). PTx before AldoST was accompanied by a greater fall in plasma \([\text{Ca}^{2+}]_o\), to 0.73 ± 0.02 mmol/l (range 0.48–0.84), which was statistically less \((P < 0.05)\) than in AldoST rats with intact parathyroid glands. This was due to a more marked fall in plasma \([\text{Ca}^{2+}]_o\) (to <0.71 mmol/l) in six PTx AldoST rats. This exaggerated decline observed in plasma \([\text{Ca}^{2+}]_o\) may be consistent with consumption of less of the \([\text{Ca}^{2+}]_o\)-supplemented diet and a decline in vitamin D stores. Reduced Ca\(^{2+}\) mobilization from bone and loss of PTH-driven renal formation of 1,25-dihydroxyvitamin D\(_3\), which serves to increase gastrointestinal absorption of Ca\(^{2+}\), result in a dependence on dietary Ca\(^{2+}\) after PTx.

Plasma PTH. The declines in plasma ionized \([\text{Ca}^{2+}]_o\) and \([\text{Mg}^{2+}]_o\) are important determinants of the parathyroid glands’ secretion of such calcitropic hormones as PTH (51) and PTH-related protein (51). An early, albeit declining, elevation in plasma PTH at 1–6 wk of AldoST, with or without the \([\text{Ca}^{2+}]_o\)-supplemented diet, was abrogated by prior PTx (Fig. 3).

PBMC \([\text{Ca}^{2+}]_i\). Cytosolic free \([\text{Ca}^{2+}]_i\) in PBMC was increased significantly at 2, 4, and 6 wk of AldoST, with or without dietary \([\text{Ca}^{2+}]_o\) supplementation (Fig. 3). PTH promotes Ca\(^{2+}\) loading of lymphocytes and monocytes, which is prevented by Ca\(^{2+}\)-channel blockers (2, 41). In support of the role of SHPT in promoting Ca\(^{2+}\) overload of PBMC, we demonstrate that prior PTx is preventive.

Oxidative stress. The Ca\(^{2+}\) overload of PBMC, both total intracellular and cytosolic free Ca\(^{2+}\) (17), is accompanied by an induction of oxidative and nitrosative stress in these cells. \(\text{H}_2\text{O}_2\) production by PBMC was increased at 1 and 2 wk of AldoST, with or without a \([\text{Ca}^{2+}]_o\)-supplemented diet (Fig. 4). PTx prevents the induction of oxidative stress in PBMC. We previously reported that increased \(\text{H}_2\text{O}_2\) production by PBMC persists over 4 wk of AldoST and can be prevented by a Ca\(^{2+}\) channel blocker or an antioxidant (2, 3).

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 \([\text{Ca}^{2+}]_o\)-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 \([\text{Ca}^{2+}]_o\) and \([\text{Mg}^{2+}]_o\) 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\(^{2+}\) and Mg\(^{2+}\). On the other hand, PTx with the Ca\(^{2+}\)-supplemented diet prevented the loss of these minerals from tibia (Fig. 5).

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

As in myocardial tissue, Ca\(^{2+}\) concentration was increased in the rectus femoris at 4–6 wk of AldoST, with or without supplemental dietary Ca\(^{2+}\), and was prevented by PTx (Fig. 6). Skeletal muscle Mg\(^{2+}\) concentration was not altered during AldoST with or without dietary Ca\(^{2+}\) 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\(^{2+}\) 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\(^{2+}\) 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\(^{2+}\) and Mg\(^{2+}\) 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\(^{2+}\)]o and [Mg\(^{2+}\)]o, each of which is a potent stimulus to the parathyroid glands’ release of calcitropic hormones (51). The decline in plasma [Ca\(^{2+}\)]o and [Mg\(^{2+}\)]o with 1–6 wk of AldoST was not prevented by our Ca\(^{2+}\)-supplemented diet or the amount of vitamin D\(_3\) available in standard chow and produced endogenously (albeit not measured). These findings suggest that supplemental calcitriol, together with a diet supplemented with Ca\(^{2+}\) and Mg\(^{2+}\), may be required to regulate PTH secretion in SHPT (68, 81). Bone resorption alone, as reflected by the fall in bone Ca\(^{2+}\) and Mg\(^{2+}\), was unable to preserve extracellular homeostasis of Ca\(^{2+}\) and Mg\(^{2+}\). We did not monitor other well-known effects of PTH, such as urinary phosphate excretion and acidification (1, 36, 67). In future studies, the importance of PTH and 1,25-dihydroxyvitamin D\(_3\) 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\(^{2+}\) and Mg\(^{2+}\) and, thereby, prevents the fall in plasma [Ca\(^{2+}\)]o and [Mg\(^{2+}\)]o, and loss of bone mineral density (17). A reduction in plasma ionized [Ca\(^{2+}\)]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\(^{2+}\)]o and [Mg\(^{2+}\)]o is accompanied by an elevation in plasma PTH, which declines over time, con-

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**Fig. 4.** Oxidative and nitrosative stress in peripheral blood mononuclear cells and their stress-induced increase in H2O2 production and in blood as shown by a decrease in plasma α1-antiproteinase (α1-AP) activity during AldoST with (\(n = 19\)) or without (\(n = 20\)) Ca\(^{2+}\)-supplemented diet. Effect was attenuated by prior PTx (\(n = 19\)). Values are means ± SE. *\(P < 0.05\) vs. controls (\(n = 20\)). Dash-dot and broken lines represent means ± SE for controls.

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**Fig. 5.** Decline in bone Ca\(^{2+}\) and Mg\(^{2+}\) concentrations in tibia induced by 4–6 wk of AldoST, with (\(n = 5\)) or without (\(n = 5\)) Ca\(^{2+}\)-supplemented diet, was prevented by Ca\(^{2+}\)-supplemented diet with prior PTx (\(n = 5\)). Values are means ± SE. FFDB, fat-free dry bone. *\(P < 0.05\) vs. controls (\(n = 5\)).
sistent with the downregulation of the parathyroid glands’ Ca\textsuperscript{2+}-sensing receptor (21). Prior PTx eliminates the rise in plasma PTH that accompanies AldoST. Elevations in plasmaPTH have been found in rats treated with deoxycorticosteroneacetate (DOCA)-salt (94), and chemical evidence of SHPT iscorrected by spironolactone or adrenal surgery in humans withprimary aldosteronism (70, 76). As in SHPT, bone Ca\textsuperscript{2+} and Mg\textsuperscript{2+} each fall significantly at 4 and 6 wk of AldoST, consistent with our previous findings for bone mineral density andbone strength (17, 18). SHPT and bone loss have also beenobserved in patients with advanced congestive heart failure(CHF), where secondary aldosteronism is expected, togetherwith the hypercalciuria and hypermagnesuria, which accompanychronic treatment with furosemide, a potent loop diuretic(6, 45, 80).

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

The Ca\textsuperscript{2+} overload of PBMC is accompanied by an induction of oxidative stress in these cells and is reflected in theirincreased production of H\textsubscript{2}O\textsubscript{2}, as shown for 1 and 2 wk ofAldoST and as previously reported at 4 wk of AldoST (2, 3).H\textsubscript{2}O\textsubscript{2} participates in the signal transduction that leads to anactivation of PBMC. As observed in the present study, priorPTx prevents the induction of oxidative stress in PBMC. Amiodipine, a Ca\textsuperscript{2+} channel blocker, and N-acetylcysteine, anantioxidant, prevent the rise in H\textsubscript{2}O\textsubscript{2} production in PBMC(2). Mitochondria take up and accumulate Ca\textsuperscript{2+} and represent arich source of reactive oxygen species. Mitochondrial Ca\textsuperscript{2+}uptake stimulates net production of reactive oxygen species throughactivation of membrane permeability transition, release ofcytochrome c, respiratory inhibition, and release ofantioxidants (84). Oxidative stress occurs in response to Ca\textsuperscript{2+}accumulation within these organelles and when antioxidantdefenses are exhausted. Another example of oxidative stressinduced by mitochondrial Ca\textsuperscript{2+} overload is found in cardiomyocytes after ischemia-reperfusion (23). In lymphocytes,H\textsubscript{2}O\textsubscript{2} acts as an intracellular messenger involved in signaltransduction and amplification to activate these cells in amanner that simulates antigen-antigen receptor binding (forreview see Ref. 72).

Oxidative and nitrosative stress at a systemic level is evidenced by the fall in plasma \( \alpha \text{1-AP} \) activity, an inverse corre-lation of oxidative and nitrosative stress, which we report here for1 and 6 wk of AldoST. Together with our previous finding,these observations demonstrate the sustained nature of oxidativeand nitrosative stress in AldoST rats (3). In the secondaryaldosteronism that accompanies CHF, oxidative and nitrosativestress has been reported in such diverse tissues as skin, skeletalmuscle, heart, PBMC, and plasma (16, 34, 55, 89). Plasmalevels of 8-isoprostane and thiobarbituric acid-reactive sub-stances are increased in rats with chronic mineralocorticoidismand can be prevented by spironolactone or tempol, a superox-ide dismutase mimetic (37, 66, 90). Here, we show that PTxprevents the decline in plasma \( \alpha \text{1-AP} \) activity that accompaniesAldoST and is related to Ca\textsuperscript{2+} loading of diverse tissues.

Another major finding of the present study is oxidative stress ininflammatory cells that invade the intramural coronary vasculatureof the right and left ventricles at 4 wk of AldoST and is expressed as increased gp91\textsuperscript{phox} 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\(^{2+}\)/H\(^{+}\)-supplemented diet but was attenuated by PTx with the Ca\(^{2+}\)/H\(^{+}\)-supplemented diet. Prior PTx has been reported to ameliorate the incidence and severity of cardiac and renal lesions that accompany DOCA-salt treatment (58). 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 (ETA) 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. ETA 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\(^{2+}\) (8). An increase in plasma norepinephrine in DOCA-salt-treated rats may result from centrally mediated increases in peripheral sympathetic neuronal activity (20, 69). Elevations in substance P, a neurotransmitter, may also contribute to chronic mineralocorticoid excess because of reductions in plasma ionized [Mg\(^{2+}\)]\(_o\) (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\(^{2+}\) excretion, reduced plasma ionized Ca\(^{2+}\), elevated plasma levels of PTH, and increased cytosolic free [Ca\(^{2+}\)]\(_i\) are found in patients with low-renin essential hypertension (11, 59, 71). High dietary Na\(^+\), 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\(^{2+}\) may explain the efficacy of Ca\(^{2+}\) 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 plasma PTH and ET-1 levels, osteopenia, and osteoporosis, is found in patients with CHF (6, 45, 80). Hypovitaminosis D has also been reported in CHF patients (80). This may further compromise Ca\(^{2+}\) homeostasis and is likely related to a lack of exposure to sunlight, which accompanies this chronic, symptomatic, and disabling disorder. The cytokine profile of primary and SHPT (i.e., 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\(^{2+}\) and Mg\(^{2+}\) 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 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 (ETA) 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. ETA 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\(^{2+}\) (8). An increase in plasma norepinephrine in DOCA-salt-treated rats may result from centrally mediated increases in peripheral sympathetic neuronal activity (20, 69). Elevations in substance P, a neurotransmitter, may also contribute to chronic mineralocorticoid excess because of reductions in plasma ionized [Mg\(^{2+}\)]\(_o\) (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\(^{2+}\) excretion, reduced plasma ionized Ca\(^{2+}\), elevated plasma levels of PTH, and increased cytosolic free [Ca\(^{2+}\)]\(_i\) are found in patients with low-renin essential hypertension (11, 59, 71). High dietary Na\(^+\), 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\(^{2+}\) may explain the efficacy of Ca\(^{2+}\) 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 plasma PTH and ET-1 levels, osteopenia, and osteoporosis, is found in patients with CHF (6, 45, 80). Hypovitaminosis D has also been reported in CHF patients (80). This may further compromise Ca\(^{2+}\) homeostasis and is likely related to a lack of exposure to sunlight, which accompanies this chronic, symptomatic, and disabling disorder. The cytokine profile of primary and SHPT (i.e., 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\(^{2+}\) and Mg\(^{2+}\) 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.

Fig. 7. Immunohistochemical study of activation of gp91phox in coronal cryostat sections of ventricle. Top: control. Middle: gp91phox, a subunit of NADPH oxidase, activity (arrows) during AldoST. AldoST-induced gp91phox activity was not altered by Ca\(^{2+}\)-supplemented diet (not shown). Bottom: AldoST-induced gp91phox activity was attenuated (arrow), but not prevented, by prior PTx.
reverses these losses and preserves bone health (78). The negative impact of non-K⁺ sparing 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-converted enzyme inhibitor and loop diuretic, with or without β-receptor blocker and digoxin (64, 65).

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Ca2+ PARADOX AND PARATHYRIDS


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