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Original Article

Evaluation of cardiac electrophysiological properties in an experimental model of right ventricular hypertrophy and failure*

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Abstract *Background:* Malignant arrhythmias are a major cause of sudden cardiac death in adults with congenital heart disease. We developed a model to serially investigate electrophysiological properties in an animal model of right ventricular hypertrophy and failure. *Method:* We created models of compensated (cHF; n = 11) and decompensated (dHF; n = 11) right ventricular failure in Wistar rats by pulmonary trunk banding. Healthy controls underwent sham operation (Control; n = 13). Surface electrocardiography was recorded from extremities, and inducibility of ventricular tachycardia was evaluated in vivo by programmed stimulation. Isolated right ventricular myocardium was analysed for mRNA expression of selected genes. *Results:* Banding caused an increased mRNA expression of both connexin 43 and the voltage-gated sodium channel 1.5, as well as a prolongation of PQ, QRS and QTc intervals. Ventricular tachycardia was induced in the majority of banded animals compared with none in the healthy control group. No differences were found between the two degrees of failure in neither the electrophysiological parameters nor inducibility. *Conclusions:* The electrophysiological properties of rat hearts subjected to pulmonary trunk banding were significantly changed with increased susceptibility to ventricular tachycardia, but no differences were found between compensated and decompensated right ventricular failure. Furthermore, we demonstrate that in vivo electrophysiological evaluation is a sensitive method to characterise the cardiac electric phenotype in an experimental rat model.

Keywords: Heart failure; ventricular tachycardia; pulmonary trunk banding; adults with congenital heart disease; right ventricle

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Malignant arrhythmias are a major cause of sudden cardiac deaths in adults with congenital heart disease. Arrhythmias in adults with congenital heart disease are heterogeneous and vary with the anatomical features specific to the individual structural lesion as well as the type of corrective surgery chosen.[1] Focusing on the malignant arrhythmia, ventricular tachycardia has proven to be the most frequent.[1,2] Ventricular tachycardia can arise from the structural lesions of the congenital heart disease itself or from the post-operative myocardial scarring of the ventricle; however, increased haemodynamic stress applied to the corrected heart may also give rise to an abnormal myocardial substrate capable of inducing ventricular tachycardia.[3]

The search for valid risk stratification to identify patients with increased risk for malignant arrhythmias has been extensive. Abnormal function of the systemic ventricle, which in many cases of congenital heart disease is the morphologic right ventricle,[4] has proven to be one of the strongest risk factors. Severe enlargement, extensive fibrosis, and decreased function of the right

*All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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ventricle are also individual risk factors for sudden cardiac death.[5–7] The maladaptation of the right ventricle causes changes in the conduction system, prolonging the QRS duration, which for a long period has served as the most used measure of risk identification.[8] Despite the vast number of risk associations found, the mechanisms linking the hypertrophied and failing right ventricle with malignant arrhythmias are still sparsely understood.

Owing to a heterogeneous patient population and low number of patients, it has proven to be challenging to obtain the power required for larger prospective follow-up studies.[2] Establishing a pre-clinical model for evaluating the electrophysiological re-modelling and arrhythmogenic properties of the hypertrophied and failing right ventricle could provide new essential knowledge for the effective designing of future clinical trials and understanding of the mechanisms of malignant arrhythmias in patients with a hypertrophic and failing right ventricle.

The aim of this study was to establish a rat model that allows serial electrophysiological evaluations from onset of acute pressure overloading of the right ventricle to chronic hypertrophy and heart failure.

Materials and methods

Study design

Male Wistar Galas rats (M&B Taconic, Ry, Denmark) were randomised to pulmonary trunk banding with a diameter of 0.6 mm (n = 11), 0.5 mm (n = 11), or SHAM surgery (n = 13); seven weeks after surgery, right ventricular function was evaluated by echocardiography, MRI, pressure/volume measurements, anatomical measures, and clinical signs of heart failure. Before invasive measures, a surface electrocardiogram was recorded, and at the end of the protocol programmed electric stimulation was performed. Finally, blood samples were collected and the animals were euthanised.

Additional research

The effects of pulmonary trunk banding and the efficacy of treatment with Losartan and Bisoprolol were investigated previously.[9]

Pulmonary trunk banding and haemodynamic evaluations

Banding of the pulmonary trunk and the haemodynamic and histological evaluations of the right ventricle were performed as described in detail previously.[9]

Electrophysiological evaluations

The inducibility of ventricular tachycardia was evaluated in vivo by programmed stimulation using an external heart stimulator (PC-EMS 4.39; Cardiotek, Maastricht, the Netherlands). Surface electrocardiography was recorded from the extremities, and durations of RR, PQ, QRS, and QTc (calculated using Bazett's formula: $QTc = QT/\sqrt{RR}$) were measured. Intra-cardiac signals were derived from the four electrodes of the pressure-volume catheter in the right ventricle (SPR-869; Millar Instruments, Houston, Texas, United States of America). The two distal electrodes were used for stimulation. Ventricular capture was achieved at 2 mA and 1 ms. The pacing protocol consisted of an 8-beat (S_1) drive train with a cycle length of 120 ms. The first extra-stimulus (S_2) was applied after 110 ms and decremental decrease in steps of 5 ms until refractoriness. Subsequently, the S_2 was set at 20 ms outside the refractory period and a second extra-stimulus (S_3) was added and decremented in a similar way. A 5 s pause was inserted between each drive train. Finally, the pacing protocol included burst pacing with a 9-beat (S_1) drive train with a cycle length staring at 120 ms with decremental decreasing steps of 5 ms. The stimulation was terminated at the completion of the protocol or at the induction of ventricular tachycardia. Arrhythmias were considered significant when they lasted >2 s equivalent to ~20 cycles.

Gene expression

A commercial purification kit (NucleoSpin® RNA II, Macherey-Nagel, GmbH & Co. KG, Neumann-Neander-Straße 6-8, Düren) was used to isolate RNA from snap-frozen right ventricle tissue (−80°C) according to the manufacturer's instructions. The concentration of RNA in the samples was determined using a spectrophotometer (Eppendorf® BioPhotometer, Eppendorf AG, Barkhausenweg 1, Hamburg, Germany). Total RNA was reverse transcribed into complimentary DNA (RevertAid First Strand cDNA Synthesis Kit; Thermo Fisher Scientific Inc., Waltham, MA, USA) following the standard protocol. Real-time quantitative polymerase chain reaction was performed using Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific) along with specific primers for the following genes: Collagen 1, collagen 3, smooth muscle actin, connexin 43, and α-subunit of the voltage-gated sodium channel 1.5. All measured transcript expression levels were normalised to the reference gene Glyceraldehyde 3-phosphate dehydrogenase and presented relative to the level in healthy control rats.

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Statistics

Unless otherwise stated, normally distributed quantitative data are expressed as mean ± standard error of mean, and non-normally distributed data are expressed as mean with 95% confidence interval. All statistical analyses were performed using GraphPad Prism 6 ($GraphPad software, La Jolla, United States of America). Data were tested for normal distribution using the Shapiro–Wilk normality test and non-parametric tests were used if not normally distributed. $p < 0.05$ was considered statistically significant.

Results

Models of compensated and decompensated right ventricular failure

Banding caused dilatation, hypertrophy, increased fibrosis, and a decrease in capillary density, which are all signs of right ventricular re-modelling. Moderate banding caused a decrease in right ventricular function, indicated by a decrease in tricuspid annular plane systolic excursion, a decrease in cardiac output, and a marked increase in right ventricular systolic pressure compared with healthy controls. Severe banding caused a further deterioration of right ventricular function on all of these parameters in addition to manifestation of extra-cardiac symptoms of heart failure, such as ascites and nutmeg liver. All haemodynamic and histological data have been presented previously.[9]

Baseline electrocardiogram

Standard surface extremity leads were used to record and evaluate baseline electrocardiogram. The RR-interval was increased in animals with decompensated heart failure compared with healthy controls (Control: 150.0 SEM 4.8 ms, dHF: 171.1 SEM 6.3, $p < 0.05$). Meanwhile, the increased RR-interval in animals with compensated failure did not reach significance (Control: 150.0 SEM 4.8 ms, cHF: 161.6 SEM 4.3, $p = 0.09$). The banding also influenced intra-cardiac conduction intervals, electrical depolarisation, and re-polarisation of the ventricles. Durations of PQ, QRS, and QTc were found to be prolonged in animals with compensated as well as decompensated failure compared with healthy controls; however, no differences were found between the two degrees of failure (Fig 1).

In vivo programmed electric stimulation

Before initiating the stimulation protocol, the effective refractory period of the myocardium was determined. Results showed a prolongation in the effective refractory period in animals subjected to both severities of banding compared with controls, whereas no difference was found between the two severities (Fig 2). Using programmed stimulation, we were able to induce sustained ventricular tachycardia in the majority of banded animals compared with none in the healthy control group (Fig 2). The inducibility did not differ between the groups of compensated and decompensated failure (Fig 2). Based on the morphology of the induced ventricular tachycardia on the surface electrocardiogram, we found a superior and left-sided axis in the frontal plane, suggesting that ventricular tachycardias originated from the right ventricle (Fig 3).

Gene expression

To investigate the aetiology of the altered electrophysiological state of the heart, we evaluated the expression of selected genes in the right ventricular myocardium. Increased pressure overload can cause the appearance of stress fibre myofibroblasts, which may cause arrhythmogenic slow conduction and ectopic activity.[10] Therefore, we analysed the expression of the smooth muscle actin at the mRNA level, specific for stress fibre myofibroblasts. No difference was, however, found between either of the failing groups compared with controls (Control: 1.00, 95% confidence interval [0.59, 1.68], cHF: 0.79, 95% confidence interval [0.68, 0.91], $p = 0.43$) (Control: 1.00, 95% confidence interval [0.59, 1.68], dHF: 1.04, 95% confidence interval [0.72, 1.52], $p = 0.90$).

Another possible aetiology of arrhythmia is the altered conduction in the myocardium. We analysed both the expression of connexin 43, an important protein in the gap junctions enabling fast inter-myocyte conduction, and the α-subunit of the voltage-gated sodium channel 1.5, which is responsible for the depolarisation of the cardiomyocyte. We found that both animals with compensated and decompensated heart failure had increased expression of both connexin 43 and the voltage-gated sodium channel 1.5 compared with controls; however, no differences were found between the two degrees of failure (Fig 4).

Finally, we investigated the expression of collagen type I and III. The results showed increased expression of collagen I in both the groups of failure compared with the control group, whereas no differences were found in the expressions of collagen III (Fig 4). The increased level of the stiffer type I collagen coincides with studies of the pressure overloaded heart.[11]

Discussion

Using two distinct severities of pulmonary trunk banding in Wistar rats, we created models of compensated and decompensated right ventricular failure with extra-cardiac manifestations. Banding caused right ventricular re-modelling with increased quantity of arrhythmogenic substrates and qualitative changes in ion channel expressions. Banding also

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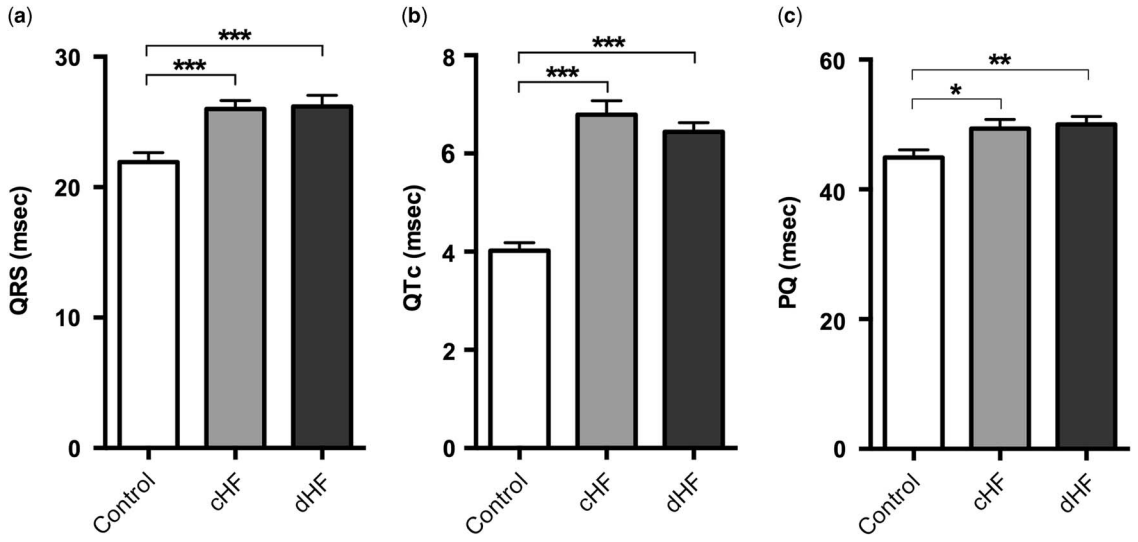

Figure 1.
*Effects of pulmonary trunk banding on the electrophysiological properties of the heart. Compared with controls, animals with compensated (cHF) and decompensated heart failure (dHF) showed elongated durations of QRS (**a**), QTc (**b**), and PQ (**c**) intervals measured in msec. Results are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.*

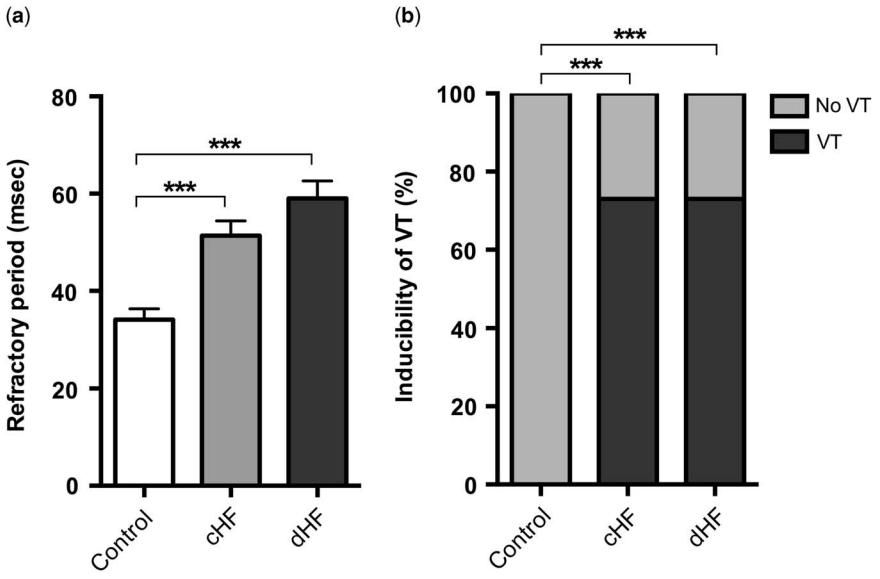

Figure 2.
*Effects of pulmonary trunk banding on the refractory period and the arrhythmogenicity of the right ventricle. Animals with compensated (cHF) and decompensated heart failure (dHF) showed a prolonged refractory period (**a**) measured in ms and depicted as mean ± SEM. The arrhythmogenicity is illustrated as the percentage of animals where induction of ventricular tachycardia (VT) was possible (**b**). *p < 0.05, **p < 0.01, ***p < 0.001.*

caused an impaired conduction in the heart and increased susceptibility to ventricular tachycardia.

Models of compensated and decompensated right ventricular failure

The deterioration of right ventricular function was more pronounced in the rats subjected to severe banding compared with rats subjected to moderate banding. Furthermore, a number of rats subjected to severe banding demonstrated decompensated right heart failure with extra-cardiac manifestations, including nutmeg liver and ascites. The distinctions between compensated and decompensated heart failure in this model have been thoroughly investigated previously.[9] In contrast with other studies of pulmonary trunk banding,[12] this allowed us to study the electrophysiological re-modelling and

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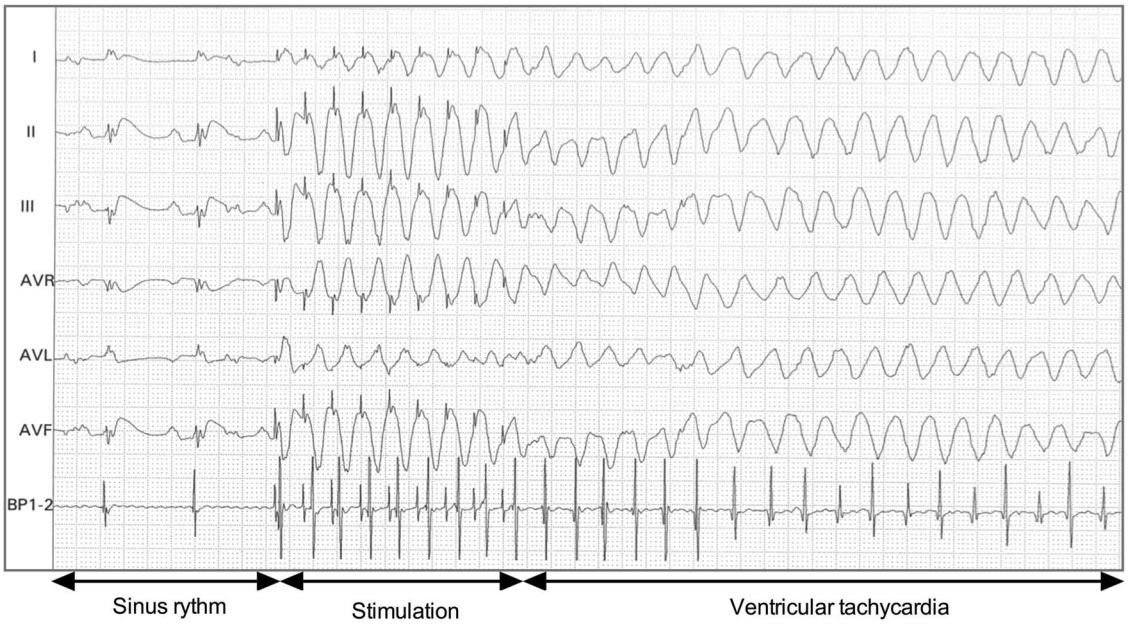

Figure 3.
Electrocardiogram of programmed electric stimulation and ventricular tachycardia. Electrocardiogram showing a representative example of a sinus rhythm followed by programmed electric stimulation and induction of ventricular tachycardia. Standard surface extremity leads are shown as well as an intra-cardiac lead derived from the four electrodes of the pressure-volume catheter in the right ventricle (BP1-2). The writing speed is 100 mm/second.

arrhythmogenicity in animals with both compensated and decompensated right heart failure. To our knowledge, the few studies that have investigated the relationship between right ventricular pressure overload and arrhythmia have used monocrotalin-induced pulmonary hypertension.[13] Monocrotalin causes an inflammatory response in the ventricle, which itself may induce substrates for ventricular arrhythmias.[14] Using pulmonary trunk banding, the afterload applied is fixed and placed at the level of the pulmonary trunk contrary to the unfixed afterload in the pulmonary vascular system induced by monocrotalin. Furthermore, the banding was applied in very young rats causing impaired right ventricular function (decreased cardiac output) as early as 1 week after surgery compared with the more gradual development of afterload in the adult rat monocrotalin models. In the aspect of creating a model of adults with congenital heart disease, the banding model may be more similar to the pathology hereof, whereas monocrotalin may cause a pathology more comparable with pulmonary arterial hypertension.

Electrophysiological properties and arrhythmogenicity

The RR-interval was increased in animals with decompensated heart failure compared with healthy controls. Although the increased RR-interval in animals with compensated failure did not reach significance, the trend, however, suggests an association between increased severity of banding and a lowering of heart rate. We found elongated durations of PQ, QRS, and QTc intervals. This shows impaired conduction throughout the heart cycle in coherence with the hypertrophy, increased fibrosis, and ion channel re-modelling. Prolonged QRS duration has for a long period served as the most used measure of risk identification for sudden cardiac death in adults with congenital heart disease.[8] QTc prolongation is known to be associated with right ventricular hypertrophy and decreased right ventricular function, and is an independent risk factor for sudden cardiac death in patients with pulmonary arterial hypertension.[15] Despite the differences in right ventricular function and levels of extracellular matrix, no difference was found in the baseline electrocardiogram between animals with compensated and decompensated right ventricular failure.

Using programmed electric stimulation, we were able to induce ventricular tachycardia in the majority of banded animals compared with none in the healthy control group. A multicentre study of adults with repaired tetralogy of Fallot found induction of sustained monomorphic ventricular tachycardia to be possible in 30.2%.[16] The higher arrhythmogenicity in our study compared with these results could be

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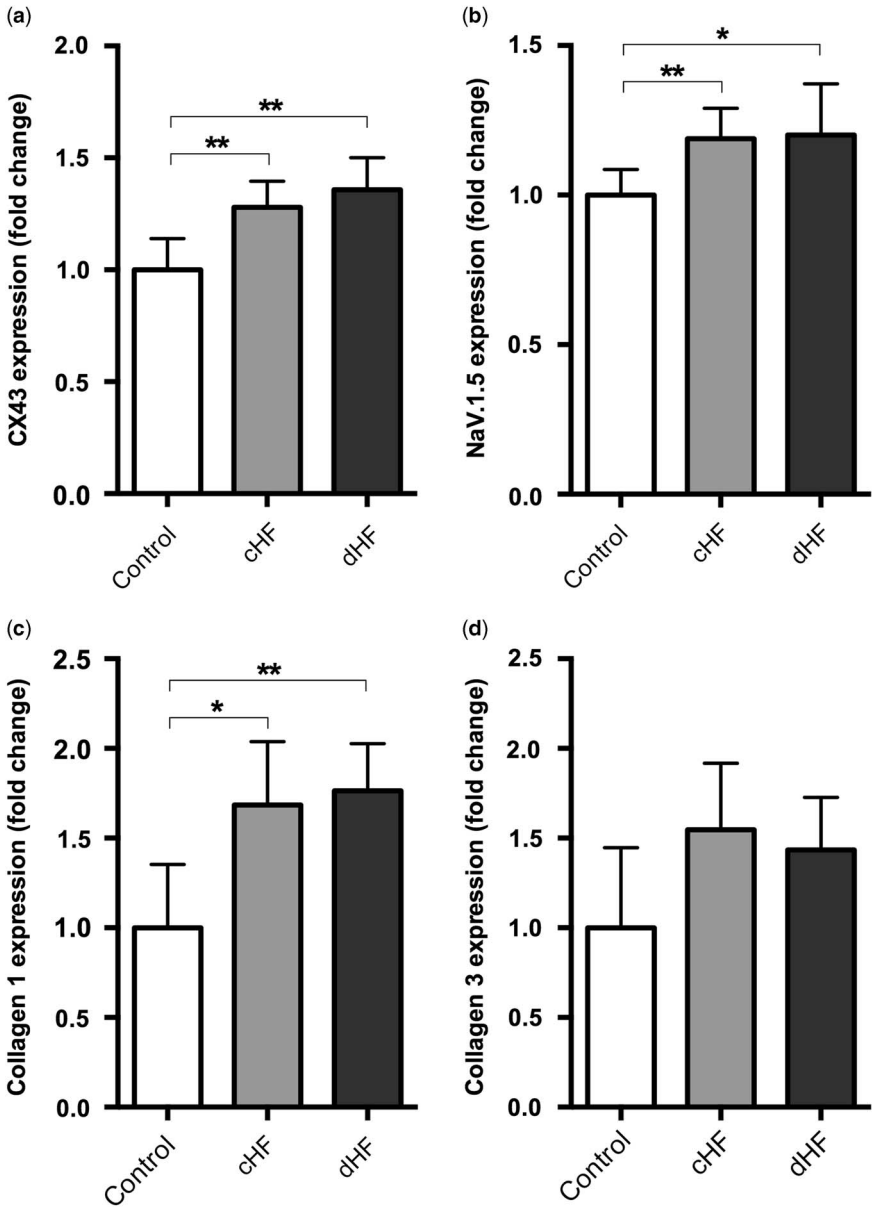

Figure 4.
*Effects of pulmonary trunk banding on gene expression levels of connexin 43, voltage-gated sodium channel 1.5, collagen 1, and collagen 3. Compared with controls, animals with compensated (cHF) and decompensated heart failure (dHF) showed increased levels of mRNA expression of (**a**) connexin 43 (CX43), (**b**) voltage-gated sodium channel 1.5 (NaV1.5), (**c**) collagen 1, and (**d**) collagen 3 qualified by real-time polymerase chain reaction and normalised to glyceraldehyde 3-phosphate dehydrogenase. Data are expressed as relative to the mRNA expression level of controls. Results are mean ± 95% confidence interval. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.*

explained by the severe deterioration of right ventricular function seen in the banded rats; however, the susceptibility did not differ between the groups of compensated and decompensated failure. This suggests that the increased quantity of arrhythmogenic substrates and qualitative changes in ion channel expressions already present in the compensated state caused increased susceptibility to arrhythmia, and that the further deterioration of right ventricular function and increased levels of fibrosis in the decompensated animals did not increase risk of arrhythmia further.

Arrhythmogenic substrates

Banding caused dilatation, hypertrophy, increased fibrosis, and a decrease in capillary density, all signs of undesirable right ventricular re-modelling seen in

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adults with congenital heart disease.[4] Increased cardiac fibrosis may be the source for electric heterogenicity, and thereby promotes re-entrant circuits and arrhytmogenesis.[17] Banding also caused an increased expression of collagen 1, whereas no difference was found in collagen 3 expression. The increased level of the stiffer type I collagen coincided with studies of the pressure overloaded heart.[11] By comparing re-modelling in the two severities of banding, we found that animals with decompensated failure developed further deterioration of right ventricular function, further dilatation, and a higher degree of extracellular matrix, suggesting an increase in arrhythmogenic substrate and arrhythmogenicity. Thus, banding caused an increase in the quantity of arrhythmogenic substrates for ventricular tachycardia comparable with that of human congenital heart disease.[3]

A consequence of the maladaptation of the right ventricle is the re-modelling of the ion channels of the myocytes, causing an altered conduction in the myocardium, and thus increased risk for arrhythmias.[18] We measured mRNA expression levels of two central proteins – connexin 43, the main protein in the gap junction complexes enabling fast inter-myocyte conduction, and the alpha-unit of the voltage-gated sodium channel 1.5, which is responsible for normal depolarisation of the cardiomyocyte. We found that mRNA expressions of both connexin 43 and voltage-gated sodium channel 1.5 were increased in both of the failing groups. Studies have shown a downregulation of functioning gap junctions in failing right ventricles of rats[19] as well as at the level of mRNA expression.[13] Surprisingly, we found an increase in connexin 43 mRNA expression. Whether this over-expression translates to increased levels of functioning gap junctions, or if it is a compensatory mechanism caused by decreasing levels of functioning channels, needs to be investigated further. The increased mRNA expression of the voltage-gated sodium channel 1.5 coincides with studies showing an upregulation of human heart failure.[20] Increased or delayed sodium current may contribute to prolongation of the action potential.[21] This may serve as a compensatory mechanism by increasing intra-cellular calcium, thus contributing to the increased contractility needed in the short-term, meanwhile increasing the risk for arrhythmias.[22] The changes in mRNA expression indicate a degree of ion channel re-modelling in animals with heart failure, which may contribute to an altered conduction in the heart, and thus increased risk for arrhythmias. Despite being associated with qualitative changes in ion channel expression at the level of mRNA, the conduction of the heart is complicated by several possible factors such as abnormal splicing, phosphorylation, and placement of the ion channels, and all these characteristics remain to be investigated.

Limitations

Outbred Wistar rats were used for this study. This should be taken into consideration and experiments in other animal models using different strains should be performed before clinical translation. The number of rats used in this study should also be taken into consideration. Rats were anaesthetised for measuring all the haemodynamic parameters. This may blunt the differences between groups. To minimise the effects of anaesthesia on the results, we followed a well-tested protocol of anaesthesia carefully.

Conclusion

The electrophysiological properties of rat hearts subjected to pulmonary trunk banding were significantly changed with increased susceptibility to ventricular tachycardia. This study demonstrates that cardiac arrhythmogenic changes develop in the hypertrophied and failing right ventricle of the banded rat heart, and that catheter-based in vivo electrophysiological evaluation is a sensitive method to characterise the cardiac electric phenotype in an experimental rat model.

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Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the Danish law for animal research and have been approved by the institutional ethics review board (authorisation number: 2012-15-2934-00384 Danish Ministry of Justice).

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