Dehydroepiandrosterone restores right ventricular structure and function in rats with severe pulmonary arterial hypertension

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Alzoubi A, Toba M, Abe K, O’Neill KD, Rocic P, Fagan KA, McMurtry IF, Oka M. Dehydroepiandrosterone restores right ventricular structure and function in rats with severe pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol 304: H1708–H1718, 2013. First published April 12, 2013; doi:10.1152/ajpheart.00746.2012.—Current therapy of pulmonary arterial hypertension (PAH) is inadequate. Dehydroepiandrosterone (DHEA) effectively treats experimental pulmonary hypertension in chronically hypoxic and monocrotaline-injected rats. Contrary to these animal models, SU5416/hypoxia/normoxia-exposed rats develop a more severe form of occlusive pulmonary arteriopathy and right ventricular (RV) dysfunction that is indistinguishable from the human disorder. Thus, we tested the effects of DHEA treatment on PAH and RV structure and function in this model. Chronic (5 wk) DHEA treatment significantly, but moderately, reduced the severely elevated RV systolic pressure. In contrast, it restored the impaired cardiac index to normal levels, resulting in an improved cardiac function, as assessed by echocardiography. Moreover, DHEA treatment inhibited RV capillary rarefaction, apoptosis, fibrosis, and oxidative stress. The steroid decreased NADPH levels in the RV. As a result, the reduced reactive oxygen species production in the RV of these rats was reversed by NADPH supplementation. Mechanistically, DHEA reduced the expression and activity of Rho kinases in the RV, which was associated with the inhibition of cardiac remodeling-related transcription factors STAT3 and NFATc3. These results show that DHEA treatment slowed the progression of severe PAH in SU5416/hypoxia/normoxia-exposed rats and protected the RV against apoptosis and fibrosis, thus preserving its contractile function. The antioxidant activity of DHEA, by depleting NADPH, plays a central role in these cardioprotective effects.

dehydroepiandrosterone; oxidative stress; pulmonary arterial hypertension; right ventricle; SU5416; Rho kinase

PULMONARY ARTERIAL HYPERTENSION (PAH) refers to a group of diseases with severe pulmonary hypertension and concentric laminar and plexiform occlusive lesions of the small pulmonary arteries and arterioles, i.e., plexogenic arteriopathy (45). If left untreated, PAH follows a progressive, refractory, and fatal course due to the development of right ventricular (RV) failure (34). The persistently high mortality rates in treated patients highlight the inadequacy of the current treatments of PAH (32). There is now general agreement that the complex pathology of PAH reflects its multifactorial nature, which means several signaling pathways are aberrant in the process (9). This accounts for the recent propensity toward the use of combination therapies for PAH (33). In addition, a recent report suggests that RV dysfunction in PAH might not be attributable solely to the pressure overload caused by increased pulmonary vascular resistance (6). Therefore, an ideal therapeutic strategy for PAH should be directed simultaneously against the multiple dysregulated signaling pathways in both pulmonary hypertension and RV dysfunction.

Dehydroepiandrosterone (DHEA) is a naturally occurring, cholesterol-derived steroid hormone secreted mainly from the adrenal cortex and serves in humans as a precursor for both estrogens and androgens (27). DHEA has been solidly linked to a wide variety of beneficial biological and physiological actions (53). Recent reports have shown that chronic DHEA treatment ameliorates experimental pulmonary hypertension in rats and mice via multiple mechanisms, including vasodilatation (30) and antiproliferative effects (31). However, the animal models utilized in these studies [chronically hypoxic (7, 21, 38) and monocrotaline-injected rats (23, 40)] do not show the full spectrum of hemodynamic and histopathological features of human PAH, including RV failure (18). In contrast, rats injected with the vascular endothelial growth factor receptor blocker Sugen(SU)5416 and then exposed to hypoxia and normoxia develop severe PAH that recapitulates the major characteristics of the human disease, i.e., plexogenic arteriopathy, chronic deterioration, RV dysfunction, and unresponsiveness to treatments (1, 47, 48). Additionally, direct beneficial effects of DHEA on dysfunctional RV in the context of severe PAH have not previously been evaluated.

It has been reported that several RV-damaging factors act in concert to cause RV decompensation and maladaptive remodeling in PAH patients. These include hemodynamic overload, neurohormonal activation, and RV inflammation and oxidative stress (5). Given the well-documented antioxidant effects of DHEA (24), we hypothesized that DHEA would restore RV structure and function in SU5416/hypoxia/normoxia (SU/Hx/Nx) rats with established severe PAH through the combined effects of a reduction in pressure overload and direct inhibition of RV oxidative stress.

METHODS

Animals. All experimental procedures were conducted in accordance with the Animal Welfare Act and approved by the Institutional Animal Care and Use Committee of the University of South Alabama. Adult male Sprague-Dawley rats (weighing 150–200 g) were assigned to three groups as follows (Fig. 1): group 1, normal controls; group 2, rats receiving a single subcutaneous injection (20 mg/kg) of SU5416 (Cayman Chemical) on day 1 and then being exposed to 3 wk of normobaric hypoxia (10% O2), followed by reexposure to normoxia (21% O2) for 5 additional wk (SU/Hx/Nx); and group 3, rats having the same characteristics as in group 2, except for receiving 1% DHEA-containing food (Teklad Custom Research Diet) from weeks 3 to 8 for a total of 5 wk (+DHEA). The dose of DHEA was based on previous studies (23).

Echocardiography. Echocardiography was performed using the Vevo770 imaging system (VisualSonics, Toronto, ON, Canada) at...
baseline and at the end of week 7 to evaluate the progression of RV function in all groups. Rats were anesthetized but kept breathing spontaneously using 2.0% isoflurane in a 1:1 O2-air mixture. Temperature, heart, and respiratory rates were monitored continuously. Two-dimensional, M-mode, and Doppler flow imaging were obtained with a 30-MHz probe. Measurements of the RV inner diameter in diastole, tricuspid annular plane systolic excursion (TAPSE), and pulmonary artery acceleration time were acquired as reported previously (43).

**Hemodynamic measurements in catheterized rats.** All rats were catheterized at the end of the 8th wk for hemodynamic measurements, as described previously (39). Rats were anesthetized with pentobarbital sodium (30 mg/kg ip). Polyvinyl catheter (PV-1, internal diameter: 0.28 mm) was inserted into the right jugular vein for measurement of RV systolic pressure (RVSP). Because of the severe RV hypertrophy and apparent change in the shape of the RV chamber in SU/Hx/Nx rats, we could not routinely catheterize the pulmonary artery, and therefore, we measured RVSP as a surrogate. In some cases, when the artery was catheterized, systolic pulmonary arterial pressure and RVSP were equal. A microtip P-V catheter (SPR-838; Millar Instruments) was inserted into the right carotid artery and advanced into the left ventricle. Heart rate, systolic arterial pressure, and cardiac output were measured. Cardiac index (CI) was calculated by dividing cardiac output by body weight (1). Total pulmonary resistance index (TPRI) was estimated by dividing RVSP by CI (38). Left ventricular (LV) end-diastolic pressure was not elevated across the three groups (2–5 mmHg).

**Dihydroethidium staining.** A qualitative evaluation of the in vivo superoxide (O2) production in the RV was done by dihydroethidium (DHE) staining, as described previously (42). Rats were injected intravenously with DHE (60 µg/kg) at the end of the catheterization procedure 5 min prior to euthanization. Hearts were then removed, dissected, weighted, frozen in optimal cutting temperature formula (Electron Microscopy Sciences) on dry ice, and stored at −70°C until sectioning. Five-micrometer sections were made in a cryostat and mounted on glass slides. DHE fluorescence was detected with excitation/emission at 518/605 nm using a Nikon Eclipse TE2000-U microscope. Data were analyzed using Metamorph 7.1.2.0 software (Molecular Devices).

Lucigenin chemiluminescence assay. Whole RV homogenates (20 µl) were added to microplate wells containing 5-µM lucigenin for the detection of superoxide, as reported previously (51). Lucigenin chemiluminescence was measured in a liquid scintillation counter (Beckman Instruments) at 37°C. The measurements were repeated in the presence of NADPH (50 µmol/l). Data are presented as counts per milligram of protein after background subtraction.

**Measurement of RV NADPH levels.** A NADP+/NADPH quantification kit (BioVision) was used for the measurement of tissue NADPH levels according to the manufacturer’s recommendations. Briefly, RV tissue homogenates were heated to 60°C for 30 min to decompose NADP content while keeping NADPH intact. Samples and NADPH standards (50 µl each, in duplicates) were incubated with 100 µl of NADP cycling mix to convert NADP to NADPH. Colorimetric reactions were started by adding 10 µl of NADPH developer, and absorbance was then measured at an occlusion density of 450 nm at 0, 15, 30, 45, and 60 min, and reactions were subsequently stopped by adding 10 µl of stop solution to each well. Data are presented as NADPH level (pmol) per milligram of protein.

**Morphometric analysis of pulmonary vascular remodeling.** Rat lungs from the three experimental groups were fixed for histology by tracheal instillation of a mixture of 1% formalin and 0.5% agarose under constant pressure (20 cm H2O). The trachea was ligated after sustained inflation, and the lungs were excised and immersed in 10% formalin for 48 h. Formalin-fixed lung tissue was cut into 5-mm-thick sections, placed in 70% ethanol, and embedded in paraffin. Paraffin sections (5 µm thick) were then serially mounted onto Superfrost Plus slides (Fisher Scientific) and stained with Verhoeff-van Gieson stain and Masson’s trichrome stain to assess the degree of fibrosis (collagen fibers stain blue) within the heart tissue or left unstained for immunohistochemistry. We assessed the vascular occlusion density by grading the small (<50 µm) pulmonary arteries, as described by Faul et al. (15) in Verhoeff-van Gieson-stained sections: grade 0 (no occlusion), grade 1 (<50% occlusion), and grade 2 (>50% occlusion). Medial wall thickening of medium-sized (50–200 µm) pulmonary arteries was calculated in α-smooth muscle actin-stained slides (primary antibody; Abcam) as a percentage of the average thickness of the medial wall to the total outer diameter of the vessel in at least 50 random but consecutive vessels per slide.

**Histological studies.** Formalin-fixed and paraffin-embedded heart tissue was stained with Masson’s trichrome stain to assess the degree of fibrosis (collagen fibers stain blue) within the heart tissue or left unstained for immunohistochemistry. Images were acquired using a Nikon E600 light microscope with digital interface and computer imaging software. Immunohistochemical staining of paraffin-embedded slides was performed using Vectastain Universal Quick Kit (Vector Laboratories) according to the manufacturer’s recommendations. Briefly, slides were heated at 60°C and then sequentially washed in xylene and gradual dilutions of ethanol for deparaffinization. Antigen retrieval was achieved by incubating the slides in heated (100°C) citrate buffer (1X, pH = 6, Dako). Endogenous peroxidase activity was then suppressed by 3% hydrogen peroxide treatment. Slides were incubated with a blocking buffer (1% universal horse serum) for 1 h at room temperature and then with the primary antibody overnight at 4°C. Incubations with a biotinylated universal secondary antibody (Dako) and then avidin-biotin complex (Dako), each for 30 min at room temperature, then followed. Detection of positive staining was done using 3,3'-diaminobenzidine substrate chromogen system (Dako), and tissues were then counterstained with hematoxylin and finally washed with xylene and ethanol. Primary antibodies used were against nitrotyrosine (Millipore), cleaved caspase 3 (Cell Signaling Technology), and CD31 (Abcam). Capillary density was expressed as CD31-positive spots per RV cross-sectional area. Capillary density was expressed as CD31-positive spots per RV cross-sectional area. Cardiomyocyte cross-sectional area was measured in ≥200 transversally cut cardiomyocytes per hematoxylin and eosin-stained sample. TUNEL assay (ROCHE) was used to detect apoptosis in the heart tissue.

**Western blotting.** Snap-frozen whole RV tissue was homogenized, and protein concentration in the supernatant was determined using the BCA protein assay (Thermo Scientific), as described previously (49). Standard methods of Western blotting were used to evaluate the expression levels of collagen I (Santa Cruz Biotechnology), cleaved
caspase 3 (Santa Cruz Biotechnology), Rho kinase I/II (BD Pharmingen), Rho kinase II/III (BD Pharmingen), myosin phosphatase-targeting subunit 1 (MYPT-1; Millipore), p-MYPT-1 (Millipore), p-STAT3 (Cell Signaling), NFATc3 (Santa Cruz Biotechnology), gp91phox (Santa Cruz Biotechnology), and β-actin (Santa Cruz Biotechnology).

Statistical analyses. Values shown are means ± SE. Analysis of variance with Bonferroni post hoc test was used for comparisons among the experimental groups. For correlations, Pearson calculations with two-tailed P value were done first, and a linear regression analysis was then used to create a best-fit line. Differences were considered significant at P < 0.05.

RESULTS

DHEA has more profound effects on cardiac output than on pulmonary arterial pressure. Consistent with previous studies (1), SU/Hx/Nx rats showed marked increases in RVSP (Fig. 2A), TPRI (Fig. 2C), and the RV hypertrophy index: RV/left ventricular (LV) + septum weight ratio (Fig. 2D) and a significant attenuation of CI (Fig. 2B). Chronic DHEA treatment restored the normal levels of CI while only moderately reducing RVSP. The beneficial effects of DHEA on cardiac output were derived from an improving RV rather than LV function, as systolic systemic arterial pressure (Fig. 2E) and heart rate (Fig. 2F) were not significantly different among the three groups. Furthermore, DHEA treatment regressed pulmonary vascular remodeling, as it reduced both the medial wall thickening of medium-sized pulmonary arteries (Fig. 2G) and, to an even greater extent, the luminal occlusion of small pulmonary arteries (Fig. 2H).

DHEA reverses PAH-induced RV dysfunction. We utilized non-invasive echocardiography to more directly address the question of whether DHEA treatment improved RV function. DHEA sig-
significantly reduced RV internal diameter during diastole (Fig. 3, A and D) and restituted the normal position and orientation of the interventricular septum, which had been otherwise flattened by the hypertrophied and/or dilated RV in the SU/Hx/Nx group. Furthermore, DHEA restored the impaired TAPSE (Fig. 3, B and E). TAPSE is widely regarded as an excellent gauge of RV ejection fraction and systolic function (16), as evidenced by the significant correlation with CI (Fig. 3F). The high pulmonary arterial pressure in the SU/Hx/Nx group was also reflected in a shorter pulmonary artery acceleration time (Fig. 3, C and G), with the appearance of systolic notching in the Doppler flow waves, compared with the normal-looking parabolic waves found in control animals. Such notching is understood to reflect systolic deceleration of flow across the pulmonary valve, indicating high pulmonary vascular resistance (50). Moreover, Arkles et al. (2) have reported that the shape and duration of RV outflow tract Doppler waves predict prognosis of PAH. Midsystolic notching, as can be seen in the SU/Hx/Nx group, corresponds to a more severe RV dysfunction, contrary to the late-systolic notching found in the DHEA-treated rats.

**DHEA protects the RV against structural maladaptive remodeling.** To evaluate the effects of DHEA treatment on RV structure, we performed a morphometric analysis of RV fibrosis, cardiomyocyte cross-sectional area, and capillary density in the three groups of rats. The RV of SU/Hx/Nx rats developed a high degree of fibrosis, as indicated by the increased

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Fig. 3. Echocardiographic assessment of RV function. RV internal diameter during diastole (RVID-Dia; A and D), tricuspid annular plane systolic excursion (TAPSE; B and E), and pulmonary artery acceleration time (PA-AT; C and G). F: a correlation analysis between TAPSE and CI in the 3 groups. Dotted red lines in A highlight the interventricular septum. White arrows in C point to systolic notching in Doppler flow waves. Data are expressed as means ± SE. *P < 0.05 vs. normal controls; +P < 0.05 vs. SU/Hx/Nx. Normal (n = 4), SU/Hx/Nx (n = 6), and +DHEA (n = 6).
collagen-specific blue color in Masson’s trichrome-stained tissue (Fig. 4A) and the increased collagen 1A expression (Fig. 4D). DHEA treatment markedly reduced the overall collagen content as well as collagen 1A expression levels in the RV. Importantly, left ventricles from the three experimental groups had minimal fibrotic areas (data not shown), indicating that the left ventricle was spared from the pulmonary hypertensive effects of SU/Hx/Nx, at least at the 8-wk time point. Moreover, the steroid treatment significantly reduced RV cardiomyocyte cross-sectional area that was otherwise enlarged in SU/Hx/Nx rats (Fig. 4, B and E). Additionally, and consistent with previous reports (6), chronic SU/Hx/Nx exposure resulted in the loss (rarefaction) of RV capillaries (Fig. 4, C and F). In contrast, DHEA treatment recovered the normal density of RV capillaries.

Because cardiac fibrosis is frequently coupled to cardiomyocyte death, we also examined the degree of RV apoptosis. RV from the SU/Hx/Nx group exhibited a significant increase in apoptosis rates, as evaluated by the TUNEL assay (Fig. 5, A and B), and active (cleaved) caspase 3 expression by immuno-histochemistry (Fig. 5C) and Western blotting (Fig. 5D). Interestingly, most apoptosis was observed in and around the coronary vessels, which might explain the observed capillary rarefaction in Fig. 4. Chronic DHEA treatment prevented RV apoptosis.

The antioxidant activity of DHEA plays a central role in its beneficial effects on RV structure and function. Since chronic pressure overload alone is not sufficient to induce RV fibrosis and apoptosis (6), it was important to explore other factors implicated in RV decompensation and maladaptive remodeling, such as RV oxidative stress. There was a substantial increase in DHE immunofluorescence in the RV of SU/Hx/Nx rats (Fig. 6A), indicating a marked increase in superoxide levels, which was completely blocked by DHEA treatment. We also evaluated the formation of nitrotyrosine in the RV, as it has been shown to be detrimental to cardiac structure and function by decreasing the bioavailability of nitric oxide, which in turn results in coronary vasoconstriction (25). As can be seen in Fig. 6B, nitrotyrosine signal intensity was increased in the RV of SU/Hx/Nx rats, most remarkably in the medial layer of coronary arteries. This was significantly attenuated...
ated by chronic DHEA treatment. To quantify these results, we used the lucigenin chemiluminescence assay (Fig. 6C). Consistent with previous reports showing a basal level of reactive oxygen species (ROS) production in normal heart tissue (28), the RV of untreated rats exhibited a baseline level of superoxide generation. Chemiluminescence was increased significantly in the RV of SU/Hx/Nx rats. In contrast, superoxide production was markedly reduced in the RV of DHEA-treated rats and even fell below baseline. Since NADPH oxidase is the main source of ROS in the heart (26), we repeated the measurements after adding NADPH, which is the preferred substrate for NADPH oxidase. Although normal and SU/Hx/Nx RV tissues had no visible increases in ROS production in the presence of exogenous NADPH, the basal high levels of ROS were recovered in the DHEA-treated RV following the addition of NADPH. Despite the increased ROS in the SU/Hx/Nx RV, the expression level of the enzyme NADPH oxidase was not different among the three experimental groups, as indicated by Western blotting of its integral subunit gp91phox (Fig. 6D). Interestingly, the high RV-tissue levels of NADPH in SU/Hx/Nx rats were markedly reduced by DHEA treatment (Fig. 6E).

The mechanism of cardioprotection by DHEA involves inhibition of Rho kinase and its downstream effectors. To further investigate the molecular mechanisms by which DHEA protects RV structure and function in the context of PAH, we studied its effects on the Rho kinase (ROCK) signaling pathway, because it is well documented that ROCK is hyperactivated in various cardiovascular diseases (11) and is involved in cardiac maladaptive structural remodeling (29). As can be seen in Fig. 7, DHEA treatment inhibited the expression levels and activity (phosphorylation of MYPT1) of ROCKI and ROCKII (Fig. 7, A–C), all of which were upregulated in the RV of SU/Hx/Nx rats. The inhibition of ROCK activity was accompanied by reductions in the
active dephosphorylated form of the nuclear factor of activated T cells/cytoplasmic 3 (NFATc3; Fig. 7D) and the active phosphorylated form of STAT3 (Fig. 7E).

**DISCUSSION**

We showed here that during 8 wk of SU/Hx/Nx exposure, rats developed very severe occlusive pulmonary hypertension. A 5-wk DHEA treatment (from weeks 3 to 8 after SU5416 injection) significantly reduced RVSP compared with the DHEA-untreated rats, although it was still much higher than that of normal rats. In contrast, DHEA restored cardiac index to normal levels, leading to a dramatic improvement of estimated total pulmonary resistance. The effect of DHEA on cardiac index is likely restoration rather than prevention/maintenance, because we have found previously that cardiac index in this model is already significantly impaired at the 2-wk time point (37). Moreover, DHEA treatment reduced the frequency and severity of occlusive neointimal lesions in small pulmonary arteries. These results demonstrated that chronic DHEA treatment significantly slowed the progression of severe occlusive PAH in SU/Hx/Nx rats and that, more impressively, it restored the impaired cardiac function to a nearly normal level. Literature alludes to several mechanisms involved in such protective effects of DHEA on the hypertensive pulmonary circulation, including improvement of endothelial function, by upregulation of endothelial nitric oxide synthase (30) and soluble guanylate cyclase (38), upregulation of potassium channel activity/expression (20), and inhibition of RhoA/Rho kinase (23) and Src/STAT3 (40) signaling pathways in pulmonary arteries.

Because the beneficial effect of DHEA on cardiac index was more profound than that on RVSP, and because a recent report...
suggests that certain factors other than pressure overload may be implicated in the progression into RV dysfunction in this rat model of PAH (6), we further examined cardiac function and structure. Functionally, DHEA improved the main echocardiographic signs of progressive RV dysfunction. TAPSE was of particular importance, because recent studies have confirmed its excellent predictive value for RV ejection fraction and systolic function (16), which we validated through the significant correlation between TAPSE and cardiac index. DHEA recovered the control levels of TAPSE, indicating a preservation of the contractile functional capacity of RV, which in turn explains the regression of signs of RV dilatation and impending failure in these animals.

Structurally, DHEA prevented the terminal features of maladaptive RV remodeling, i.e., RV capillary rarefaction, apoptosis, and fibrosis. It is hypothesized that in the early stages of PAH, the high pulmonary arterial pressure and pulmonary vascular resistance and stiffness are compensated by an adaptive “concentric” RV hypertrophy (22) to maintain normal levels of cardiac output. During the latter stages of the disease, however, the continued pressure overload, in concert with other RV-damaging factors, results in inhibition of angiogenesis and vascular cell apoptosis and rarefaction, which ultimately lead to reduction in number of cardiomyocytes (6). Given the low rates of cardiac cell regeneration (3), fibrosis is considered the main repair process in the heart. The resulting “eccentric” remodeling, where parts of the RV are dilated and others are hypertrophied, is a sign of impending RV failure. DHEA treatment recovered the normal density of coronary capillaries and inhibited both RV apoptosis and fibrosis, but in contrast, it only moderately reduced the degree of RV hypertrophy. Therefore, we propose that DHEA shifts the RV from

Fig. 7. Western blot analyses of Rho kinase (ROCK)I/β (A), ROCKII/α (B), phosphorylated myosin phosphatase-targeting subunit 1 (p-MYPT1)/total (T)-MYPT1 ratio (C), nuclear factor of activated T cells/cytosplasmic 3 (NFATc3; D), and p-STAT3 (E) in whole RV tissue homogenates. Western blot bands were rearranged as indicated by the black dividing lines. *P < 0.05 vs. normal controls; +P < 0.05 vs. SU/Hx/Nx. Normal (n = 4), SU/Hx/Nx (n = 5), and +DHEA (n = 5).
the stage of partially compensated eccentric remodeling seen in SU/Hx/Nx rats into a fully compensated concentric RV hypertrophy phenotype.

Although the precise mechanisms of the progression into decompensated RV failure in PAH are not fully understood, there is convincing evidence that ROS play a key role in driving RV apoptosis and fibrosis (8). RV oxidative stress could potentially result from several factors in PAH, including sustained neurohumoral activation, inflammation, and chronic pressure overload. Redout et al. (41) and Bogaard et al. (6) have reported that antioxidant treatment protects monocrotaline-injected and SU/Hx/Nx-exposed rats, respectively, against RV fibrosis and failure. Our results show that DHEA treatment significantly inhibited the degree of RV oxidative stress, most notably in and around the coronary arteries, where most apoptosis and fibrosis can be seen. This finding corresponds to recent reports showing aberrant morphology, density, and structure of the RV microcirculation in SU/Hx/Nx rats with RV dysfunction (6).

Potential sources of ROS in the heart are NADPH oxidase, xanthine oxidase, and cytochrome P450 (17). The low constitutive activity of the nonphagocytic NADPH oxidase can be upregulated in response to several pathological stimuli, including cytokines, mechanical stress, and neurohormones (44). In turn, the resulting oxidative stress drives cardiac hypertrophy, apoptosis, and fibrosis. In this context, Gupta et al. (19) have reported that glucose-6-phosphate dehydrogenase-derived NADPH is the principal fuel for the production of superoxide by NADPH oxidase in the failing left heart. DHEA is documented to be a potent inhibitor of glucose-6-phosphate dehydrogenase (20), and thus it regulates the redox state of the cell by reducing the availability of NADPH. Collectively, our current findings indicate that DHEA treatment suppressed NADPH production and consequently inhibited oxidative stress in the RV of PAH rats.

Mechanistically, we found that DHEA inhibited the expression and activity of ROCK in the RV. There is substantial evidence that the ROCK signaling pathway is activated in various cardiovascular diseases, among which are PAH (35) and heart failure (10). Previously, we have shown that DHEA treatment of left-pneumonectomized/monocrotaline-injected rats inhibited activation of lung RhoA/Rho kinase signaling, arrested progression of PAH, increased cardiac output, and strikingly, increased survival of the rats (23). Moreover, Takeshima et al. (46) have recently reported a cardioprotective effect of ROCK inhibitors in rats with systemic hypertension and left ventricular failure. We propose that the antioxidant activity of DHEA on the RV is central to its inhibitory effect on ROCK signaling pathway, which in turn could mediate the suppression of several downstream effectors, including the cardiac remodeling-related transcription factors STAT3 (36) and NFATc3 (52), in addition to the activity of the integral apoptosis regulator caspase-3 (54).

There are other possible mechanisms of direct beneficial effects of DHEA on cardiac function. For instance, recent reports point to a possible role for the DHEA-specific, G protein-coupled sigma 1 receptors in cardioprotection through regulation of various cardiac ion channels (4). However, the definitive involvement of this axis in the protection against RV remodeling and dysfunction is unclear. The likely peripheral conversion of DHEA into estrogens and androgens could also contribute to the observed cardioprotection in pulmonary hypertensive rats. However, the potential beneficial effects of sex hormones, particularly estrogen, on PAH are still controversial.

The dose of DHEA used in this study is very high. However, effective doses in humans might be much lower because of their very high basal DHEA levels compared with those in rodents. DHEA has not been tested as a therapeutic option in human PAH, and therefore, optimal dosing is not known. However, a recent preliminary report of an ongoing clinical trial in chronic obstructive lung disease-associated pulmonary hypertension patients revealed that chronic DHEA treatment (200 mg/day for 12 wk) improved their exercise tolerance and hemodynamic parameters (12).

Although our results support the hypothesis that DHEA exerts a direct beneficial effect on RV structure and function independent of the decrease in pressure overload, we will need to address this issue more definitively in our future work. For example, it may be helpful to use the exclusive RV pressure-overload animal model, i.e., the pulmonary artery-banding model, although this model provides variable results on RV dysfunction and its mechanisms, depending on the severity and duration of banding (13, 14).

CONCLUSION

The results of this project provide a foundation for an effective and safe oral therapeutic approach for severe PAH. Our findings suggest that DHEA has anti-pulmonary hypertensive as well as potent direct RV protective effects both structurally and functionally. Besides being novel, these findings are highly significant given the fact that RV failure is the main cause of morbidity and mortality in PAH patients and that current treatments are expensive and minimally effective.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.A. participated in the design of the study and the data interpretation, performed the experiments and the statistical analyses, and wrote the manuscript; M.T. and K.A. carried the in vivo hemodynamic experiments and participated in data interpretation. K.D.O., P.R., and K.A.F. participated in data interpretation; I.F.M. participated in the overall design of the study and data interpretation and helped to draft the manuscript; M.O. supervised the overall study design and coordination.

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