Increased Reactive Oxygen Species, Metabolic Maladaptation, and Autophagy Contribute to Pulmonary Arterial Hypertension–Induced Ventricular Hypertrophy and Diastolic Heart Failure

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Abstract—Pulmonary arterial hypertension (PAH) is a debilitating and deadly disease with no known cure. Heart failure is a major comorbidity and a common cause of the premature death of patients with PAH. Increased asymmetrical right ventricular hypertrophy and septal wall thickening compress the left ventricular cavity and elicit diastolic heart failure. In this study, we used the Sugen5416/hypoxia/normoxia-induced PAH rat to determine whether altered pyridine nucleotide signaling in the failing heart contributes to 1) increased oxidative stress, 2) changes in metabolic phenotype, 3) autophagy, and 4) the PAH-induced failure. We found that increased reactive oxygen species, metabolic maladaptation, and autophagy contributed to the pathogenesis of right ventricular remodeling and hypertrophy that lead to left ventricular diastolic dysfunction. In addition, arterial elastance increased in PAH rats. Glucose-6-phosphate dehydrogenase is a major source of pyridine molecule (nicotinamide adenine dinucleotide phosphate), which is a substrate for nicotinamide adenine dinucleotide phosphate oxidases in the heart. Dehydroepiandrosterone, a 17-ketosteroid that reduces pulmonary hypertension and right ventricular hypertrophy, inhibited glucose-6-phosphate dehydrogenase, decreased oxidative stress, increased glucose oxidation and acetyl-coA, and reduced autophagy in the hearts of PAH rats. It also decreased arterial stiffness and improved left ventricular diastolic function. These findings demonstrate that pyridine nucleotide signaling, at least partly, mediates PAH-induced diastolic heart failure, and that reduction of glucose-6-phosphate dehydrogenase-derived nicotinamide adenine dinucleotide phosphate is beneficial to improve left ventricle diastolic function. **(*Hypertension*. 2014;64:1266-1274.)** ● **Online Data Supplement**

Key Words: dehydroepiandrosterone ■ free radicals ■ heart function tests ■ lung

Heart failure is a major comorbidity and a common cause of the premature death in patients with pulmonary arterial hypertension (PAH).[1,2] The number of PAH cases is increasing around the world, and, despite recent advances, current medical treatment remains inadequate.[3] Elevated pulmonary arterial pressure increases afterload and decreases right ventricular (RV) function.[4–6] It is noteworthy, however, that for many years, relatively little effort has been made to study mechanisms of RV hypertrophy (RVH) and failure.[4] As a result, the molecular mechanisms involved in the pathogenesis of RVH remain unknown, and no therapy has yet been identified to prevent or reverse the development of heart failure in PAH.

Left heart failure, an outcome of increased afterload and ischemia or other conditions, is determined by the balance between cell death– and cell survival–promoting mechanisms. Significant progress has been made in understanding the molecular causes of left ventricular (LV) hypertrophy and failure. It is now known that in the failing heart 1) oxidative stress is increased, 2) metabolic phenotype is altered, 3) myocardium is inflamed, 4) the rate of cardiomyocyte apoptotic and autophagic death is augmented, and 5) myocardial fibrosis is induced. Activation of these processes causes myocardial stiffening, dilatation, and dysfunction.

Solid evidence indicates that production of reactive oxygen species (ROS), because of an imbalance between superoxide-generating and antioxidant systems, is elevated in the failing left heart.[7–9] The nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Nox-2 and Nox-4) mediate, respectively, neurohumoral and pressure-overload–induced LV hypertrophy.[10] Interestingly, ROS derived from Nox-4

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localized in the mitochondrial matrix also contributes to cardiac myocyte apoptosis and autophagy.[11] In the failing heart, uncoupled nitric oxide synthase—a condition caused when tetrahydrobiopterin is reduced by increased oxidative stress—also generates superoxide anion instead of nitric oxide by donating an electron from NADPH to molecular oxygen.[12] It now seems that Nox- and mitochondria-derived ROS levels go up in the heart before the development of dilated cardiomyopathy.[11] These observations imply that there is a cause–effect relationship between increased myocardial ROS production and heart failure. As in left-sided failure, an association between ROS production and RVH induced by pulmonary hypertension has been observed in humans and rats.[13,14] Therefore, one goal of this study was to determine whether ROS production goes up in the RV and LV and contributes to the pathogenesis of heart failure in PAH.

NAD(P)H is a cosubstrate for oxidoreductases, such as Nox and nitric oxide synthase in the heart. NAD(P)H/NAD(P)$^+$-dependent signaling pathways are emerging as important players in controlling myocardial function, as well as cardiac myocyte survival and death, and more studies are needed to understand the precise role of pyridine nucleotide signaling in causing the failure,[15,16] as well as the signaling pathways downstream of NADPH-dependent and Nox-derived ROS that reduces the heart function.[10] Although dehydroepiandrosterone is a pleiotropic steroid that can potentially affect multiple biochemical pathways simultaneously in the cell to exert beneficial effects, it is a potent inhibitor of glucose-6-phosphate dehydrogenase (G6PD), which is a major supplier of NADPH in many cells, including the cardiac myocytes.[17] To our knowledge, dehydroepiandrosterone is the only safe and nontoxic drug available in the market at present that can be used to study the role of pyridine nucleotide signaling in vivo. It reversibly blocks extramitochondrial and mitochondrial G6PD activity and NADPH synthesis, which reduces NADPH supply to Nox in the myocardium, and thereby lowers the myocardial oxidative stress in diabetic and failing hearts.[18–20] Dehydroepiandrosterone is constitutively secreted from healthy human hearts, where it inhibits the development of cardiac myocyte hypertrophy. In failing hearts, secretion of dehydroepiandrosterone is diminished. Therefore, it is suggested that dehydroepiandrosterone or a metabolite protects the heart by exerting antihypertrophic effects on myocytes.[21] In this regard, dehydroepiandrosterone attenuates L-type Ca2+ channel function, which is implicated in activating nuclear factor of activated T-cells, cytoplasmic signaling that causes hypertrophy and failure,[22] in rat cardiac myocytes (R. Ochi and S.A. Gupte, unpublished data, 2013). In addition, epiandrosterone (5β-androstan-17-one), a metabolite of dehydroepiandrosterone, also decreases L-type Ca2+ currents, reduces diastolic dysfunction, and protects the heart from reoxygenation injury.[23] Studies, including ours,[24–27] have demonstrated that dehydroepiandrosterone treatment ameliorates pulmonary hypertension and reduces RVH in chronically hypoxic, monocrotaline-injected, left pneumonectomized/monocrotaline-injected, and Sugen/hypoxia/normoxia-exposed rats. Therefore, another objective was to determine whether dehydroepiandrosterone prevents the heart from failing by reducing 1) NADPH signaling-dependent ROS production, 2) improving cardiac metabolism, and 3) decreasing autophagy, in the remodeled RV and LV of PAH rats.

Methods

For detailed description see Methods in the online-only Data Supplement.

Results

ROS Are Increased in the RV but Not in the LV of PAH Rats

Because activation of ROS-dependent signaling pathways has been known to suppress myocardial function, we determined ROS production in RV and LV isolated from control and PAH rat hearts. ROS production, as determined by the lucigenin chemiluminescence method, increased by 400% (P<0.05) and decreased by 12% (NS), respectively, in RV and LV of PAH when compared with control rats (Figure 1A and 1B). Consistently, incubation of PAH RVs with tempol (1 mmol/L), a superoxide dismutase mimic that scavenges superoxide, ebselen (100 μmol/L), a glutathione peroxide mimic that scavenges hydrogen peroxide, apocynin (50 μmol/L), a NADPH oxidase inhibitor, or antimycin (10 μmol/L), a mitochondrial complex III inhibitor, decreased ROS in RV but not in LV. Acpocynin (50 μmol/L) did not

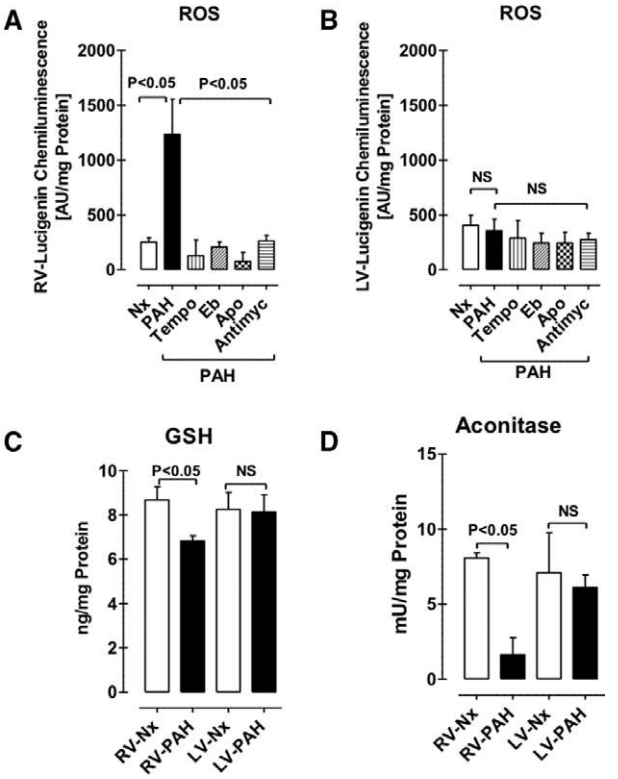

Figure 1. Reactive oxygen species (ROS) are elevated in right (**A**) but not in left (**B**) ventricle of pulmonary arterial hypertension (PAH) rats. Right ventricular ROS are decreased by incubation with tempol (1 mmol/L), a spin trap, ebselen (100 μmol/L), a glutathione(GSH) mimetic, apocynin (50 μmol/L), a NOX inhibitor and ROS scavenger, and antimycin (50 μmol/L), a mitochondrial complex III inhibitor. Reduced GSH (**C**) and aconitase activity (**D**) are decreased in right but not in left ventricle of PAH rats. n=5 to 7.

reduce lucigenin chemiluminescence (control, 6155±602 versus apocynin, 6156±501 AU; n=5) signals elicited by xanthine (2.5 mmol/L)+xanthine oxidase (0.06 U/mL).

Reduced Glutathione Level and Aconitase Activity Are Decreased in the RV but Not in the LV of PAH Rats

Glutathione detoxifies hydrogen peroxide and hence we measured reduced glutathione levels as a marker of ROS production in RV and LV isolated from PAH and control rats. Reduced glutathione was decreased ($P<0.05$) in RV but not in the LV of PAH rats (Figure 1C).

Aconitase is the first enzyme in the Krebs cycle and it is inhibited by elevated mitochondrial ROS production in the failing hearts[28]; therefore, we measured aconitase activity as a surrogate marker for ROS production in RV and LV from PAH and control rats. Aconitase activity was decreased in the RV but not in the LV of PAH rats (Figure 1D).

G6PD Activity and ROS Production Are Decreased by Dehydroepiandrosterone Treatment in the RV but Not in the LV of PAH Rats

Dehydroepiandrosterone, a 17-ketosteroid, is an inhibitor of G6PD. Because G6PD is the major source of NADPH, which fuels ROS production from Noxs in the heart and blood vessels,[10,29–31] we treated PAH and control rats with dehydroepiandrosterone (1% daily diet) for 3 weeks and then measured G6PD activity and ROS production in RV and LV. Although G6PD activity was not significantly increased either in RV or in LV of PAH when compared with control rats, dehydroepiandrosterone treatment decreased G6PD activity in RV (by 58.1%) and in LV (by 52.6%) of PAH when compared with untreated PAH rats (Figure 2A and 2B), as well as reduced activity and NADPH levels by 53.5% in treated versus nontreated normal hearts. Concomitantly, dehydroepiandrosterone also suppressed ROS production in the RV but not in the LV of PAH when compared with untreated PAH rats (Figure 2C and 2D). Dehydroepiandrosterone also reduced (15%–20%; NS) basal ROS production in the RV or in the LV of the control rat hearts (data not shown).

Pyruvate and Acetyl-CoA Levels Are Increased by Dehydroepiandrosterone Treatment in the RV and in the LV of PAH Rats

In the normal adult heart, acetyl-CoA is derived from fatty acid and glucose oxidation in the mitochondria. Because cardiac metabolism is altered in failing hearts,[32] we estimated pyruvate and acetyl-CoA levels in the RV and in the LV of control, PAH, and PAH+dehydroepiandrosterone–treated rats. The levels of pyruvate (by 88±12%) and acetyl-CoA (by 53±18%) were less in the RV than in the LV of control rats. Furthermore, pyruvate (Figure 3A and 3B) and acetyl-CoA (Figure 3C and 3D) were decreased ($P<0.05$) in the LV but not in the RV of PAH rats when compared with control rats. Interestingly, levels of pyruvate and acetyl-CoA were increased by 744% and 730%, respectively, in the RV and by 134% and 178%, respectively, in the LV of PAH rats treated with dehydroepiandrosterone. Tissue lactate:pyruvate ratio, a surrogate measure of cellular NADH-to-NAD^+ ratio and levels of NADH that is used by mitochondrial electron transport chain to produce ATP, was increased in the RV (PAH, 0.05±0.0001 and PAH+dehydroepiandrosterone, 0.2±0.08; $P<0.05$; n=5) and in the LV (PAH, 0.06±0.06 and PAH+dehydroepiandrosterone, 0.37±0.08; NS; n=5) by dehydroepiandrosterone treatment when compared with untreated PAH rats.

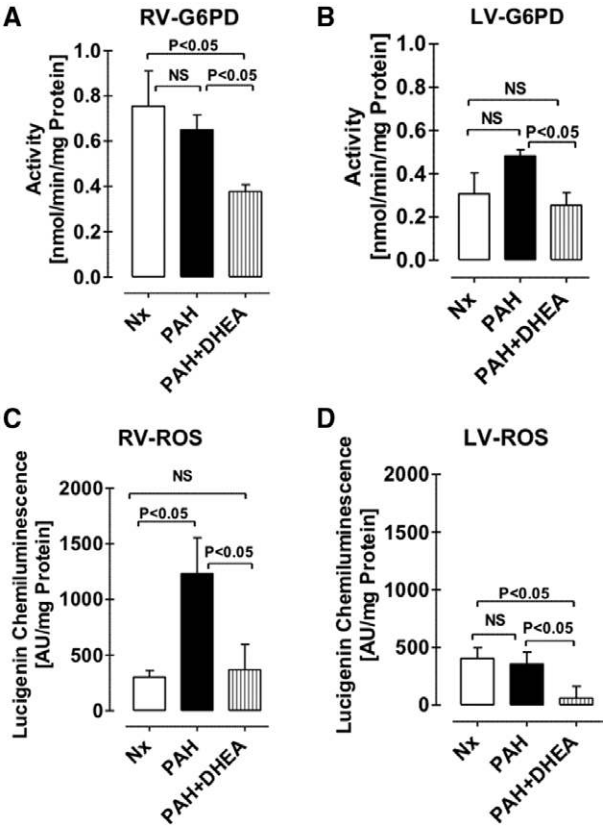

Figure 2. Dehydroepiandrosterone (DHEA) treatment decreased glucose-6-phosphate dehydrogenase activity (G6PD; **A** and **B**) and reactive oxygen species (ROS; **C** and **D**) in the right and left ventricles (RV and LV, respectively) of pulmonary arterial hypertension (PAH) rats. n=5 in PAH and PAH+DHEA groups.

Autophagy Is Triggered in the RV but Not in the LV of PAH Rats

Autophagy plays a role in cell repair and, if uncontrolled, promotes cell death. It is triggered by excess ROS production and by starvation.[30,33,34] In the heart, autophagy plays a role in hypertrophic remodeling of ventricles, an adaptive response to pressure overload, and also increases cardiac myocyte death and causes heart failure.[35]

Therefore, we performed electron microscopy to determine whether autophagy is triggered in the cardiac tissue of rats by increased ROS production. Electron microscopy of cardiac myocytes in which ROS was elevated showed vacuolar changes partly because of dilated sarcoplasmic cisternae, variably sized and shaped mitochondria, and an autophagic vacuole that contains fragments of organelles when compared with control hearts (Figure 4A and 4B).

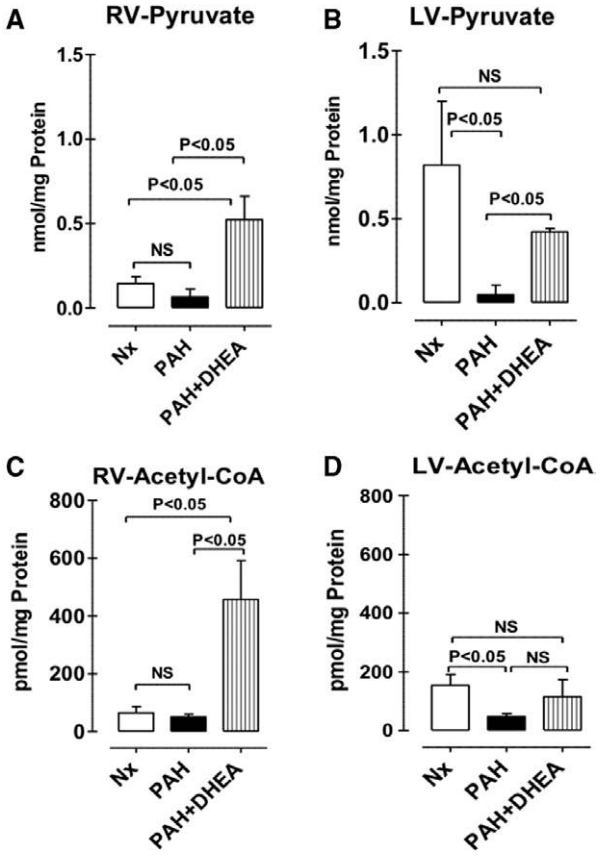

Figure 3. Dehydroepiandrosterone (DHEA) treatment increased pyruvate (**A** and **B**) and acetyl-CoA (**C** and **D**) levels in the right and left ventricles (RV and LV, respectively) of pulmonary arterial hypertension (PAH) rats. n=5.

We also compared the expression of LC3A/B, an autophagy marker, in the RV and LV from PAH and control rats by Western blot analysis. We detected LC3A/B using antibody from Cell Signaling (Cat # 4108), which predominantly detects type II LC3A/B, and found that the expression of LC3A/B-II increased (P<0.05) in the RV but not in the LV of PAH rats when compared with control rats (Figure 4C and 4D).

Autophagy Is Decreased by Dehydroepiandrosterone in the RV but Not in the LV of PAH Rats

Because elevated ROS and decreased metabolism trigger autophagy, we determined whether autophagy was reduced in RV and LV by dehydroepiandrosterone. The increased expression of LC3A/B-II was clearly and significantly reduced in the RV (Figure 4C) of PAH+dehydroepiandrosterone–treated when compared with PAH untreated rats. Dehydroepiandrosterone treatment did not alter the normal level of LC3A/B-II in the LV (Figure 4D) of PAH rats.

Diastolic Heart Failure Is Decreased by Dehydroepiandrosterone in PAH Rats

Our previous studies show that the RV is severely hypertrophic in PAH rats.[36–38] The remodeled RV and septum compress the LV and reduce the volume of the LV cavity. Therefore, we measured LV hemodynamics in PAH+dehydroepiandrosterone–treated, PAH untreated, and control rats. The hemodynamic results are shown in the Table. RV pressure and remodeling were moderately decreased by treating PAH rats with dehydroepiandrosterone.[38] Although treatment of PAH rats with dehydroepiandrosterone did not affect dp/dt_{max} (Figure 5A), it normalized dp/dt_{min} when compared with untreated PAH rats (Figure 5B). In addition, dehydroepiandrosterone significantly reduced elevated Tau_g (Figure 5C), end-diastolic pressure (Figure 5D), and arterial elastance (E_a=end-systolic pressure/systolic volume=[heart rate]×resistance; Figure 5E) when compared with untreated PAH rats. Ratio of myocardial elastance (E_{es}) to arterial elastance decreased from 0.106 (control) to 0.016 (PAH). Dehydroepiandrosterone treatment increased E_{es} to 0.058 and also increased stroke work (Figure 5F).

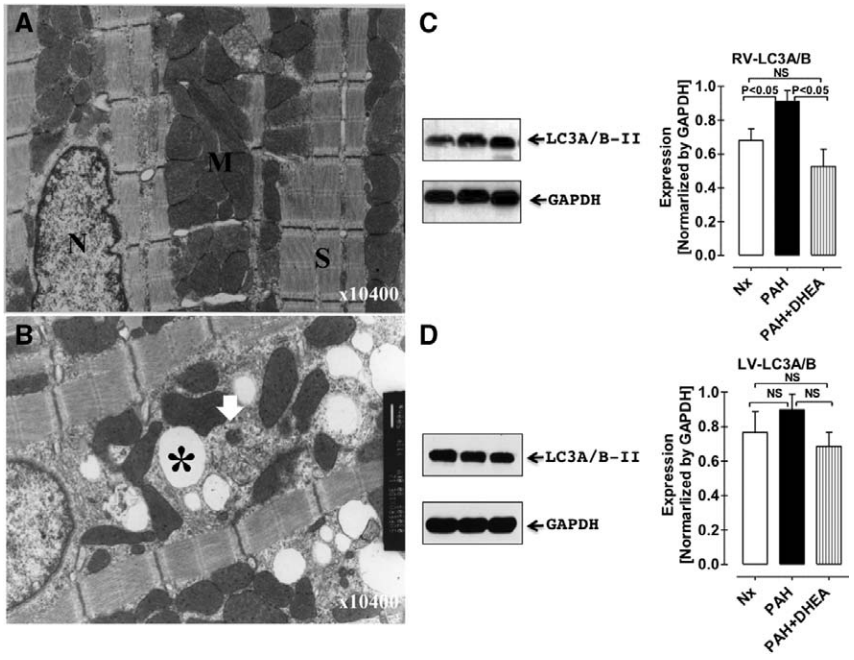

Figure 4. Electron micrograph of control (**A**) and increased reactive oxygen species producing (**B**) right ventricles (RV) showing autophagy in the latter. Asterisk indicates vacuolar changes and white arrow indicates an autophagic vacuole in the pulmonary arterial hypertension (PAH) RV. Expression of LC3A/B-II, an autophagy marker protein, was increased in RV (**C**) but not in left ventricle (LV; **D**) of PAH rats. Dehydroepiandrosterone (DHEA) treatment decreased LC3A/B expression in RV but not in LV. n=7 in control and n=5 in PAH and PAH+DHEA groups.

Table. Left Ventricular Hemodynamic Parameters Recorded by Cardiac Catheterization in Control, PAH, and PAH+DHEA Rats

Parameter	Normal	SU/Hx/Nx	SU/Hx/Nx+DHEA
$BP_{systole}$, mm Hg	135.8±16.4	158.4±5.3	151.5±6.8
$BP_{diastole}$, mm Hg	99.6±10.7	126.2±2.8*	117.5±5.2
CI, mL/min per kilogram	118.8±12.2	65.6±6.7*	111.1±9.6†
End-systolic volume, μL	107.6±22.4	114.7±21.6	114.0±22.3
End-diastolic volume, μL	178.5±13.1	144.9±3.9*	163.1±18.0
End-systolic pressure, mm Hg	134.6±8.2	161.1±7.2*	150.2±4.8
Stroke volume, μL	93.3±10.0	63.2±4.1*	80.3±3.4†
EF	53±7	52±8	52±6
dV/dt max, μL/s	3727±783	3424±483	3455±240
dV/dt min, μL/s	−3327±171	−4692±974	−3834±546
V@dP/dt max, μL	182.3±13.6	154.0±10.2	164.6±19.0
V@dP/dt min, μL	106.8±20.1	116.8±16.3	113.3±21.0
Tau_w, ms	9.1±0.8	10.4±0.8	9.6±0.6
Maximal power, mW	50.1±2.2	73.4±13.0*	45.9±3.4
Preload adjusted maximal power, mW/μL^2	16.8±2.8	35.6±5.2*	20.6±5.0

BP indicates blood pressure; CI, cardiac index; DHEA, dehydroepiandrosterone; EF, ejection fraction; Hx, hypoxia; Nx, normoxia; PAH, pulmonary arterial hypertension; and SU, Sugen.

*P<0.05 vs normal.

†P<0.05 vs SU/Hx/Nx.

Cardiac Myocyte L-Type Ca^{2+} Channel Function and Myocardial Contractility Are Reduced by Dehydroepiandrosterone

The next questions were does dehydroepiandrosterone affect myocardial G6PD activity and NADPH levels and cardiac function? To determine this, we perfused hearts isolated from normal rat with dehydroepiandrosterone for 30 minutes and measured 1) G6PD activity, 2) NADPH levels, and 3) LV hemodynamics. We found that dehydroepiandrosterone dose dependently decreased (P<0.05) G6PD activity and NADPH levels in the whole heart homogenate (Figure 6A and 6B). Dehydroepiandrosterone (100 μmol/L) also decreased left ventricular developed pressure and dp/dt_{max} (Figure 6C and 6D). Furthermore, dehydroepiandrosterone suppressed L-type Ca2+ channel function (Figure 6E–6L). It decreased currents/current density (Figure 6E and 6F) without affecting steady-state activation (Figure 6G) but by shifting steady-state inactivation curve to the left by 15.4 mV (control, −29.4±0.1 and dehydroepiandrosterone, −44.8±0.9 mV; Figure 6H). Also, dehydroepiandrosterone decreased currents in a reversible manner (Figure 6I and 6J). Interestingly, the dehydroepiandrosterone-induced reduction of I_{CaL} amplitude, but not steady-state inactivation curve (not shown), was reversed by dialyzing NADPH (100 μmol/L; Figure 6K) and not by dialyzing NADH (100 μmol/L; Figure 6 L) into the cardiac myocyte isolated from control rats.

Discussion

The salient findings of our study are 1) ROS production and autophagy were increased in the severely hypertrophied RV of PAH rats, 2) metabolism was not increased in the hypertrophied RVs, 3) PAH rats were in diastolic heart failure, 4) dehydroepiandrosterone treatment attenuated ROS production and autophagy, increased metabolic substrates—pryuvate and acetyl-CoA—and prevented diastolic heart failure, 5) dehydroepiandrosterone decreased G6PD activity in the RV and in the LV of PAH rats, and 6) dehydroepiandrosterone reduced myocardial G6PD activity and NADPH, myocardial contractility, and cardiac myocyte L-type Ca2+ currents.

RV heart hypertrophy and failure, a comorbidity, ensues in patients with PAH and in animal models of PAH. Heart failure is a major cause of death of patients with PAH. A PAH rat model that mimics clinical features of patients with PAH[37] has elevated RV pressure and develops severe RVH.[36,38,39] Interestingly, ROS are also elevated in the severely remodeled RV of these rats.[38] ROS generated by Noxs and mitochondria are elevated in the RV of patients with PAH and rats,[13,14] and it is well known that ROS produced by extramitochondrial Noxs, mitochondrial electron transport chain and Nox-4, and uncoupled eNOS are increased in the failing left heart.[10,29,30] Moreover, ROS derived from mitochondrial Nox-4, elevated mainly by pressure overload/afterload, and from extramitochondrial Nox-2, elicited by angiotensin-II, contribute to the pathogenesis of LV hypertrophy.[29,30] Because apocynin, a Nox-2 inhibitor, and antimycin, a mitochondrial complex III inhibitor, decreased ROS levels in the RV, this suggests that ROS were generated by Nox-2 and mitochondrial electron transport chain/Nox-4 activated by elevated neurohumoral factors and pressure overload. Moreover, our findings that ROS production was not upregulated in the LVs of PAH when compared with control rats suggest that increased afterload is, at least partly, essential to increase ROS production in the RV of PAH rats. Although the signaling pathways that cause RV and left heart failure are incompletely understood, this report highlights that RV and LV share common sources of ROS that cause hypertrophy and failure.

NADPH fuels ROS production from Nox in failing hearts and in cardiac myocytes cultured in high glucose.[19,29,30] In addition, uncoupled eNOS oxidizes NADPH and increases ROS production in myocardial tissue of failing dog and human hearts.[18,40] Recently, other authors have proposed a similar mechanism to explain, at least in part, the cardiac oxidative stress in mice with defective glycolytic pathway and aortic constriction.[41] In addition, G6PD-derived NADPH increases reductive stress and plays a significant role in the pathogenesis of protein aggregation cardiomyopathy and heart failure.[42] Therefore, from these observations, it would not be out off context to suggest that increased NADPH cellular content amplifies reductive and oxidative stress in the cardiac myocytes in failing heart. In the adult heart, 60% to 70% of NADPH is produced by extramitochondrial and mitochondrial G6PD and ≈30% to 40% of NADPH is derived from mitochondrial malic enzyme and isocitrate dehydrogenase.[43] For this reason, it is speculated that NADPH derived from G6PD-independent sources increase oxidative stress in the LV of G6PD-deficient mice after coronary ligation or transverse aortic constriction.[44] Consistently, although G6PD activity did not increase in the RV or LV (Figure 2), Alzoubi et al[38] reported 4-fold higher NADPH levels in the RV of PAH than that of control rats. This indicates that increased NADPH is derived from either malic enzyme or isocitrate dehydrogenase in the remodeled RVs. Nonetheless, dehydroepiandrosterone treatment lowers NADPH and oxidative stress in the RV of

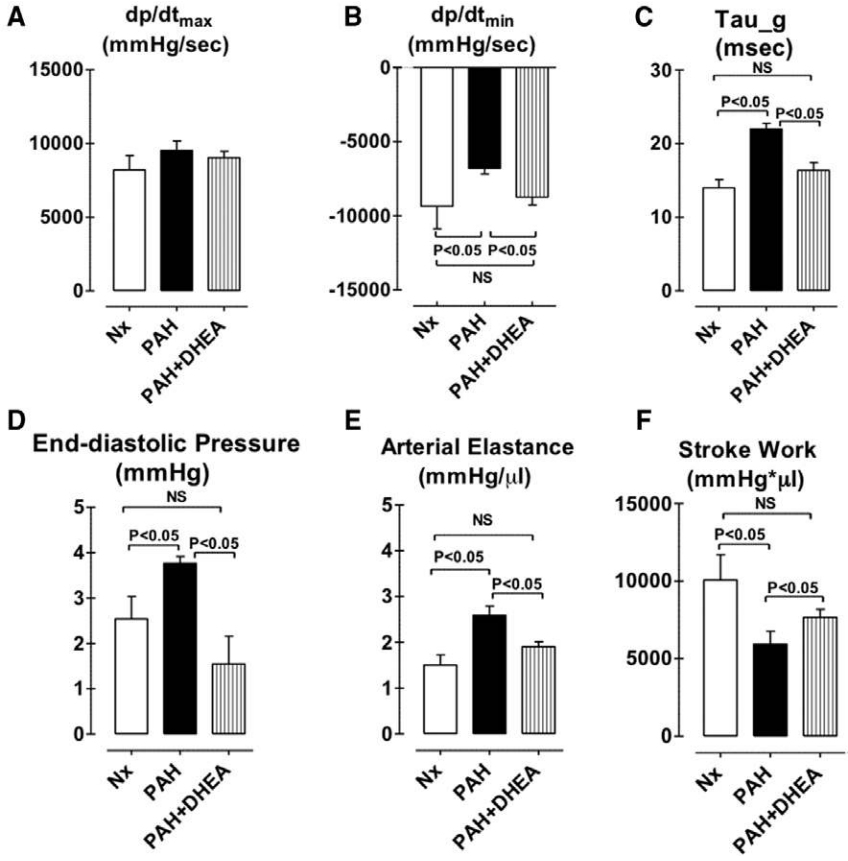

Figure 5. Left ventricle hemodynamic changes. **A**, Rate of contraction, dp/dt$_{max}$, (**B**) rate of relaxation, dp/dt$_{min}$, (**C**) isovolumic relaxation time, Tau_g, (**D**) end-diastolic pressure, (**E**) arterial elastance, and (**F**) stroke work; determined by left ventricle catheterization of normal and pulmonary arterial hypertension (PAH) rats are shown. Dehydroepiandrosterone (DHEA) treatment improved diastolic function. n=7 in control and n=5 in PAH and in PAH+DHEA groups.

PAH rats.[38] Because dehydroepiandrosterone decreased G6PD activity in the RV and in the LV from PAH rats (Figure 2), our findings suggest that lowering G6PD-derived NADPH was beneficial to blunt cardiac oxidative stress, which damages myocardium and compromises myocardial function,[29] in the severely hypertrophied RV of PAH rats. However, because dehydroepiandrosterone is a pleiotropic steroid that can potentially affect multiple biochemical pathways simultaneously in the cell to exert beneficial effects, it is plausible that dehydroepiandrosterone reduced cardiac hypertrophy and dysfunction via other pathways in addition to decreasing G6PD/NADPH-dependent ROS generation. Consistent with this speculation, other authors have also reported that G6PD-dependent ROS production plays a role in the pathophysiology of RVH and septal hypertrophy induced by pressure overload.[45] Pyridine nucleotides, NADP(H) and NAD(H), are now emerging as important signaling molecules in controlling cardiac myocyte survival and death, as well as myocardial function.[15,16] Therefore, the current findings—inhibition of G6PD-reduced hypertrophy and failure in PAH—underscore the notion that the pyridine nucleotide signaling has clinical relevance in cardiovascular medicine.[15]

Along with augmented oxidative stress, cardiac metabolic phenotype is altered in failing hearts.[17,32,46] Pryuvate, an end product of glycolysis and lactate oxidation, and acetyl-CoA, a product of pyruvate and fatty acid oxidation, substrates required for energy production were not increased, but the activity of aconitase, the first enzyme that converts citrate to isocitrate in the Krebs cycle, was decreased in the hypertrophic when compared with normal RV. These observations suggest that the metabolic need of the remodeled RV was inadequately compensated. Studies show that cardiac free fatty acid oxidation, the main source of energy in the normal adult heart, is downregulated in failing left hearts, with consequent higher glucose consumption.[17,32,46] But glucose is inefficiently catabolized in the glycolytic pathway because of the downregulation of phosphofructose kinase in the failing left hearts and is thus unable to meet the energy demand of cardiac myocytes in heart failure.[47] Likewise, the gene profile of proteins involved in regulating fatty acid metabolism and mitochondrial function is downregulated in the dysfunctional RV from PAH rats,[48] and glucose oxidation is decreased in RV from pulmonary artery banding-induced RV overloaded and monocrotaline-induced PH rats.[49,50] Interestingly, dehydroepiandrosterone treatment enhanced pyruvate and acetyl-CoA levels and increased lactate:pyruvate ratios in the RV and LV of PAH rats. These results suggest that blockade of G6PD activity and ROS production presumably redirected glucose-6-phosphate entry into glycolytic pathway and, at least partly, increased glucose oxidation. Vimercati et al[51] have recently made similar observations in failing dog hearts in which they found that acute inhibition of the pentose phosphate pathway with 6-aminonicotinamide enhanced cardiac glucose oxidation. Therefore, these studies suggest that the inhibition of G6PD may be beneficial to improve metabolism and heart function.

Cardiac myocyte apoptosis or autophagy is triggered by increased oxidative stress and inadequate metabolic compensation. In the heart, autophagy contributes to the pathogenesis of both hypertrophy and failure.[35] Recent studies found that autophagy induced by mitochondrial Nox-4–derived ROS and mitochondrial dysfunction causes remodeling of the RV and LV in pulmonary artery banding[52] and in transverse aortic

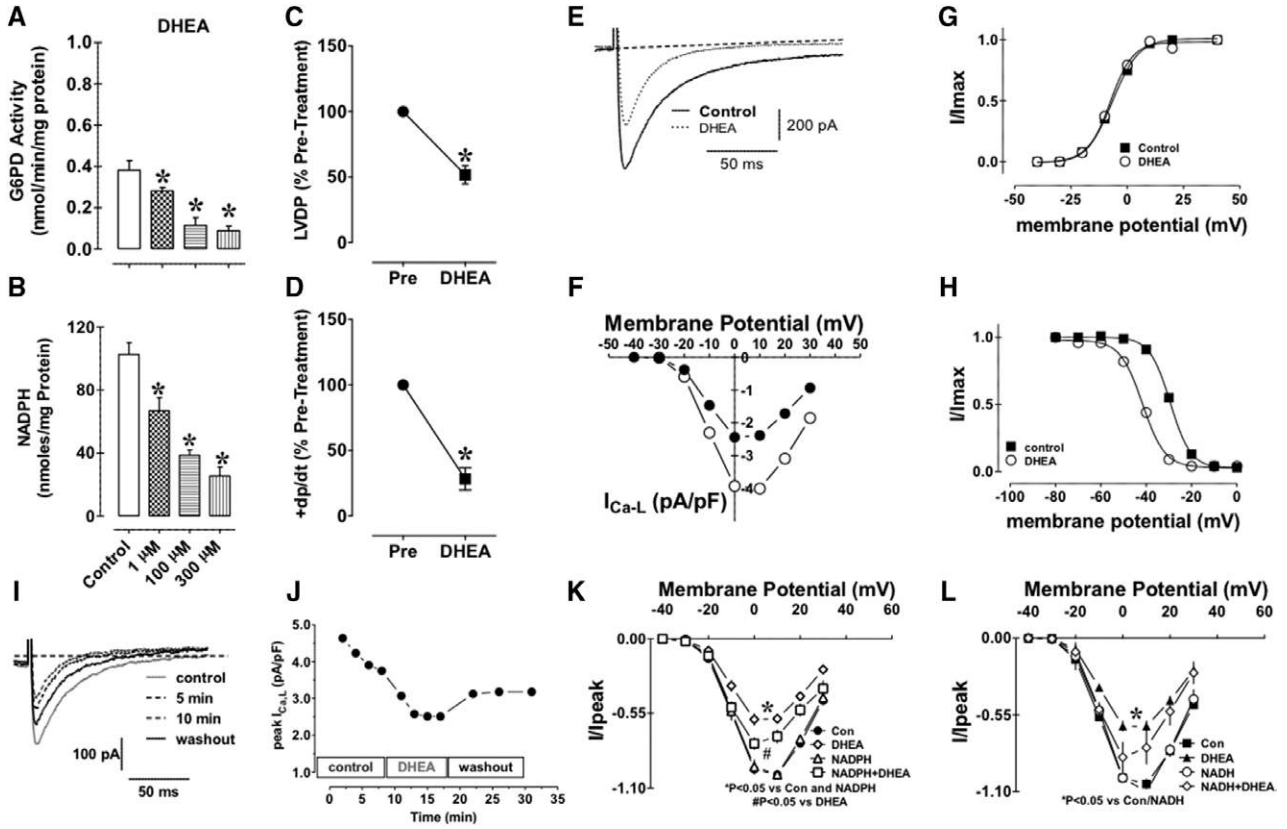

Figure 6. Perfusion of isolated rat hearts with dehydroepiandrosterone (DHEA) dose dependently decreased. **A**, Glucose-6-phosphate dehydrogenase (G6PD) activity and (**B**) nicotinamide adenine dinucleotide phosphate (NADPH) levels. DHEA (100 μmol/L) attenuated: (**C**) left ventricular developed pressure (LVDP) and (**D**) dp/dt_{max}. **E** and **F**, DHEA (100 μmol/L) decreased cardiac myocyte L-type Ca2+ currents (I_{CaL}). **G** and **H**, It shifted the steady-state inactivation curve to the negative potentials and did not affect the steady-state activation. **I** and **J**, DHEA-induced inhibition of I_{CaL} was partially reversible on wash out. **K** and **L**, Dialysis of NADPH and not NADH in the pipette solution reversed DHEA-induced suppression of I_{CaL}. n=5.

constriction[53] models. Consistently, we detected increased autophagy in the hypertrophic RV but not in the LV of PAH rats. Dehydroepiandrosterone, which decreased oxidative stress and increased energy substrates, suppressed increased expression of LC3A/B-II—a marker of autophagy—in the RV of PAH rats. Therefore, we suggest that autophagy, possibly induced by NADPH-dependent increase in oxidative stress, played a role in the pathogenesis of RVH induced by PAH.

Maladaptive RVH is a hallmark of PAH. Although increased afterload, secondary to pulmonary hypertension, is one of the main causes of RVH, elevated neurohumoral factors also contribute to the pathogenesis of heart failure.[39] In patients with markedly increased RV pressures, LV function is often compromised.[54] Increased asymmetrical RVH and septal wall thickening compress the LV cavity and cause diastolic heart failure in patients with PAH.[55] Along these lines, we found that PAH rats were in left heart diastolic failure as indicated by preserved LV ejection fraction, increased dp/dt_{min} and isovolumic relaxation time, and elevated filling pressures. Diastolic dysfunction and heart failure are caused by 1) structural changes, 2) inefficient Ca2+ sequestration by sarcoplasmic reticulum in diastole, 3) increased fibrosis, and 4) elevated filing pressures because of decreased lusitrophy. Interestingly, dehydroepiandrosterone treatment reversed diastolic dysfunction and increased stroke work. In this regard, the human heart synthesizes dehydroepiandrosterone, and dehydroepiandrosterone secretion is reduced in failing human hearts, which leads to the speculation that dehydroepiandrosterone protects the heart by exerting antihypertrophic effects on myocytes.[21] We attribute the cardioprotective action of dehydroepiandrosterone to its ability to reduce myocardial G6PD-derived NADPH and decrease myocardial NADPH-dependent prohypertrophic signals, such as ROS and I_{CaL}, which decrease Ca2+ entry and left ventricular developed pressure/contractility. Elevated ROS and mitochondrial dysfunction increase intracellular Ca2+ and Ca2+-dependent signaling that induces hypertrophy and failure. For example, nuclear factor of activated T-cells, cytoplasmic 3, is activated by increased Ca2+ influx through L-type Ca2+ channel, and nuclear factor of activated T-cells, cytoplasmic 3–dependent signaling pathway mediates pressure-overload–induced LV failure.[22] Dehydroepiandrosterone treatment inhibits nuclear factor of activated T-cells, cytoplasmic 3 activation and reduces RVH in PAH rats.[38] In addition, dehydroepiandrosterone blocks aldosterone-induced neonatal rat cardiac myocyte hypertrophy by reducing T-type Ca2+ channel expression and function.[56] Furthermore, dehydroepiandrosterone, which antagonizes endothelin-1–induced vasoconstriction and relaxes systemic arteries,[57] may have indirectly reduced diastolic failure by normalizing the increased elastance/stiffening in PAH rats. Arterial stiffness is increased because of the elevation of circulating vasoconstrictors, such as endothelin-1 or serotonin, which rise in PAH. Because G6PD inhibition relaxes pulmonary arteries[58,59] and dehydroepiandrosterone treatment reduces the severity of pulmonary hypertension in PAH

rats,[38] we suggest that dehydroepiandrosterone also reduced maladaptive RVH by decreasing pulmonary arterial pressure-induced afterload. Although contractility that reduced acutely by dehydroepiandrosterone could potentially be detrimental in heart failure, our results suggest a long-term treatment with dehydroepiandrosterone, which reduces hypertrophy and compensates the diminished contractility that improves filling and protects the heart from failing severely in PAH. Therefore, G6PD-derived NADPH signaling, which is reduced by dehydroepiandrosterone, seems to be an important regulator of the triggers of maladaptive hypertrophy, such as ROS and I_{CaL}, in the RV and in the LV of PAH rats.

Perspective

Overall, our novel findings demonstrate that 1) ROS production from extramitochondrial and mitochondrial sources are increased in the remodeled RV but not in the LV of PAH rats, 2) dehydroepiandrosterone treatment of PAH rats reduces G6PD activity and NADPH-dependent L-type Ca2+ currents, and 3) dehydroepiandrosterone treatment improves diastolic function in hearts of PAH rats. Therefore, we propose that dehydroepiandrosterone improves function and attenuates hypertrophy[38] by improving metabolism and decreasing ROS production to prevent excess autophagy and by reducing L-type Ca2+ channel function to reduce prohypertrophic transcription factor signaling in the RV of PAH rats. Thus, dehydroepiandrosterone or congeners that suppress NADPH (pyridine nucleotide) signaling may be a beneficial therapy to reduce diastolic heart failure in the PAH syndrome.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Pyridine nucleotide and reactive oxygen species signaling plays a critical role in pulmonary arterial hypertension–induced arterial stiffness and diastolic heart failure.
- Glucose-6-phosphate dehydrogenase inhibitor DHEA treatment decreased pulmonary arterial hypertension–induced arterial stiffness and diastolic heart failure.
- Glucose-6-phosphate dehydrogenase inhibitor DHEA reduced NADPH and reactive oxygen species production in right but not in left heart.
- Glucose-6-phosphate dehydrogenase inhibitor dehydroepiandrosterone improved glucose oxidation and metabolism and prevented autophagy in right but not in left heart.

What Is Relevant?

- Heart failure is a hallmark of pulmonary arterial hypertension.
- Rat model of pulmonary arterial hypertension is used.
- Pulmonary arteries undergo severe occlusive remodeling that produces right heart hypertrophy, arterial stiffness, and left heart diastolic failure.

Summary

Inhibition of glucose-6-phosphate dehydrogenase suppressed pyridine nucleotide-dependent signaling and reduced myocardial reactive oxygen species and autophagy, improved myocardial glucose metabolism, decreased arterial stiffness, and ameliorated left ventricular diastolic failure.