GRK2-Mediated Inhibition of Adrenergic and Dopaminergic Signaling in Right Ventricular Hypertrophy

Therapeutic Implications in Pulmonary Hypertension

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Background—The cause and consequences of impaired adrenergic signaling in right ventricular failure/hypertrophy (RVH) are poorly understood. We hypothesized that G protein–coupled receptor kinase-2 (GRK2)–mediated uncoupling of β-adrenergic receptor signaling impairs inotropic reserve. The implications of right ventricular (RV) adrenergic remodeling for inotrope selection and the therapeutic benefit of interrupting Gβγ–GRK2 interaction, using gallein, were tested.

Methods and Results— Chamber-specificity and cellular localization of adrenergic remodeling were compared in rodent RVH associated with pulmonary arterial hypertension (PAH-RVH; SU5416+chronic-hypoxia or Monocrotaline) versus pulmonary artery banding–induced RVH (PAB-RVH). Results were corroborated in RV arrays from 10 PAH patients versus controls. Inotropic reserve was assessed in RV- and left ventricular–Langendorff models and in vivo. Gallein therapy (1.8 mg/kg/day ×2-weeks) was assessed. Despite similar RVH, cardiac output (58.3±4.9 versus 82.9±4.8 mL/min; P<0.001) and treadmill distance (41.5±11.6 versus 244.1±12.4 m; P<0.001) were lower in PAH-RVH versus PAB-RVH. In PAH-RVH versus PAB-RVH there was greater downregulation of β1-, α1- and dopamine-1 receptors, more left ventricular involvement, and greater impairment of RV contractile reserve. RV GRK2 activity increased in parallel with a reduction in both adrenergic receptor expression and inotrope-stimulated cAMP levels (P<0.01). β1-receptor downregulation also occurred in human PAH-RVH. Dobutamine was superior to dopamine as an RV inotrope, both ex vivo and in vivo.

Conclusions—GRK2-mediated desensitization-downregulation of adrenergic and dopaminergic receptors impairs inotropic reserve in PAH-RVH. Acute inotropic support in RVH is best accomplished by dobutamine, reflecting its better coupling to adenylyl cyclase and the reliance of dopamine on dopamine-1–receptor signaling, which is impaired in RVH. Inhibiting Gβγ–GRK2 interactions has therapeutic benefit in RVH. (Circulation. 2012;126:2859-2869.)

Key Words: SU5416 ■ dopamine receptors ■ hypertension, pulmonary ■ right ventricular hypertrophy ■ β1-adrenoreceptor ■ β-adrenergic receptor kinase

Right ventricular hypertrophy (RVH) occurs in response to pressure overload in congenital heart diseases, such as pulmonic stenosis, and in pulmonary arterial hypertension (PAH). Severe and prolonged right ventricular (RV) pressure overload often results in RV failure (RVF), which is a leading cause of death in PAH1 and congenital heart disease.2 We hypothesized that reduced contractile reserve in RVH, particularly when associated with PAH (PAH-RVH), reflects a broad downregulation and desensitization of adrenoreceptors and dopamine receptors by G protein–coupled receptor kinase-2 (GRK2). Using rodent models of RVH and human ventricular tissue from PAH patients with RVF, we explored the molecular basis for impaired RV adrenergic signaling. In addition, we assessed whether changes in adrenergic signaling involved the left ventricle (LV). Throughout the study, we compared findings in RVH induced by pulmonary artery banding (PAB-RVH) with those in PAH-RVH. This comparison was chosen because PAB-RVH is well tolerated, suggesting it is adaptive, whereas PAH-RVH is poorly tolerated with a greater predisposition to RVF and premature death, suggesting it is maladaptive. Likewise, a human equivalent of PAB-RVH, as occurs in pulmonic stenosis, is better tolerated...
than the RVH associated with PAH. Because there is little scientific guidance for the selection of an optimal inotrope to support the failing RV in PAH, we also evaluated the consequences of adrenergic remodeling in RVH for the choice of inotropic agent. Finally, we tested a new therapeutic strategy to improve adrenergic signaling and RV function in experimental RVH, namely interruption of Gβγ–GRK2 signaling using a small molecule inhibitor, gallein.

The finding that CO and exercise capacity can be increased by targeting GRK1–Gβγ interaction may be clinically relevant. Key results in the rodents were confirmed in human tissues.

Methods and Materials
All authors have read and agreed to the manuscript as written.

Experimental Protocols
The University of Chicago Institutional Animal Care and Use Committee approved all protocols. Three RVH models were created in adult male Sprague-Dawley rats: (1) PAB-RVH, (2) PAH-RVH, induced by SU5416+chronic hypoxia (CH+SU) or Monocrotaline (MCT). End points were studied after 4 weeks in each model (n=8–13). The infusion of inotropes was primarily performed in additional cohorts of Control and MCT (n=7–12/group), although results were confirmed in CH+SU (n=5) and PAB (n=2). In additional cohorts, gallein (1.8 mg/kg/day, Tocris Bioscience, Ellieville, MO) was injected intraperitoneally for 2 weeks, beginning 2 weeks after Monocrotaline injection or PAB surgery (n=6–9).

Experimental Models
The PAB model has been described previously12 (see online-only Data Supplement). In CH+SU model, rats (260–280 g) were injected with the VEGF receptor antagonist SU5416 (20 mg/kg, subcutaneously) and then transferred to hypoxic cages (~10% oxygen, Biospheres, LA, NY) for 4 weeks. In the MCT model, rats (260–280 g) were injected with monocrotaline (60 mg/kg, subcutaneously; Sigma, St. Louis, MO).

Treadmill Distance
Exercise capacity was tested by measuring maximal distance run on a motorized treadmill, as described12 (see online-only Data Supplement).

Echocardiography
A Vevo 2100 (Visual Sonics, Ontario, Canada) was used to assess CO, stroke volume (SV), and RV function, as described13 (see online-only Data Supplement).

Right Ventricular Hypertrophy
RVH was measured postmortem as the ratio of RV/(LV+septum) weight.

RV and LV Langendorff Models
The Langendorff models were performed as previously described12 (see online-only Data Supplement).

Thermodilution Cardiac Output
Thermodilution CO was measured as previously described13 (see online-only Data Supplement).

Right Heart Catheterization With Infusion of Dopamine and Dobutamine
Rats were anesthetized (3% isoflurane with 95% oxygen), intubated, and placed on a heated surgical table (37°C). A 1.9F pressure–volume catheter (Sciensense Inc, London, Ontario, Canada) was inserted into RV via the right jugular vein to monitor the RV systolic pressure (RVSP) and volume. After stabilization, a pressure–volume signal was continuously recorded at sampling rate of 1000/s using an MPVS-300 (ADInstruments; Colorado Springs, CO) coupled to a PowerLab8/30 converter (ADInstruments). Dopamine or dobutamine was infused via the left jugular vein in 1 mL over 5-minute at clinically relevant doses14 (11 and 22 μg/kg/min), using a syringe pump (Cole-Parmer, Vernon Hills, IL). Heart rate (HR), RVSP, CO, ejection fraction, and SV were computed using pressure–volume analysis software (Labchart7.2; ADInstruments).

In initial experiments, we defined the molecular basis for impaired adrenergic and dopaminergic signaling in RVH and profiled receptor expression in both ventricles. The functional significance of adrenergic and dopaminergic receptor down-regulation was assessed using RV and LV Langendorff preparations. Next, we compared inotropes that are commonly used in clinical practice to determine which is optimal for acute support of the hypertrophied RV. These experiments were conducted ex vivo (using RV- and LV-Langendorff preparations) and in vivo. Subsequently, we assessed the role of GRK2 in mediating adrenergic receptor downregulation. In left ventricular failure (LVF), circulating catecholamine levels are elevated and excess Gβγ signaling recruits cytosolic GRK2 to agonist-stimulated β-adrenoceptors (β-AR), promoting receptor downregulation and desensitization.3 GRK2 also regulates the expression and function of α-adrenoceptors and dopamine-1A receptors.4,5 Likewise, in LV hypertrophy, interaction between GRK2 and Gβγ desensitizes β-ARs and, together with Gq-coupled receptor signaling, contributes to the pathophysiology.7 Although much is known about the adrenergic system in LVF/LV hypertrophy, a recent National Heart, Lung, and Blood Institute position paper emphasized therapeutic value of interrupting the interaction between Gβγ–GRK2 signaling using a small molecule inhibitor, gallein.

We discovered that GRK2-mediated adrenergic remodeling, notably downregulation and desensitization of the β1-AR, impairs inotropic reserve. In adaptive RVH models the adrenergic remodeling is largely confined to the RV; however, in PAH-RVH the abnormalities affect the LV. In all models dobutamine is a superior inotrope versus dopamine.
RV tissue was embedded in OCT compound (Sakura Finetek, Torrance, CA) and stored at −80°C. RV sections 7 μm thick were stained with the Arcturus HistoGene frozen-section staining kit (Applied Biosystems, Carlsbad, CA). RV myocytes and intramyocardial RV coronary arteries were harvested from fresh cryosections using the Palm MicroBeam system (Carl Zeiss, Thornwood, NY), and mRNA was isolated immediately. Each sample consisted of 20 to 30 RV myocytes or intramyocardial coronary arteries. Tissue localization of mRNA expression was guided visually and confirmed by enriched expression of tissue-specific reporters (α-myosin heavy chain for cardiomyocytes versus CD31 for vasculature).

qRT-PCR, Immunoblot, and Immunofluorescence

These techniques were performed as described previously (see online-only Data Supplement).12

Human Tissue Microarrays

Immunohistochemistry was performed on 1-mm RV cores from formalin-fixed, paraffin-embedded archival material of autopsied PAH patients (n = 10) or age-matched non-PAH patients (n = 8). Briefly, samples were arranged on a microscope slide, creating a tissue microarray (see text and Table I in the online-only Data Supplement for immunofluorescence technique and patient demographics). Consent was obtained for each autopsy, and the institutional review board was notified of the research performed on anonymized specimens from deceased individuals.

GRK2 Activity (Rhodopsin Phosphorylation) and cAMP levels (ELISA)

The heart was isolated from anesthetized rats, mounted in the Langendorff apparatus, and perfused with oxygenated Krebs–Henseleit buffer for 10 minutes. Hearts were then perfused for 2 minutes with vehicle or dobutamine or dopamine (10 μmol/L each). The RV was isolated and stored at −80°C for measurement of cAMP levels and GRK2 activity. These assays were performed as previously described13 (see online-only Data Supplement).

Statistics and Sample Size

Values were expressed as mean±SEM. Sample sizes are shown in each figure. Prism 5 (GraphPad Software, La Jolla, CA) was used for data analysis. Comparisons between groups used an ANOVA or unpaired Student t test, as appropriate. Post hoc testing used a Bonferroni correction for multiple comparisons. If the test for normality failed or if the sample was <5, a Fisher exact test was used. A P<0.05 was considered statistically significant.

Results

Depressed RV Contractile Reserve in RVH

RV mass was similarly increased in all 3 RVH models. In the RV Langendorff model, basal RVSP was greater in PAB than CH+SU or Monocrotaline (98±26 versus 71±7 and 59±4 mm Hg, Figure 1). All RVH models had higher RVSP than control (29±6 mm Hg, P<0.01). The inotropic reserve (whether defined as the fold- or absolute-increase in RVSP caused by 10 nmol/L dobutamine; Figure 1D and Figure I in the online-only Data Supplement) was lower in all RVH groups versus Control and was the lowest in the PAH-RVH models (Figure 1D).

Although basal LVSP remained unchanged in PAB, CH+SU and Monocrotaline (95±4, 96±2, 117±9 mm Hg) versus Control (105±5 mm Hg; Figure IA in the online-only Data Supplement), the LV inotropic reserve in response to dobutamine (10 mmol/L) was reduced in the PAH-RVH models (P>0.05; Figure IB in the online-only Data Supplement).

CO was reduced in PAB, CH+SU, and MCT (82.9±4.8, 71.5±8.0, 58.3±4.9 mL/min) versus control (154.2±16.2 mL/min; Figure 1E). Likewise, treadmill walking distance was significantly decreased in PAB, CH+SU, and MCT (244.1±12.3, 180.0±25.7, 41.5±11.6) versus control (406.3±54.4 m; Figure 1F). MCT rats had the lowest CO and the shortest treadmill distance.

Comparative Inotropic Potency

Whereas the inotropic response of the RV to dobutamine and dopamine was reduced in all RVH groups versus control...
The inotropic reserve of LV was only depressed in the PAH-RVH models (Figure 2E–2H). The dose-response to dobutamine was left-shifted versus dopamine in both the LV and RV in all RVH models (Figure 2B–2D and 2F–2H), reflecting the superior potency of dobutamine. A similar maximal pressure could be obtained with dopamine (albeit at ~1-log higher dose).

In the RV Langendorff, 10 nmol/L dobutamine, a dose in the clinically-relevant range, increased RVSP more than equimolar dopamine (Figure IIA in the online-only Data Supplement). Consistent with this, the EC50 of dobutamine was significantly lower than the EC50 of dopamine in all RVH groups (Figure IIB in the online-only Data Supplement). The EC50s were highest in PAH-RVH models, consistent with reduced potency. In the LV, the EC50 of dobutamine was also lower than that of dopamine, but it was unaltered by RVH (Figure IIC and IID in the online-only Data Supplement).

A dose–response curve to isoproterenol and phenylephrine in control and MCT (Figure III in the online-only Data Supplement) confirmed that contractile reserve to these inotropes was also reduced in RVH. The potency of inotropes in the RV Langendorff model in MCT hearts was as follows (in descending order): dobutamine = isoproterenol > dopamine > phenylephrine.

**Dobutamine Versus Dopamine In Vivo**

Baseline HR, CO, and SV were each reduced in MCT versus Control (HR, 294±5 versus 320±7 bpm, P<0.01; CO, 75±5 versus 110±5 mL/min, P<0.001; SV, 0.30±0.02 versus 0.37±0.03 mL, P<0.05; Figure 3A). In contrast, RVSP was increased in MCT versus control (RVSP, 61±3 versus 27±1 mm Hg, P<0.001). Dobutamine (22 μg/kg/min) caused a greater fold-increase in HR, CO, and SV than dopamine (22 μg/kg/min) in MTC (Fold increase: HR, 1.30±0.01 versus 1.22±0.05; CO, 1.7±0.1 versus 1.2±0.1; SV, 1.4±0.1 versus 1.2±0.1; Figure 3B–3D), suggesting greater efficacy of dobutamine versus dopamine. There were no differences in baseline HR, RVSP, CO, dP/dt, or SV in the 2 groups of MTC rats before infusion of dobutamine versus dopamine (Figure IVA–IVF in the online-only Data Supplement). Similar results, showing the superiority of dobutamine, were obtained at 11 μg/kg/min (data not shown).

Detailed hemodynamics, obtained using an RV conductance catheter, are summarized in Table II (in the online-only Data Supplement). MTC rats had a markedly elevated RV end diastolic pressure versus control (13±2 versus 3±1 mm Hg; P<0.01). However, RV dP/dt was not different in MTC versus control (1221±72 versus 1557±134 mm Hg/s), and increased further with inotrope infusion (Table II in the online-only Data Supplement).

As in the MTC group, dobutamine increased CO and SV more than dopamine in the PAB and CH SU groups, although some values were not statistically significant because of the smaller sample sizes (Figure V in the online-only Data Supplement).

**The D1-R Antagonist SCH23390 Selectively Reduces the RV Inotropic Reserve in Response to Dopamine in RVH**

SCH23390 (10 μmol/L) did not change basal RV contractility or the contractile response to dobutamine in either control or MTC (P>0.05 versus baseline; Figure 4). How-
ever, SCH23390 inhibited dopamine-induced RV inotropy in MTC without altering the response of control RV to dopamine (Figure 4). These findings are consistent with a role for the D1-R in dopamine-induced RV inotropy in RVH.

Figure 3. Dobutamine increases CO more than dopamine in vivo. A, Representative pressure–volume loops before and after dobutamine or dopamine (each infused at 22 μg/kg/min) for 10 minutes in CTR and MCT rats. B, Dobutamine infusion increases HR more than dopamine in MCT. C and D, Dobutamine causes greater increases in CO and SV than dopamine in MCT. RV indicates right ventricular; CO, cardiac output; CTR, control; MCT, monocrotaline; HR, heart rate; and SV, stroke volume.

Downregulation of β1-AR, Dopamine Receptors, and α1-AR in RVH

RV β1-AR mRNA expression was significantly decreased in all RVH models versus control (Figure 5A), whereas RV
β1-AR and D1-R are preferentially downregulated in the RV in RVH. A, RV β1-AR is downregulated in all RVH models, and D1-R mRNA is downregulated in the RV in PAH-RVH models. B, β1-AR mRNA is downregulated in the LV only in CH+SU, whereas LV D1-R mRNA expression is unchanged in all models. C, RV myocytes and the vessels in the RV were collected by laser capture microdissection. D, mRNA for β1-AR and D1-R is decreased in RV myocytes, but not coronary arteries, in all RVH groups. E and F, Representative immunoblot and mean data show decreased expression of β1-AR and D1-R in the plasma membrane fraction of homogenized RV tissue. The downregulation of β1-AR and D1-R is more severe in PAH-RVH models than in PAB-RVH. RV indicates right ventricular; LV, left ventricular; AR, adrenoceptor; PAH, pulmonary arterial hypertension; PAB, pulmonary arterial banding; RVH, right ventricular hypertrophy; MCT, monocrotaline; CTR, control; and CH+SU, SU5416+chronic hypoxia.

Human RV Tissue Microarray

The expression of β1-AR was downregulated in the hypertrophied RV myocytes in RV tissue microarray specimens from PAH patients versus age- and sex-matched controls (Figure 6C and 6D and Table I in the online-only Data Supplement).

Uncoupling of Adrenergic Receptor Signaling in RVH

Basal RV intracellular cAMP production was similarly decreased in all RVH groups (Figure 7A). Increases in cAMP concentrations in response to dobutamine and dopamine (10 nmol/L) were reduced in MTC versus control (Figure 7B). Dobutamine caused significantly greater increases in cAMP than dopamine in control and MTC RVs, consistent with better coupling to adenylyl cyclase (Figure 7B).

RV GRK2 activity increased in all RVH groups (Figure 7C). In control, both inotropes increased GRK2 activity (Figure 7D), whereas in MTC, neither inotrope increased GRK2 activity beyond its already elevated basal level, suggesting maximal basal desensitization of the β1-AR signaling pathway in this model (Figure 7D).

Serial measurements revealed that RV GRK2 activity and expression increased by week 3 and 5 after monocrotaline...
injection, respectively (Figure IXA–IXC in the online-only Data Supplement). RV expression of β1-AR and D1-R mRNA began to decrease at week 3 and continued to decline thereafter (Figure IXE and IXF in the online-only Data Supplement). Thus, GRK2 activation and receptor downregulation occur concomitantly, consistent with the proposed role for GRK2 in adrenergic remodeling.

In Vivo Gallein Therapy

In PAB, 2 weeks of gallein treatment increased treadmill distance and cardiac index. Gallein caused a statistically insignificant trend toward increased tricuspid annular plane systolic excursion, a measure of RV function (P>0.05; Figure 8A–8D). In MTC, gallein significantly increased CO and tricuspid annular plane systolic excursion on echocardiography (P<0.01; Figure 8C and 8D); however, RV/LV+septum ratio, the catheterization and treadmill data, though trending toward benefit, were not statistically significant (P>0.05; Figure 8A and 8B and Figure XA and XB in the online-only Data Supplement). To confirm that gallein indeed had an effect on GRK2, we measured phospho-670 GRK2 and total GRK2 protein. RV expression of both proteins was reduced by gallein in both PAB and MTC (Figure 8E–8H and Figure XC–XF in the online-only Data Supplement). Phospho-ERK1/2 and total ERK1/2 expression, which were increased in MTC, were decreased by gallein (P<0.01, P<0.05 versus MTC; Figure 8I and 8J and Figure XG and XH in the online-only Data Supplement). Gallein treatment did not restore β1-AR protein expression in MTC (P>0.05 versus untreated MTC; Figure XIA and XIB in the online-only Data Supplement).

Discussion

The molecular basis for reduced RV inotropy and impaired adrenergic signaling in severe RVH was studied in 2 types of experimental RVH, 1 associated with PAH (CH+SU or MCT) and the other a model of pure RVH (PAB). The use of these different RVH models allowed identification of the derangements of adrenergic and dopaminergic signaling that are common to all forms of RVH versus those that are model-specific. In this regard, although the β1-AR downregulation was universal, it was more severe and extensive (involving the LV) in the maladaptive models, those with PAH-RVH. We refer to these models as being maladaptive because exercise capacity and CO were more depressed than in PAB-RVH, even though the severity of the RVH was similar (Figure 1).
Molecular Basis and Chamber Specificity of Impaired Adrenergic and Dopaminergic Signaling in RVH

In the current study, both mRNA and protein expression of adrenergic and dopaminergic receptors were downregulated in all RVH models (Figures 5 and 6). Downregulation and desensitization of adrenergic (β1- and α1-adrenoreceptors) and dopaminergic receptors (D1-R) primarily occurred in RV myocytes (Figures 5 and 6 and Figures VI and VIII in the online-only Data Supplement). Although it may seem intuitive that the downregulation of β1-AR and D1-R would occur in RV myocytes, there are other candidate cells in the RV (vascular cells, fibroblasts, and inflammatory cells). Laser capture microdissection provided the precision to identify the affected cell type as the RV myocyte (Figure 5C and 5D and Figure VII in the online-only Data Supplement). Decreased expression of the β1-AR is also evident in the RV myocytes of humans with PAH (Figure 6C and 6D), confirming previous studies in rodents17 and human.18 The pathological relevance of this adrenergic remodeling is supported by the observation that the magnitude of the reduction in β1-AR expression is greatest in PAH-RVH models, and these models have the greatest impairment of contractile reserve (Figures 5 and 6).

Bristow et al18 examined the β-adrenergic system in patients with idiopathic PAH who either had RVF or were well compensated. Only those with RVF had decreased RV β-AR density. This PAH-RVF subset displayed depletion of norepinephrine and decreased adenylyl cyclase responsiveness to β-agonists, consistent with our findings in rats with PAH-RVH. Noontens et al19 demonstrated high circulating catecholamine levels in PAH-RVF patients, noting the loss of the normal ability to augment catecholamine levels with exercise. Likewise, in PAH-RVH rats, we found that inotropic stimulation could not further augment RV GRK2 activity beyond the elevated basal levels (Figure 7). Bristow et al reported that in humans with PAH, adrenergic impairment affected only the RV (not the LV).18 A similar finding was made in a canine RVF model (induced by severe PAB plus tricuspid insufficiency).20 Consistent with these studies, we report that in PAB-RVH, adrenergic and dopaminergic receptor downregulation is confined to the RV and the depression of RV inotropic reserve is mild. However, in rodent PAH-RVH models, downregulation of adrenergic and dopaminergic receptors was more severe and occurred in both the RV and LV, resulting in biventricular impairment of contractile reserve (Figure 2).

By evaluating the inotropic responses in both RV and LV Langendorff preparations we were able to assess contractility in each chamber, without the confounding effects of heart rate and afterload. This allowed us to address a longstanding controversy regarding the functional consequences of β1-AR downregulation to LV function in PAH. The reduced LV response to dobutamine in MTC and CH SU (Figure 2E–2H) confirms the physiological relevance of observed downregulation and uncoupling of adrenergic receptors in the LV in these models. The finding of decreased β-AR expression in the LV in the PAH-RVH models is consistent with previous studies of monocrotaline rats.17,21 LV involvement in PAH-RVH, but not PAB-RVH, likely reflects fundamental differences in how the models were created rather than differences in RVH severity. PAH-RVH models were induced by endothelial toxins (SU5416 or monocrotaline), which likely have vasculotoxic effects beyond the pulmonary circulation, per-
haps including the RV vasculature. The concept that the stimuli that initiate PAH may also induce the RV coronary vascular injury is intriguing, particularly as many forms of PAH have evidence of systemic arterial endothelial damage. For example, in scleroderma-associated PAH, patients have circulating cytotoxic autoantibodies that are directed against epitopes in endothelial cells. Interestingly, compared with patients with idiopathic PAH, these scleroderma patients have more severe RV dysfunction, potentially suggesting an effect of the endothelial-targeted antibodies on the RV circulation.

In addition to β1-AR downregulation, most dopamine receptor isoforms are also reduced in RVH (Figures 5 and 6 and Figure VI and VIII in the online-only Data Supplement). Dopamine receptors have been intensively studied in blood vessels and neural and renal tissues; however, only recently was the expression of D1, D2, D4, and D5 receptors reported in human hearts. Their function remained uncertain, although Li et al showed that D1-R activation modulates ischemia/reperfusion-induced apoptosis in neonatal rat cardiomyocytes. Dopamine receptors are pharmacologically classified into two classes: D1-like (which stimulate adenylyl cyclase) and D2-like (which inhibit adenylyl cyclase). The pattern of depressed dopamine receptor expression observed in RVH would be predicted to reduce D1-signaling and might contribute to the observed impairment in cAMP production. To test the relevance of D1-R downregulation to impaired contractile reserve we administered a selective D1-R antagonist, SCH23390. This inhibitor selectively reduced dopamine contractility in RVH without affecting the response of dopamine in control hearts or altering the response to dobutamine (Figure 4). This reveals for the first time that D1-R contributes to dopamine-induced RV inotropy. Thus, the loss of dopamine receptors in experimental RVH likely contributed to the reduced contractile response to dopamine, both in vitro and in vivo (Figures 2 and 3 and Figures I, II, and IV in the online-only Data Supplement). As with the β1-AR, D1-R expression is most depressed in PAH-RVH models, although in contrast D1-R expression is not reduced in the LV in any model (Figures 5 and 6).

**Optimal Inotropic Support in RVH**

Dobutamine was shown to be more effective in increasing CO than dopamine in all RVH models, as a result of both superior coupling to adenylyl cyclase (as indicated by the greater evoked increase in cAMP) and the loss of D1-R expression in RVH, which exclusively impairs dopamine contractility (Figures 2–4 and 7). In RV and LV Langendorff models, the dose–response curves of dobutamine were left-shifted compared with those of dopamine (Figure 2). At equimolar doses (10 nmol/L), dobutamine induced greater contractility than dopamine in all RVH models (and even in the normal RV; Figure II in the online-only Data Supplement). These data indicate that dobutamine is superior to dopamine as an RV inotrope. Consistent with observations in left heart failure, dobutamine caused a greater increase in CO (resulting from greater increases in SV and HR) than dopamine (Figure 3). However, dopamine is better at increasing dP/dt in vivo (Table II in the online-only Data Supplement). Ejection fraction is severely reduced in MTC-RVH,
and dobutamine tends to increase ejection fraction more than dopamine (P > 0.05; Table II in the online-only Data Supplement). Although dobutamine is a better inotrope than dopamine, this comes at some cost of increased heart rate (Figure 3). However, a significant portion of the net effect of dobutamine on CO is related to its ability to increase stroke volume. The greater increase in RV contractility in the RV Langendorff preparation also proves that increased heart rate is not the only reason for the superior augmentation of CO by dobutamine. The reduced RV response to dopamine reflects the combined consequence of alterations in adrenergic signaling in RVH, some of which uniquely or differentially impair the response to dopamine versus dobutamine. Impaired RV responses to dopamine in PAH-RVH reflect the combination of decreased β1-AR expression (which also effect dobutamine; Figures 5 and 6), inferior coupling of dopamine to adenyl cyclase (less cAMP production; Figure 7), and the loss of D1–R–dependent contractility (which only affects dopamine; Figure 4). The greater elevation of cAMP concentrations in response to dobutamine versus dopamine, which we have also noted in human heart failure.27

Role of GRK2 in Mediating Receptor Downregulation in RVH

We identified a key role for GRK2 in the adrenergic and dopaminergic receptor downregulation in RVH. GRK2 activity was markedly increased in RVH and was maximal in MTC (Figure 7 and Figure IV in the online-only Data Supplement), consistent with the severe RVF noted in this model (RVEDP 13±2 mm Hg; Table II in the online-only Data Supplement). Our findings support those of Leineweber et al,28 who reported increased GRK2 activity in monocrotaline-RVF.

The Reduced RV Response to Dopamine Reflects Therapeutic Value of Interrupting Gβγ–GRK2 Interaction In Vivo

We explored the therapeutic potential of correcting the adrenergic remodeling. One possible intervention would be the use of an inhibitor of β1-AR. The α- and β-AR inhibitor, carvedilol, improves LV function and survival in patients with LVF. Although β-AR antagonists are not approved for treatment of PAH, carvedilol improves RV function in the CH+SU5416 model.9 Because of the central role of GRK2 we chose to inhibit Gβγ–GRK2 interaction, using gallein, as an alternative to a β-AR antagonist. Gallein inhibits Gβγ subunit–dependent signaling and has been used for interrupting the interaction between GRK and the Gβγ subunit of activated G proteins in vitro.3 Gallein and other peptide Gβγ inhibitors have been shown to reduce GRK2 expression and improve cardiac function in experimental LVF.3 In the current study, gallein improved cardiac function, as evidenced by improved treadmill distance, tricuspid annular plane systolic excursion, and CO in both PAB-RVH and PAH-RVH (Figure 8). Consistent with its proposed mechanism of action, gallein decreased RV GRK2 expression. Further evidence that the beneficial effects of gallein related to its actions on the GRK2 pathway came from the demonstration that it decreased expression of activated (phosphorylated) ERK1/2, a kinase that regulates GRK2 activity (Figure 8I and 8J and Figure XG and XH in the online-only Data Supplement). The relationship between ERK1/2 and GRK is complex. Some studies suggest that ERK is upstream and phosphorylates β-arrestin and GRK,29 whereas others place ERK downstream from GRK.30

Limitations

We did not explore the mechanism for GRK2 activation. However, previous studies suggest that in states of hypertrophy and autonomic activation, protein kinase C phosphorylates and activates GRK2.15

There are some limitations to the gallein experiments. First, gallein undoubtedly has effects in addition to Gβγ inhibition. Second, at the doses used, gallein did not restore β1-AR protein expression, although it did inhibit the expression of GRK2. The regimen that restored β1-AR expression in LV failure (30 mg/kg/d for 3–4 weeks3) was more intense than what we used (1.8 mg/kg/d for 2 weeks). However, whereas gallein (0.1 μmol/L) acutely increases contractility in Control and PAB, it slightly decreased contractility in MTC (Figure XIC and XID in the online-only Data Supplement), suggesting lower doses may be required in PAH-RVH. Third, although gallein increased CO in PAB rats (a model devoid of pulmonary or systemic vascular disease), studies are needed to assess possible effects of gallein on the pulmonary and systemic vasculature.

Conclusion

GRK2-mediated adrenergic remodeling of the RV and LV contributes to impaired cardiac function in PAH-RVH. Acute RV inotropic support in PAH-RVH is best accomplished with dobutamine. Inhibition of Gβγ–GRK2 interaction may have promise as a therapy in RVH.

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Disclosures

None.

References

Right ventricular (RV) failure in pulmonary arterial hypertension is associated with adrenergic activation. Clinicians are often confronted with two questions: (1) Which is the optimal inotrope in RV failure? (2) Is there a long-term role for β-adrenergic receptor downregulation/desensitization, and GRK2 inhibitors improve adrenergic signaling and function. We explored the molecular basis and therapeutic relevance of adrenergic abnormalities in RV failure and RV hypertrophy (RVH).

Using human tissues and rodent models (of maladaptive and adaptive-RVH), we show that RVH results in downregulation of α- and β1-adrenergic receptors and dopamine receptors. These changes are confined to the RV in adaptive RVH, but in the more clinically relevant maladaptive models, the receptor downregulation also involves the left ventricle. Receptor downregulation is functionally important, reducing inotropic reserve. The basis for the adrenergic changes in RVH is activation of GRK2, and disrupting the interaction between Gβγ–GRK2 in vivo (using gallein) is beneficial, improving cardiac output and exercise tolerance. The comparison of dobutamine and dopamine showed better efficacy for dobutamine in all models. This largely reflects its superior coupling to adenylyl cyclase. In addition, we discovered a new role for the D1-dopamine receptor in RV contractile reserve. In RVH, dopamine interacts with this receptor to augment adrenergic signaling, and its loss contributes to the inferior performance of dopamine. We conclude that adrenergic remodeling in the RV is worse in maladaptive RVH, is mediated by GRK2, and contributes to RV failure. Adrenergic signaling and interactions between Gβγ–GRK2 are promising therapeutic targets.
GRK2-Mediated Inhibition of Adrenergic and Dopaminergic Signaling in Right Ventricular Hypertrophy: Therapeutic Implications in Pulmonary Hypertension

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**Supplemental Material**

**Methods**

**Experimental models**  The PAB model: Briefly, rats (200–230g) were anesthetized with 3% isoflurane, intubated and subjected to a limited median sternotomy. A 1.3mm diameter needle was placed parallel to the main PA, and ligated with a 4-0 silk suture. The needle was withdrawn to create a uniform, fixed stenosis.

**Treadmill running distance**  Exercise capacity was tested by measuring maximal distance run on a motorized treadmill (Simplex II Instrument; Columbus Instruments, Columbus, OH). The initial treadmill speed was 10 m/min and increased 5 m/min every 5 min for 30 minutes or until the rat fatigued. The treadmill distances in control (CTR) versus the three RVH models were measured after 4-weeks (Fig 1) versus after 3 weeks in the gallein therapy experiments (to reduce the number of IP injections of gallein and minimize animal discomfort).

**Echocardiography**  A Vevo 2100 (Visual Sonics, Ontario, Canada) was used to assess cardiac output (CO), stroke volume (SV) and RV function. Transthoracic echocardiogram was performed on lightly anesthetized but spontaneously breathing rats (isoflurane 2.0% in medical gas) using the Vevo 2100 animal echocardiogram (VisualSonics Inc, Ontario, Canada). Analysis was performed offline. Cardiac output was quantified through the pulmonary artery by measuring maximal diameter of pulmonary artery (PAd) and velocity time integral (VTIpa) of pulsed wave Doppler obtained from the short-axis view. Cardiac output was then calculated from the formula Cardiac Output = Stroke Volume (SV) x heart rate, where SV = 3.14 x (PAd/2)^2 x VTIpa. Tricuspid annular plane systolic
excursion (TAPSE) was measured as a marker of right ventricle function. TAPSE of the tricuspid annular plane was measured using M-mode through the lateral portion of the tricuspid annular plane in the apical four-chamber view\(^2\).

**Thermodilution cardiac output** After anesthesia and intubation, a 2 cm-long vertical incision was made in the middle of the neck. The underlying tissues were carefully dissected to expose the right carotid artery and jugular vein. After the distal part of the carotid artery was ligated, a small incision was made just below the ligature. A thermistor probe catheter (Physitemp Instruments, Inc., Clifton, NJ) was then gently inserted into the same incision to aorta via the carotid artery and a 22-gauge catheter was gently introduced into jugular vein via cut-down technique. A bolus of iced saline (0.3ml) was injected and a thermodilution curve (temperature vs time) was recorded. PowerLab was used for data acquisition. Cardiac output was then determined by calculating the area under the temperature curve and cardiac index was calculated by normalizing CO to body weight.

**RV and LV Langendorff** Briefly, the heart was isolated from the anesthetized, intubated rat. The aorta was mounted on a Langendorff perfusion apparatus and perfused with oxygenated Krebs–Henseleit buffer (95% O\(_2\)+5% CO\(_2\)) at a constant pressure of 75–85 cm H\(_2\)O. The AV node was ablated, and the heart was paced at a cycle length of 200 ms. RV systolic pressure (RVSP) and LV systolic pressure (LVSP) were measured using Millar pressure catheters (Millar Instruments, Houston, TX) inside RV and/or LV balloons inserted.

**qRT-PCR** Tissue total RNA was extracted using the PureLink Micro-to-midi total RNA
isolation kit (Invitrogen, Carlsbad, CA). mRNA of RV myocytes and RV vessels were isolated using the PicoPure RNA isolation kit (Applied Biosystems, Carlsbad, CA). Briefly, mRNA levels of α-myosin heavy chain (MHC), β1, β2, dopamine 1-5 receptors (D1-5Rs) and α-adrenoreceptors were measured using an ABI PRISM 7900HT PCR system. mRNA expression was normalized to β2 microglobulin mRNA expression.

**Immunoblot** Membrane proteins from RV tissues were isolated following the manufacturer’s instructions (Thermo Scientific, Rockford, IL, #89826 for membrane protein). Western blots were performed using standard procedures as we described previously\(^3\). Immunoblotting for receptors was performed on membrane fractions, as described previously\(^3\).

**Immunofluorescence** Frozen sections were fixed in methanol, blocked with albumin (Sigma, St. Louis, MO), and incubated with primary antibodies for 1 hour at 25ºC (mouse monoclonal anti-dystrophin, 1:400 dilution, rabbit polyclonal anti-β1-AR 1:500 dilution, Abcam, Cambridge, MA). Samples were then incubated with secondary anti-rabbit (Alexa-568) and anti-mouse (Alexa-488) antibodies (Invitrogen, Carlsbad, California). The antifade reagent (Prolong Gold; Invitrogen) contained 4',6-diamidino-2-phenylindole (DAPI) to stain the nuclei of the cells. Images were acquired with the Zeiss LSM 510 META confocal microscope (Carl Zeiss Inc; Thornwood, NY) using the 488 nm and 561 nm lasers. DAPI was visualized with the Chameleon Ultra 2 photon laser (Coherent Inc; Santa Clara, CA) at 770 nm. Immunofluorescence intensities were calculated with ImageJ (NIH; Bethesda, MD) after background subtraction.
**Human Tissue Microarray** The tissue microarray was constructed using 4 cores (1 mm in diameter) from each specimen using an Automated Tissue Microarrayer ATA-27 (Beecher Instruments, Sun Prairie, WI). The region to be sampled was selected based on myocyte orientation and absence of any discrete lesions (such as infarction).

PAH patients were female and had a race ratio (African-American:Caucasian:Hispanic) of 3:5:2. Control patients were predominantly female (7/1) and had a similar race ratio (4:4:0). PAH patients had an average disease duration of 8±3 years, a mean PA pressure (PAP) of 58.9±3.0 mmHg and 80% died from RVF (Supplemental Table 1).

**Immunofluorescence:** After antigen retrieval (95-100°C for 30 min in citrate buffer), human RV tissue microarrays were incubated in anti-β1-AR (dilute ratio 1:100, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and anti-dystrophin (1:100; Abcam; Cambridge, MA) overnight at 4°C. Subsequent processing was the same as for rat RV. The images were captured on a Zeiss LSM META using 100x 1.46 NA objective.

**GRK2 Activity by Rhodopsin Phosphorylation** The measurement of GRK2 activity has been described previously^4^. Flash-frozen RV tissue (50mg) samples were homogenized in 10 volumes of 0.1M HCl and centrifuged at >600g for 10 minutes. Supernatants were assayed and cAMP content was measured by a Direct ELISA kit (Assay Designs, Ann Arbor, MI, USA). RV tissue was homogenized in lysis buffer containing the following (mM): Tris-HCl (pH 7.5) 25, EDTA 5, EGTA 5, MgCl₂ 10, and phenyl-methylsulfonyl fluoride 1 with 10µg/ml leupeptin, 20µg/ml aprotinin. Tissue lysates (150µg total protein) were incubated with rhodopsin-enriched rod outer-segments in 60µL lysis buffer with 10 mM MgCl₂ and 0.1 mM ATP containing [γ-³²P]ATP. After an incubation period
of 30 minutes in white light at room temperature, reactions were quenched with ice-cold lysis buffer and centrifuged at 13,000g for 15 min. Sedimented proteins were re-suspended in 25µl of protein gel-loading dye and subjected to 12% SDS-PAGE electrophoresis. Phosphorylated rhodopsin was visualized by autoradiography of dried polyacrylamide gels. GRK2 activity is expressed in arbitrary units and measurements are made on a standardized mass of muscle and extracted total protein (150µg). Standardize loading ensure that differences in density between lanes is proportional to differences in activity, permitting comparison amongst experimental groups. To confirm that the total activity in these assays was exclusively that of GRK2 and not GRK5, GRK2 or GRK5 antibodies (1µg) were added to the reaction mix to inhibit the respective activity of each GRK. Rod outer segments were obtained from Invision BioResources (Seattle, WA, USA). Each kinase activity reaction mixture contained 50µg of purified rhodopsin.
### Supplemental Table 1. Clinical data of PAH patients and non-PAH patients

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Cause of PAH</th>
<th>Duration of PAH</th>
<th>Treatment</th>
<th>PAP*</th>
<th>PVR†</th>
<th>Cause of death</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>53</td>
<td>F</td>
<td>AA‡</td>
<td>Idiopathic</td>
<td>23 years</td>
<td>PG§ x 18 years</td>
<td>63</td>
<td>8</td>
<td>Adenocarcinoma</td>
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<tr>
<td>2</td>
<td>54</td>
<td>F</td>
<td>Hispanic</td>
<td>Lupus</td>
<td>1 year</td>
<td>Bosentan x 3 months, Sildenafil x 3 months</td>
<td>NA‡</td>
<td>NA</td>
<td>RV failure</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>F</td>
<td>Caucasian</td>
<td>Idiopathic</td>
<td>NA</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>RV failure</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>F</td>
<td>Caucasian</td>
<td>Idiopathic</td>
<td>7 years</td>
<td>PG x 6 years, Sildenafil x 6 years</td>
<td>59</td>
<td>16</td>
<td>RV failure</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>F</td>
<td>AA</td>
<td>HIV</td>
<td>NA</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>Sepsis</td>
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<tr>
<td>6</td>
<td>29</td>
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<td>Hispanic</td>
<td>Idiopathic</td>
<td>7 years</td>
<td>PG x 6 years, Sildenafil x 1 year</td>
<td>52</td>
<td>11</td>
<td>RV failure</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>F</td>
<td>Caucasian</td>
<td>Anorexigen</td>
<td>10 years</td>
<td>PG x 7 years, Sildenafil x 3 years</td>
<td>54</td>
<td>NA</td>
<td>RV failure</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>F</td>
<td>AA</td>
<td>Lupus</td>
<td>6 years</td>
<td>PG x 5 years</td>
<td>54</td>
<td>16</td>
<td>RV failure</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>F</td>
<td>Caucasian</td>
<td>Anorexigen</td>
<td>NA</td>
<td>PG x 1 years</td>
<td>75</td>
<td>21</td>
<td>RV failure</td>
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<tr>
<td>10</td>
<td>45</td>
<td>F</td>
<td>Caucasian</td>
<td>Sjogren’s</td>
<td>6 months</td>
<td>PG x 3 months</td>
<td>55</td>
<td>15</td>
<td>RV failure</td>
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</table>

1. 38 F AA Pneumonia
2. 61 F Caucasian Cholangiocarcinoma
3. 48 F Caucasian Hemoperitoneum
4. 38 F AA Acute pulmonary embolism
5. 21 F AA Malaria
6. 43 F Caucasian Pneumonia
<table>
<thead>
<tr>
<th></th>
<th>17 M AA</th>
<th>Pneumonia</th>
<th>8 60 F Caucasian</th>
<th>Aortic dissection</th>
</tr>
</thead>
</table>

*PAP = pulmonary arterial pressure (mm HG), †PVR = pulmonary vascular resistance (Wood units), ‡AA= African American, §PG = prostaglandin, †NA = not available

**Supplemental Table 2. Hemodynamics in in vivo dobutamine and dopamine Infusion**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Monocrotaline</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Dobutamine</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>320±7</td>
<td>406±8###</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>0.37±0.03</td>
<td>0.61±0.12##</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>110±5</td>
<td>247±17###</td>
</tr>
<tr>
<td>SW (mmHg*ml)</td>
<td>4.29±0.24</td>
<td>6.73±0.57###</td>
</tr>
<tr>
<td>Pmax (mmHg)</td>
<td>27±1</td>
<td>39±2###</td>
</tr>
<tr>
<td>Pes (mmHg)</td>
<td>25±1</td>
<td>31±1</td>
</tr>
<tr>
<td>Ped (mmHg)</td>
<td>3±1</td>
<td>2±1</td>
</tr>
<tr>
<td>dP/dt Max (mmHg/s)</td>
<td>1221±72</td>
<td>1633±222</td>
</tr>
<tr>
<td>dP/dt Min (mmHg/s)</td>
<td>858±58</td>
<td>1220±137##</td>
</tr>
<tr>
<td></td>
<td>Ved (ml)</td>
<td>0.63±0.03</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>Ves (ml)</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>dV/dt Max (ml/s)</td>
<td></td>
<td>12.17±1.00</td>
</tr>
<tr>
<td>dV/dt Min (ml/s)</td>
<td></td>
<td>11.87±1.16</td>
</tr>
<tr>
<td>EF (%)</td>
<td></td>
<td>61±4</td>
</tr>
<tr>
<td>τ (ms)</td>
<td></td>
<td>15.40±1.46</td>
</tr>
</tbody>
</table>

Values are set as means±SE. HR, heart rate; SV, stroke volume; CO, cardiac output; SW, stroke work; Pmax, maximum RV pressure; Ped, RV end-diastolic pressure; Pes, RV end-systolic pressure; dP/dt max, maximum value of dP/dt; dP/dt min, minimum value of dP/dt; Ved, RV end-diastolic volume; Ves, RV end-systolic volume; dV/dt max, maximum value of dV/dt; dV/dt min, minimum value of dV/dt; EF, RV ejection fraction; τ, relaxation time constant; Ea, effective arterial elastance. *P<0.05, **P<0.01, ***P<0.001 vs. CTR baseline; #P<0.05, ##P<0.01, ###P<0.001 vs. baseline in the same group; &P<0.05 vs. dobutamine in the same group.
The absolute increase in RVSP and LVSP by 10nM dobutamine

A

LVSP (mm Hg)

CTR  PAB  CH+SU  MCT

B

Increase in LVSP (fold)

CTR  PAB  CH+SU  MCT

10 nM dobutamine

P<0.001

n=4-6 in each group

C

RVSP (mmHg)

CTR  PAB  CH+SU  MCT

P<0.001  P<0.01  P<0.01  P<0.01

D

LVSP (mm Hg)

CTR  PAB  CH+SU  MCT

P<0.001  P<0.05

n=3-4 in each group
RV Langendorff

A

Increased RVSP (fold)

P<0.001  P<0.01  P<0.01  P<0.01

CTRL  PAB  CH+SU  MCT

10 nM Dobutamine 10 nM Dopamine

B

LogEC50

P<0.05  P<0.05  P<0.01  P<0.005

CTRL  PAB  CH+SU  MCT

LV Langendorff

C

Increased LVSP (fold)

P<0.05  P<0.05  P<0.05

CTRL  PAB  CH+SU  MCT

10 nM Dobutamine 10 nM Dopamine

D

LogEC50

P<0.05  P<0.001  P<0.001  P<0.05

CTRL  PAB  CH+SU  MCT

n=4-7 in each group
Supplemental Fig 3

(A) RV

(B) LV

n=3-4 in each group
Supplemental Fig 4

Baselines before dobutamine or dopamine infusion

**A**
HR (bpm) vs. CTR and MCT
- CTR: Black bars
- MCT: White bars

**D**
dP/dt (mmHg/s) vs. CTR and MCT
- CTR: Black bars
- MCT: White bars

**G**
Increase in RVSP (fold)
- Black bars: CTR
- White bars: MCT

**B**
RV/LV+Septum vs. CTR and MCT
- CTR: Black bars
- MCT: White bars

**E**
CO (ml/minute) vs. CTR and MCT
- CTR: Black bars
- MCT: White bars

**H**
Increase in dP/dt (fold)
- Black bars: CTR
- White bars: MCT

**C**
RVSP (mmHg) vs. CTR and MCT
- CTR: Black bars
- MCT: White bars

**F**
SV (ml) vs. CTR and MCT
- CTR: Black bars
- MCT: White bars

**I**
22 µg/Kg/min Dobutamine
- RV Pressure (mmHg)
- RV Volume (ml)

**J**
22 µg/Kg/min Dopamine
- RV Pressure (mmHg)
- RV Volume (ml)

Legend:
- Black bars: 22 µg/Kg/min Dobutamine
- White bars: 22 µg/Kg/min Dopamine

n=7-12 in each group
n=7 in each group in CTR
n=2 in each group in PAB
n=4-5 in each group in CH+SU

A  Increase in HR (fold)
B  Increase in RVSP (fold)
C  Increase in dP/dt (fold)

D  Increased in CO (fold)
E  Increased in SV (fold)

22 µg/Kg/min Dobutamine
22 µg/Kg/min Dopamine
Supplemental Fig 6

RV

A  β2-AR

B  D2-R

C  D3-R

D  D4-R

E  D5-R

Normalized mRNA Expression

P<0.05

P<0.01

P<0.001

n=4-7 in each group

LV

F  β2-AR

G  D2-R

H  D3-R

I  D4-R

J  D5-R

Normalized mRNA Expression

P<0.05

P<0.01

P<0.001

P<0.01

P<0.001
Supplemental Fig 7

(A) α-MHC

(B) CD31

n=5-7 in each group
**Supplemental Fig 9**

A. GRK2 activity

- **CTR**
- **3W**
- **5W**

P < 0.05

B. GRK2 mRNA

C. GRK2 protein

D. α-MHC mRNA

P < 0.05

E. β1-AR mRNA

P < 0.05

F. D1-R mRNA

P < 0.01

n = 3-5 each group
Catheterization

A

Cardiac Index (ml/min*g)

P<0.05

0.0 0.1 0.2 0.3

PAB PAB+Gallein MCT MCT+Gallein

B

RVSP (mm Hg)

0 50 100

PAB PAB+Gallein MCT MCT+Gallein

n=3-5 in each group

Supplemental Fig 10

C

PAB PAB+gallein

GRK2

Actin

D

Normalized Protein Expression

GRK2/actin

PAB PAB+gallein

n=6-9 in each group

E

MCT MCT+gallein

GRK2

Actin

F

Normalized Protein Expression

GRK2/actin

MCT MCT+gallein

G

CTR MCT MCT+gallein

ERK1/2

Actin

H

Normalized Protein Expression

ERK1/2

CTR MCT MCT+gallein

p<0.05

n=5 in each group
Supplemental Fig 11

A

<table>
<thead>
<tr>
<th></th>
<th>CTR</th>
<th>MCT</th>
<th>MCT+gallein</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1-AR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td></td>
<td></td>
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</tbody>
</table>

B

![Normalized Protein Expression Chart]

C

![Graph C]

D

![Graph D]

E

![Graph E]
Figure Legends:

Supplemental Fig 1. The changes of RVSP and LVSP in three RVH models. A. Unstimulated LVSP is unaltered in RVH. B. LV inotropic reserve is only decreased in MCT. C. In RV, PAB has the highest absolute response to 10 nM dobutamine. Even though the baseline RVSP is high in CH+SU and Monocrotaline (MCT), the two groups did not have as high response to dobutamine as PAB. D. In LV, CTR and PAB presented with the highest absolute response to dobutamine.

Supplemental Fig 2. Dobutamine is more effective in elevating systolic pressure than dopamine in both LV and RV in Langendorff system.

Dobutamine produces more of an increase in RVSP (A) and LVSP (C) than dopamine at the same dose (10 nM) in CTR and all RVH groups. The half maximal effective concentration (EC50) of dobutamine is lower than the EC50 of dopamine in both RV (B) and LV Langendorff (D) in all groups.

Supplemental Fig 3. The responses of RVSP to isoproterenol and phenylephrine are decreased in RVH induced by MCT in both RV (A) and LV (B).

Supplemental Fig 4. The baseline parameters and pressure-volume curves in infusion experiments.
A-F. The baseline parameters are not different between dobutamine and dopamine infusion groups.

G-H. Dobutamine and dopamine increase RVSP and dP/dt.

I-J. The original traces of RV pressure and volume changed by 22 μg/Kg/min dobutamine and 22 μg/Kg/min dopamine in RV in MCT group.

Supplemental Fig 5. Dobutamine in vivo infusion improves cardiac function more than dopamine in PAB and CH+SU induced RVH.

A-C. 22 μg/Kg/min obutamine and 22 μg/Kg/min dopamine cause similar increase in heart rate (HR), RVSP and dP/dt in both PAB and CH+SU.

D. There is a trend that dobutamine causes greater increases in CO than dopamine in PAB and CH+SU.

E. Dobutamine causes greater increase in SV than dopamine in PAB. There is a trend that dobutamine increases SV more than dopamine in CH+SU group.

Supplemental Fig 6. The changes of mRNA levels of β2-AR and dopamine receptors (D2-5Rs) in RV and LV in RVH groups.

A, RV β2-AR mRNA is downregulated only in CH+SU.
**B-E**, D2-5Rs are downregulated in RV in MCT model. D1-3Rs and D5Rs are downregulated in RV in CH+SU model.

**F**, LV β2-AR mRNA expression is unchanged in all models.

**G-J**, D2-5Rs are not downregulated in LV in RVH models. D2-4Rs are actually increased in CH+SU and MCT models.

**Supplemental Fig 7.** The expression of α-myosin heavy chain (α-MHC) and CD31 confirm successful capture of myocytes versus intramyocardial coronary arteries by LCM in the RV. Receptor downregulation is confined to the RV myocytes.

**Supplemental Fig 8.** α adrenergic receptors (α1a, α1b and α1d) are downregulated in RV in RVH groups.

**A-C**, The gene expression of α1a, α1b and α1d are downregulated in RV in all RVH models.

**D-E**, The gene expression of α1a, α1b are not downregulated in LV in RVH models.

**F**, The gene expression of α1d is downregulated in LV in all RVH groups.

**G-H**, Western blot results and bar graph showing the protein expressions of α1-ARs at cell membrane are downregulated in RV in all RVH models.
**Supplemental Fig 9.** The temporal changes of GRK2 activity and the relative gene and protein expressions in RV in monocrotaline-induced RVH.

**A.** GRK2 activity is increased in RV at 3 weeks of monocrotaline injection.

**B-C.** GRK2 mRNA and protein expression tend to be increased in RV at 5 weeks after MCT injection.

**D-E.** α-MHC, β1-AR and D1-R mRNA expression are gradually reduced in RV after MCT injection.

**Supplemental Fig 10.** The effects of gallein on total GRK and ERK1/2 protein expressions and on RVSP in RVH models.

**A.** Cardiac output is increased by gallein treatment in PAB model.

**B.** RVSP is not changed by gallein in both PAB and monocrotaline models.

**C-F.** Western blot bands and mean data show that the protein levels of total GRK are slightly reduced in RV in PAB and MCT.

**G-H.** Western blot bands and mean data show that the protein levels of phospho-ERK1/2 and total ERK1/2 are normalized by gallein in RV in MCT.

**Supplemental Fig 11.** The effects of gallein on β1-AR and on RVSP in RVH models.
**A-B.** Western blot bands and mean data show that the protein level of β1-AR is not altered by gallein in RV in MCT.

**C.** In RV Langendorff, acute treatment with 0.1 μM gallein increases RVSP in control heart.

**D.** In RV Langendorff, acute gallein treatment increases RVSP in PAB heart.

**E.** In RV Langendorff, acute gallein treatment decreases RVSP in MCT heart.
References:


