様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　４ 年　 ６ 月 １３ 日現在

機関番号：８４４０４
研究種目：若手研究
研究期間：2019～2021
課題番号：１９Ｋ１６４９８

研究課題名（和文）Understanding the pathogenesis of right heart failure in pulmonary arterial hypertension

研究課題名（英文）Understanding the pathogenesis of right heart failure in pulmonary arterial hypertension

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：肺高血圧症（PAH）ラットの右心室（RV）において、経時的に進行するRV機能障害の悪化が認められた。さらに、actin-myosin cross-bridge（CB）レベルの変化や筋フィラメント、PAHの全身性RV機能障害の根本的な要因であることが判明した。
予防的モデルとして、PAHラットにヘキサレリン(HEX)を慢性的に投与し、交感神経系の過剰な駆動を抑制したところ、PAHラットのRVのRV機能には影響を与えなかった。さらに、心筋細胞やCBのレベルではHEXの効果は観察されなかった。
最後に、GRK-2阻であるパロキセチンをPAHラットにところ、RVの収縮機能と肺の血行動態が改善された。

研究成果の学術的意義や社会的意義
In this project, we were able to reveal that the changes at the level of the myofilaments are closely linked with changes in global right ventricle (RV) function in the context of pulmonary arterial hypertension. GRK2-inhibition improved RV function in PAH, and will be further investigated.

研究成果の概要（英文）：In the right ventricle (RV) of rats with pulmonary arterial hypertension (PAH), it was found that progressive worsening of RV dysfunction occurred across the time course of PAH. Importantly, we revealed that changes at level of the actin-myosin cross-bridge and myofilament post-translational modifications were an underlying factor of global RV dysfunction in PAH.
Using a prevention model approach, PAH rats were chronically treated with hexarelin to attenuate sympathetic nervous system overdrive. Hexarelin treatment of PAH rats had no affect on RV remodelling or global RV function in PAH rats. Moreover, no effects of hexarelin were observed at the level of the cardiomyocyte or actin-myosin cross-bridge.
Lastly, intervention treatment with the GRK-2 inhibitor, paroxetine, in PAH rats was able to significantly improve RV systolic function and pulmonary hemodynamics compared to the vehicle-treated rats.

研究分野：Cardiovascular Physiology

キーワード：Pulmonary Hypertension　Right Ventricle　Myocardium　Myofilament　X-ray Diffraction　Pressure -Volume Loop

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Pulmonary atrial hypertension (PAH) is a rare but devasting disease where the pulmonary vessels significantly remodel that in turn, substantially increases the afterload upon the right ventricle (RV). The RV initially adapts to this increased afterload, although sustained increases in RV afterload inevitably results in overt RV dysfunction and eventual failure. Previous studies indicate that overactivation of the sympathetic nervous system (SNS) and alterations to cardiomyocyte metabolism are mechanisms known to initially support RV output, although induce severe pathological changes to the RV myocardium leading to RV failure. A direct link between the SNS/metabolic pathways and changes at the level myofilament is yet to be established in the RV and no studies have examined these changes across the progression of PAH.
G protein-coupled receptor kinase 2 (GRK2) is a protein that has been implicated in reducing β-adrenoceptor density and sensitivity in the context of left heart failure. In addition to this, GRK2 has been shown to reduced nitric oxide bioavailability in the myocardium and impair cardiomyocyte metabolism through the CD36 receptor. Ultimately, SNS desensitization, changes in nitric oxide signaling and alterations of cardiomyocyte metabolism can directly influence sarcomeric and myofilament function, leading to RV dysfunction and eventual failure. Therefore, blockade of the SNS with the ghrelin analog, hexarelin, or a GRK2 inhibitor may be novel therapeutic strategies for RV dysfunction and failure in PAH.

２．研究の目的

In this project, I aimed to develop a "whole heart to myofilament approach" to address the research questions. We utilized multiple approaches where global RV function and *in vivo* sarcomere dynamics (actin-myosin cross-bridges), isolated cardiomyocyte mechanics and myofilament proteomics to directly evaluate the role of the SNS and alterations to metabolic pathways in the development of RV dysfunction and failure in PAH. To do this, the project was divided into 3 main studies.

① **Longitudinal study.** This study was undertaken to examine the progressive development of global RV dysfunction in a rat model of PAH and relate this back to changes at the level of the myofilament and sarcomere. **Hypothesis:** Changes in global RV function over the time-course of PAH will be associated with impaired myofilament/sarcomeric function and alterations at the level of myofilament and cytoskeletal proteins. **Aim:** To characterize progressive changes in RV function in PAH and relate these changes back to functional and proteomic alterations in the RV.

② **Prevention study.** As a proof-of-principle, a prevention study design was implemented to assess if hexarelin or the GRK2-inhibitor, paroxetine, could prevent the development of progressive RV dysfunction and failure in a rat model of PAH. **Hypothesis:** Chronic hexarelin or paroxetine treatment will prevent the development of RV dysfunction in PAH. **Aims:** 1) To assess if SNS blockade with hexarelin or GRK2-inhibition with paroxetine will prevent the development of RV systolic and diastolic dysfunction in a rat model of PAH. 2) To determine if the effects of hexarelin and paroxetine are potentiated through direct actions on the cardiac myofilaments/sarcomeres.

③ **Intervention study.** To enhance the clinical relevance of this project, I also proposed an intervention study design where the treatment of hexarelin or paroxetine would begin once PAH and RV dysfunction has been established. **Hypothesis:** Intervention with hexarelin or paroxetine will halt or reverse the progression of RV dysfunction in PAH. **Aims:** 1) Evaluate if intervention treatment with hexarelin or paroxetine will stop or reverse RV myocardial remodeling associated with PAH. 2) Establish if intervention treatment with hexarelin or paroxetine can enhance RV function in PAH. 3) Identify key mechanisms at the level of cardiomyocyte by which hexarelin or paroxetine can improve RV structure and function in PAH.

３．研究の方法

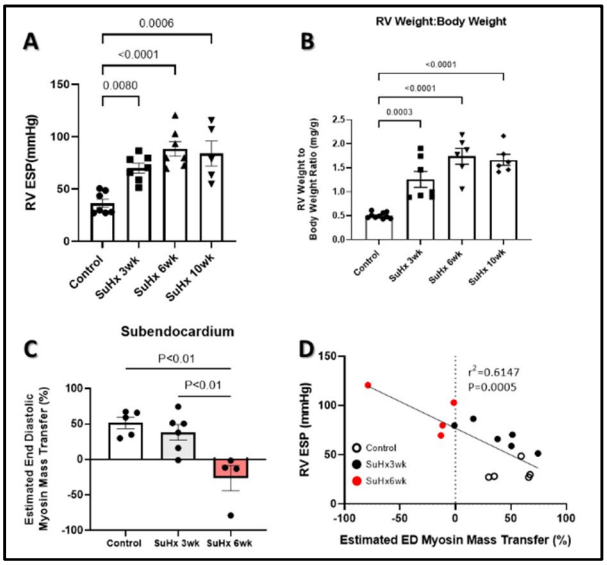
① Longitudinal study. PAH was induced in male Sprague-Dawley (SD) rats using the Sugen5416/10% O_2 hypoxia method, whereby rats received a single injection of Sugen5416 (20mg/kg, s.c.) and were house in 10% O_2 (hypoxia) for 3-weeks. After the hypoxia period, rats were house in normoxic (room air) conditions for a further 3 (SuHx3wk), 6 (SuHx6wk) or 10 weeks (SuHx10wk). Control rats received an equivalent volume of Sugen5416 vehicle (0.5% CMC, 4mL/kg, s.c.) and were housed in normoxic conditions throughout the study. At the end of the normoxic period, rats from the Control, SuHx3wk and SuHx6wk group were anesthetized for the simultaneous measurement of global RV function by pressure-volume (PV) loops and actin-myosin cross-bridge dynamics in the *in situ* beating heart using synchrotron radiation (SR) x-ray diffraction (SAXS). Briefly, once deep surgical anesthesia was established, a full thoracotomy was performed to expose the heart to make an unobstructed path for the x-ray beam. A PV-catheter was inserted into the RV along its long-axis using the apical stab method. SR SAXS and PV-loop recordings were then acquired in different layers of the RV free wall under baseline (lactate, 2mL/h) and β-adrenergic stimulation (dobutamine, 8μg/kg/min) conditions. At the conclusion of the experiments, the cardiac muscle quiescence was induced with an infusion of KCl (0.1M, i.v.) to allow for the calculation of estimated myosin mass transfer to actin. In a separate group of rats, control and SuHx10wk rats were anesthetized and were subjected to RV pressure-volumetry only.

② Prevention study. The prevention study design has been detailed in Waddingham et al. Frontiers in Physiology, 2022. Briefly, PAH was induced in male SD rats as stated above. SuHx3wk and SuHx6wk rats were randomized to receive hexarelin (100μg/kg/day) or an equivalent volume of vehicle (0.9% saline) by a subcutaneously implanted osmotic minipump and daily subcutaneous injections for the final 2 (SuHx3wk) or 5 weeks (SuHx6wk). Control rats received an equivalent volume of sugen vehicle (4mL/kg) and had vehicle solution by osmotic pump/subcutaneous injection and were housed in normoxia for the duration of the study. After the treatment period, rats were subjected to SR SAXS with simultaneous RV pressure-volumetry as stated above and at the conclusion of the experiments, cardiac muscle quiescence was induced with an infusion of KCl (as above).

③ Intervention study. For the intervention study, only the SuHx6wk model was used due to it developing more overt RV dysfunction. PAH was induced in male SD rats as stated above (Waddingham et al. Frontiers in Physiology, 2022). Two weeks after the hypoxic period (i.e. 2 weeks into the normoxic period), SuHx rats were randomized to receive paroxetine (5mg/kg/day) or vehicle (10% DMSO) by a subcutaneously implanted osmotic mini-pump and followed for a further 4 weeks. Control rats were administered the sugen vehicle (0.5% CMC), underwent a sham osmotic minipump implantation and were housed under normoxic conditions throughout the study. After the treatment period, RV hemodynamics and PV-loops were acquired via an open-chest approach using the apical stab method. RV volumes were normalized to stroke volume measured by a flow probe placed around the aorta.

４．研究成果

① Longitudinal study. Over the time course of PAH in rats, there was a progressive and significant increase in RV weight, Fulton Index (RV weight relative to LV + septum weigh) and RV weight relative to body weight. Interestingly, comparing the SuHx6wk and SuHx10wk groups, there were only small differences in RV morphology (Fig. 1) RV systolic pressure also increased over the time course of PAH from 3 to 6 weeks post hypoxia, yet did not significantly change from 6 to 10 weeks post hypoxia (Fig. 1). The RV diastolic relaxation time constant (Tau Logistic) progressively increased over the time course of PAH, indicating a progressive development of diastolic dysfunction (Fig. 1). At the level of the myofilament, SR SAXS in the *in situ* beating heart revealed that in the deeper layers of the RV myocardial free wall, that myosin transfer to actin at end diastole was significantly reduced compared to the both the Control and SuHx3wk rats (Fig. 1). Moreover, end diastolic myosin transfer to actin was negatively correlated with RV systolic pressure (Fig. 1), suggesting that the severity of PAH is



linked the degree of myosin head displacement from actin at end diastole.

Figure 1: RV end systolic pressure (ESP; A) and RV weight to body weight ratio (B) at terminal points in control and SuHx (PAH) rats. SR SAXS estimated end diastolic myosin mass transfer in deeper layer (subendocardium, C) of Control, SuHx3wk and SuHx 6wk rats. The degree of displacement significantly correlated with increasing RV ESP (D). A two-way ANOVA with a Bonferroni's post hoc test was performed to assess differences. Date presented as mean ± SEM.

RV myofilament protein analysis showed reduced phosphorylation and isoform switching of the giant protein, titin, in both SuHx3wk and SuHx6wk rats when compared to controls as well as reduced phosphorylation of MLC-2v at the serine 15 site.

② **Prevention study.** Compared to the control rats, SuHx rats presented with lower body weight, significantly increased heart weight, RV weight and RV weight relative to body weight, consistent with the PAH phenotype. Chronic hexarelin treatment had no significant effect on body weight, heart weight, RV weight or RV weight relative to body weight. As expected, RV end systolic pressure was significantly higher in SuHx rats compared to the control rats and chronic hexarlein treatment had no RV end systolic pressure. Given that the RV contractility index was virtually identical between the groups, this strongly suggests that RV systolic function was maintained in the SuHx3wk model, further highlighting the notion that this model is still in the adaptive phase of RV remodeling in response to PAH. Two-way ANOVA analysis of the SR SAXS revealed differences between the groups with respect to end diastolic myosin mass transfer to actin. In all myocardial layers of the RV free wall, SuHx3wk rats exhibited an end diastolic myosin mass transfer ratio closer to the quiescent value than control rats, but this difference was more pronounced in the deeper myocardial layers (Fig. 2). Chronic hexarelin treatment in the SuHx3wk and SuHx6wk rats had no effect on diastolic myosin mass transfer (Fig. 2). For the myosin interfilament spacing, SuHx3wk rats treated with hexarelin had a ~1nm smaller interfilament spacing when compared to controls. There was no difference between the SuHx6wk hexarelin treated rats or any other group. These data indicate that SuHx3wk rats were operating at a longer sarcomere lengths relative to control rats. These findings have been published with the following citation: Waddingham MT et al. *Frontiers in Physiology*. 12:766818 2022.

	Control vehicle	SuHx3wk vehicle	SuHx3wk hexarelin
Hemodynamics			
MAP	112 ± 6	114 ± 6	106 ± 9
Heart rate (BPM)	345 ± 15	302 ± 19	310 ± 18
RV systolic indices			
ESP (mmHg)	46 ± 3	**88 ± 9***	**101 ± 11****
dP/dt_{max} (mmHg/s)	1,732 ± 191	3,064 ± 484	**3,752 ± 506***
Contractility Index (1/s)	64 ± 5	67 ± 5	69 ± 3
RV diastolic indices			
EDP (mmHg)	7.4 ± 0.7	10.2 ± 2.0	12.4 ± 1.8
dP/dt_{min} (mmHg/s)	−1,056 ± 123	**−2,713 ± 369***	**−3,351 ± 422****

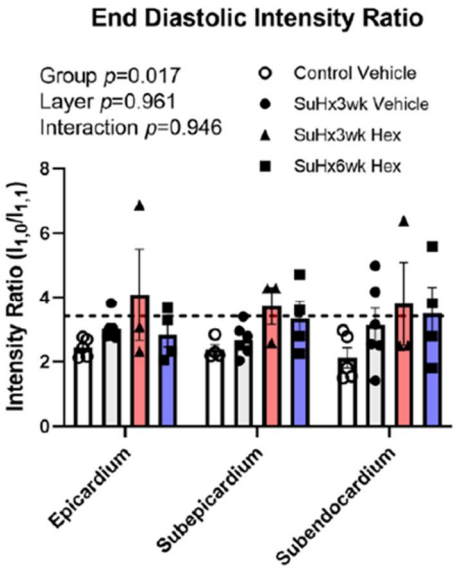


Figure 2: Hemodynamics and RV function indices from the prevention study (Left panel). Bolded values indicate significant differences. End diastolic myosin mass transfer from SR SAXS in the prevention study. Data presented as mean ± SEM. Adapted from Waddingham et al. Frontiers in Physiology, 2022.

③ Intervention Study. As in the *Longitudinal study*, SuHx6wk rats exhibited a significant increase in normalized heart and RV weights and Fulton index when compared to the control group. Moreover, SuHx6wk rats demonstrated increased RV cardiomyocyte size, RV interstitial fibrosis, myosin heavy chain and titin isoform switching that are all consistent with overt RV dysfunction and the development of RV failure. Chronic paroxetine treatment for 4-weeks did not affect heart or RV weights, Fulton index, cardiomyocyte size, myosin heavy chain or titin isoform switching. However, chronic paroxetine treatment did have a variable effect on reducing RV interstitial fibrosis but did not reach statistical significance. When compared to control rats, SuHx6wk rats developed RV systolic dysfunction as evidenced by significantly lower stroke volume and cardiac output and RV diastolic dysfunction with elevated RV end diastolic pressure and prolonged Tau relaxation time constant (Fig. 3). Unsurprisingly, SuHx rats were also found to have pronounced elevations in RV afterload and total pulmonary resistance by comparison to control rats (Fig. 3). Paroxetine treatment in SuHx rats significantly improved RV systolic function along with reducing RV afterload. At the level of the myofilament, PAH in rats was associated with a significant reduction in titin and myosin light chain-2 phosphorylation, which also significantly enhanced with chronic paroxetine treatment. Improvements in RV systolic function in SuHx rats treated with paroxetine may be attributed to elevated myofilament protein phosphorylation status.

	Control (n=6)	SuHx+Vehicle (n=7)	SuHx+Paroxetine (n=6)
Hemodynamics			
Mean Arterial Pressure (mmHg)	87±6	88±6	76±7
Heart Rate (BPM)	305±16	255±6	271±14
Arterial Elastance ($mmHg \cdot \mu L^{-1}$)	0.12±0.01	**0.84±0.05**	**0.64±0.07**
Total Pulmonary Resistance ($mmHg \cdot mL^{-1} \cdot min^{-1}$)	0.28±0.02	**2.10±0.17**	**1.38±0.25**
RV Systolic Function			
End Systolic Pressure (mmHg)	24±1	**96±3**	95±4
Stroke Volume (μL)	204±9	**116±6**	**162±19**
Cardiac Output ($mL \cdot min^{-1}$)	63±6	**30±2**	**44±6**
RV Diastolic Function			
End Diastolic Pressure (mmHg)	4.2±0.6	**7.1±0.6**	6.2±0.7
Tau Logistic (ms)	7.3±0.4	**9.9±0.4**	8.8±0.7

Figure 3: Hemodynamic and RV functional parameters from Control, SuHx+Vehicle and SuHx+Paroxetine rats in the Intervention Study. Values highlighted in red indicate P<0.05 vs. Control and values highlighted in green indicate P<0.05 vs. SuHx+Vehicle. Chronic paroxetine treatment improved RV systolic function and reduced RV afterload. Data presented as mean ± SEM.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Waddingham Mark T.、Tsuchimochi Hirotsugu、Sonobe Takashi、Asano Ryotaro、Jin Huiling、Ow Connie P. C.、Schwenke Daryl O.、Katare Rajesh、Aoyama Kohki、Umetani Keiji、Hoshino Masato、Uesugi Kentaro、Shirai Mikiyasu、Ogo Takeshi、Pearson James T.	４．巻 12
２．論文標題 Using Synchrotron Radiation Imaging Techniques to Elucidate the Actions of Hexarelin in the Heart of Small Animal Models	５．発行年 2022年
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掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fphys.2021.766818	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Waddingham MT, Tsuchimochi H, Sonobe T, Pearson JT, Ogo T
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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関