Early changes in rat hearts with developing pulmonary arterial hypertension can be detected with three-dimensional electrocardiography

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Henkens IR, Mouchaers KTB, Vliegen HW, van der Laarse WJ, Swenne CA, Maan AC, Draisma HHM, Schalij I, van der Wall EE, Schalij MJ, Vonk-Noordegraaf A. Early changes in rat hearts with developing pulmonary arterial hypertension can be detected with three-dimensional electrocardiography. Am J Physiol Heart Circ Physiol 293: H1300–H1307, 2007. First published May 25, 2007; doi:10.1152/ajpheart.01359.2006.—The study aim was to assess three-dimensional electrocardiogram (ECC) changes during development of pulmonary arterial hypertension (PAH). PAH was induced in male Wistar rats (n = 23) using monocrotaline (MCT; 40 mg/kg sc). Untreated healthy rats served as controls (n = 5). ECCs were recorded with an orthogonal three-lead system on days 0, 14, and 25 and analyzed with dedicated computer software. In addition, left ventricular (LV)-to-right ventricular (RV) fractional shortening ratio was determined using echocardiography. Invasively measured RV systolic pressure was 49 (SD 10) mmHg on day 14 and 64 (SD 10) mmHg on day 25 vs. 25 (SD 2) mmHg in controls (both P < 0.001). Baseline ECCs of controls and MCT rats were similar, and ECCs of controls did not change over time. In MCT rats, ECC changes were already present on day 14 but more explicit on day 25: increased RV electromotive forces decreased mean QRS-vector magnitude and changed QRS-axis orientation. Important changes in action potential duration distribution and repolarization sequence were reflected by a decreased spatial ventricular gradient magnitude and increased QRS-T spatial angle. On day 25, LV-to-RV fractional shortening ratio was increased, and RV hypertrophy was found, but not on day 14. In conclusion, developing PAH is characterized by early ECC changes preceding RV hypertrophy, whereas severe PAH is marked by profound ECC changes associated with anatomical and functional changes in the RV. Three-dimensional ECC analysis appears to be very sensitive to early changes in RV afterload.

PULMONARY ARTERIAL HYPERTENSION (PAH) is a rare and severe disease of the afferent pulmonary vasculature, characterized by a progressive increase in pulmonary vascular resistance and overloading of the right side of the heart (6). In patients with developing PAH, there is generally a considerable delay between the onset of pulmonary vasculature loss and the onset of PAH-related symptoms (12, 18, 27, 32). Diagnosis of PAH is therefore often delayed (25). Hence, a simple noninvasive diagnostic test for PAH is warranted to allow earlier detection of the disease (25). The routine electrocardiogram (ECC) is a very simple test but has proven to be of limited value in the evaluation of patients with suspected PAH (2, 25). In rats it has been demonstrated that pulmonary hypertension precedes right ventricular hypertrophy, where the latter can be detected with sequentially recorded ECCs (5). The vectorcardiogram (VCG) has been considered of additional value to ECC analysis, since it renders different information and allows calculation of parameters that cannot be computed from separate ECC leads (11, 13, 14). However, the potential value of sequentially recorded VCGs for detection of changes in developing pulmonary hypertension has not been studied. Information recorded by three orthogonally oriented bipolar leads can be readily reconstructed into a three-dimensional VCG with the help of dedicated software. Since the right ventricle (RV) has a lower mass than the left ventricle (LV) in both rats (5, 21) and humans (22), RV electrical activity is largely masked by the LV electrical activity under normal conditions (28). We hypothesized that an increasing RV workload, elicited by progressive loss of pulmonary vasculature in PAH, would trigger a corresponding degree of RV hypertrophy, inducing three-dimensional body surface ECC changes (7). We chose to investigate the evolution of three-dimensional body surface ECC abnormalities in a rat model of pulmonary hypertension. In addition, we evaluated RV and LV contractility using echocardiography and determined RV hypertrophy by measuring mean cross-sectional area of RV cardiomyocytes. We investigated whether ECC abnormalities precede echocardiographic abnormalities and RV hypertrophy. Invasively measured RV systolic pressure served as the gold standard for presence of PAH.

MATERIALS AND METHODS

Experimental setup. This study was performed in accordance with the national guidelines and with the permission of our institutional animal ethics and welfare committee. Male Wistar rats (Harlan Laboratories, Horst, The Netherlands) weighing 180–200 g were used in this study (n = 28). PAH was induced by a single subcutaneous injection of monocrotaline (MCT; 40 mg/kg, n = 23; Sigma-Aldrich, Steinheim, Germany) dissolved in 0.9% NaCl (8 mg/ml) at pH 7.4. Untreated healthy rats received an equal volume of saline alone and served as controls (n = 5). The local animal ethics and welfare committee ruled that an experiment involving rats exposed to 40 mg/kg MCT should not be extended beyond 25 days, since comparable doses had led to end-stage heart failure and premature death in other experimental setups (8). Animals were housed two per cage with a 12:12-h light-dark cycle. Food and water were available ad libitum. This study protocol was performed parallel to an ongoing project.
aimed at elucidating changes in pulmonary vasculature in PAH and the effects of medication on these changes. As such, this protocol was designed as a reversal study in which rats injected with MCT received either placebo (n = 10) or one of three drugs: the dual endothelin receptor antagonist bosentan (100 mg·kg\(^{-1}\)·day\(^{-1}\), n = 4), the phosphodiesterase-5 inhibitor sildenafil (1 mg·kg\(^{-1}\)·day\(^{-1}\), n = 4), or the Rho-kinase inhibitor fasudil (30 mg·kg\(^{-1}\)·day\(^{-1}\), n = 5). Drugs were dissolved in 2 ml of commercially available vanilla pudding, which served as vehicle. Drugs were administered orally from day 14 onward. Untreated healthy and MCT rats received vehicle alone. On day 0 (before MCT injection), on day 14, and on day 25, a body surface ECG and echocardiogram were recorded. We chose to perform measurements on day 14, since elevated pulmonary arterial pressures were reportedly present at this time after administration of similar doses of MCT (23). Before ECG recording and echocardiography, rats were anesthetized by inhalation of 4% isoflurane. Anesthesia was maintained under 2% isoflurane administration. All rats breathed spontaneously throughout this procedure.

**RV pressure measurements.** After completion of ECG and echo recordings, right ventricular systolic pressure (RVSP) was measured in 8 MCT rats on day 14 and in 15 MCT rats and 5 controls on day 25. Before the procedure, rats were intubated with a 16-gauge plastic venflon that was inserted directly into the trachea. Animals were subsequently attached to a mechanical microventilator (UNO, Zevenaar, The Netherlands), ensuring a breathing frequency of 75 breaths/min with an intermittent positive pressure ventilation/positive end-expiratory plateau (IPPV/PEEP) of 15-5 mbar (control) or 8-2 mbar (MCT). PEEP was kept lower in MCT rats to avoid ventilator-induced lung injury. Pressure measurements were performed using a Millar pressure catheter (Millar, Houston, TX) that was directly inserted through the apical RV free wall after right lateral thoracotomy through the fifth intercostal space. RVSP was measured for 10 s and averaged. Data were obtained using a PowerLab setup (ADInstruments, Castle Hill, NSW, Australia). After RV pressure measurement, rats were killed. Before animal death, isoflurane administration was increased again to 4% and the absence of reactivity to external stimuli was verified. During the entire procedure, body temperature was monitored and maintained at 37°C with a controlled heating pad.

**RV hypertrophy.** Hematoxylin and eosin staining was performed on cross sections of each heart as described by des Tombe et al. (9) to determine the degree of cardiomycocyte hypertrophy in both ventricles. The cross-sectional area (CSA) of 40 randomly chosen cardiomycocytes in the RV was measured. In addition, sarcomere length was randomly determined in five areas of each ventricle, where cardiomycocytes were cut along their longitudinal axis. CSA was then normalized on a sarcomere length of 2 μm to correct for variation between sarcomere lengths, which makes comparison between different hearts feasible. In addition, the occurrence of enduring ischemia was determined by staining for cytosolic cytochrome c release (4).

**Echocardiography.** Body surface ECGs were made with rats in the supine position. ECGs were recorded using five subcutaneously placed needle electrodes: one on each limb and one chest electrode centrally placed over the fourth intercostal space (Fig. 1).

A resistance network was used to derive three ECG leads, equivalent to Einthoven’s lead I, lead aVF, and the inverted average of leads V1 and V2. In the following, these three leads are treated as vectorcardiographic leads X (right to left), Y (cranio-caudal direction), and Z (anteroposterior direction). Orientation of the X-, Y-, Z-axis is according to the American Heart Association recommendations (20). The fifth electrode on the right hindlimb functioned as a reference electrode. The three orthogonal ECG signals were recorded with a 2,000-Hz sampling rate. The registration setup (PowerLab) was optimized with respect to grounding and shielding to keep background noise to a minimum. Registration was performed with a minimum duration of 1 min to allow for beat selection and subsequent averaging, to further improve the signal-to-noise ratio. All ECGs were recorded without electronic filters and were processed off-line.

**Electrocardiographic analysis.** ECGs were analyzed using LEADS, our dedicated electrocardiography analysis program (11). In short, LEADS automatically selects beats for averaging on the basis of signal quality criteria (baseline, noise). This selection of beats is then reviewed and edited by the investigator. The beats are then averaged by LEADS. After the onset and end of QRS-complex and the end of T-wave in the averaged beat are manually reviewed and edited, vectorcardiographic calculations are automatically performed.

**Electrocardiographic parameters.** Depolarization was characterized by QRS duration, the orientation of the QRS-axis, and the mean QRS vector magnitude. QRS-axis orientation with unit radius was decomposed into its X, Y, and Z components for comparison of orientation over time. Concordance/discordance of depolarization and repolarization on the ECG was characterized by the spatial QRS-T angle (the angle between the spatial orientation of the QRS- and T-axis) (26). Action potential duration heterogeneity was characterized by the spatial ventricular gradient (VG) magnitude (10). All parameters were derived from the averaged beat, using information from the three orthogonal leads.

**Echocardiography.** The average heart rate of a rat is approximately five times higher than that of humans, precluding real-time appreciation of cardiac function with echocardiography. We chose to perform echocardiography with a straightforward, easily reproducible approach, capturing both LV and RV during the cardiac cycle. RV and LV short-axis images were made in B-mode and M-mode, using a ProSound SSD-4000 PureHD echo machine ( Aloka, Tokyo, Japan). End-diastolic and end-systolic diameters (EDD and ESD, respectively) for both RV and LV were measured perpendicularly to the interventricular septum at midseptal level. EDD and ESD were used to calculate fractional shortening with the following formula: fractional shortening = [(EDD - ESD)/EDD] × 100%. Since comparison of individual RV and LV fractional shortening over time is sensitive to changes in echo probe positioning along the longitudinal cardiac axis, we used the ratio of LV fractional shortening to RV fractional shortening (LV/RV fractional shortening) to describe changes in cardiac function.

**Statistical analysis.** All data sets were randomized before analysis by observers (I. R. Henkens and K. T. B. Mouchaers) who were blinded to treatment groups. SPSS for Windows software (version 12.0.1; SPSS, Chicago, IL) was used for data analysis. Normality distributed values are presented as means and their standard deviations (SD) in parentheses. Values not normally distributed are presented as medians and their minimum and maximum values in parentheses. Independent t-tests were used for comparison of controls and MCT rats. Sequential measurements within groups of similarly treated rats were compared with paired t-tests. A P value <0.05 was considered to be statistically significant.

**RESULTS**

**RV systolic pressure and hypertrophy.** On day 14 all MCT rats already had elevated RVSP compared with controls (Fig. 2A). However, RV hypertrophy was not yet present at this time, as demonstrated by a mean cross-sectional cardiomycocyte area of 286 (SD 23) μm\(^2\), which was not different from 274 (SD 44) μm\(^2\) in controls (P = 0.52) (Fig. 2, B and C). On day 25, all MCT rats, regardless of therapy, had severe PAH (Fig. 2A). At this time MCT rats showed marked RV hypertrophy, with a considerably higher mean CSA of RV cardiomycocytes of 476 (SD 65) μm\(^2\) compared with both controls.
and MCT rats on day 14 (both $P < 0.001$) (Fig. 2, B and C). LV cardiomyocyte dimensions were not different between MCT rats and controls. MCT rats were negative for cytochrome c release, indicating that myocardial perfusion was adequate despite marked hypertrophy in MCT rats.

**Body surface ECGs and echocardiograms.** Of the 76 recorded body surface ECGs, 2 (2.6%) were not interpretable because of 50-Hz background noise. Suitable for analysis were 28 registrations on day 0, 28 registrations on day 14, and 18 registrations on day 25. Out of 76 echocardiographic registrations performed, 72 (94.7%) were suitable for interpretation of RV and LV fractional shortening.

**ECGs at baseline.** There was no difference at baseline between rats receiving saline and rats receiving MCT with respect to heart rate, QRS duration, orientation of the QRS-axis, mean QRS vector magnitude, QRS-T spatial angle, or VG magnitude. There were no rats with a bundle branch block configuration in the ECG.

**ECGs after 14 and 25 days.** Controls did not show ECG changes on day 14 or day 25. MCT rats, however, showed marked changes in ECG characteristics on day 14 compared with baseline, which had further evolved on day 25 (Table 1). ECG changes were not different for MCT rats receiving treatment compared with MCT rats receiving placebo. In addition, ECG changes were also not different between rats receiving different medications (bosentan, sildenafil, or fasudil). New onset bundle branch block was not observed.

On day 14, heart rate was lower in MCT rats than on day 0. Furthermore, depolarization changes were present in MCT rats,
as well as changes in concordance of depolarization and repolarization and changes in action potential duration heterogeneity. The increased RV contribution to the electromotive forces was demonstrated by an important decrease in mean QRS vector magnitude. Furthermore, there was a change in three-dimensional QRS-axis orientation, most notably in the Z direction. The suggested evolutionary mechanism for the observed changes on day 14 and day 25 is presented in the DISCUSSION. Of note, VG magnitude declined, whereas QRS-T spatial angle increased, signifying an alteration in both action potential duration heterogeneity and repolarization sequence.

On day 25, ongoing development of PAH had resulted in marked changes in both depolarization and repolarization characteristics in MCT rats, compared with both baseline and day 14 (Table 1).

The sphere plot of QRS-axes (orientation and projections on the transverse, frontal, and sagittal plane) illustrates the changes in spatial orientation 14 and 25 days after administration of MCT compared with baseline (Fig. 3).

Echocardiography. A typical illustration of echocardiographic images obtained at baseline, on day 14, and on day 25 is shown in Fig. 4A. LV/RV fractional shortening was un-

<table>
<thead>
<tr>
<th>3-D ECG Variables</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 25</th>
<th>Day 0 vs. Day 14</th>
<th>Day 0 vs. Day 25</th>
<th>Day 14 vs. Day 25</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>419 (25)</td>
<td>400 (24)</td>
<td>328 (27)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>QRS duration, ms</td>
<td>15.6 (2.3)</td>
<td>16.5 (2.2)</td>
<td>17.9 (3.1)</td>
<td>0.19</td>
<td>0.066</td>
<td>0.085</td>
</tr>
<tr>
<td>QRS X component</td>
<td>0.08 (0.17)</td>
<td>0.18 (0.28)</td>
<td>0.03 (0.15)</td>
<td>0.01</td>
<td>0.521</td>
<td>0.052</td>
</tr>
<tr>
<td>QRS Y component</td>
<td>0.29 (0.27)</td>
<td>0.27 (0.36)</td>
<td>0.28 (0.32)</td>
<td>0.689</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>QRS Z component</td>
<td>-0.78 (0.49)</td>
<td>-0.55 (0.56)</td>
<td>0.44 (0.81)</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>QRS vector magnitude, µV</td>
<td>318 (169)</td>
<td>175 (98)</td>
<td>274 (170)</td>
<td>&lt;0.001</td>
<td>0.247</td>
<td>0.167</td>
</tr>
<tr>
<td>QRS-T spatial angle, °</td>
<td>32 (30)</td>
<td>49 (46)</td>
<td>146 (45)</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VG magnitude, mV·ms</td>
<td>12.2 (4.3)</td>
<td>9.8 (3.6)</td>
<td>4.4 (2.0)</td>
<td>0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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Values are means (SD) of variables derived from 3-dimensional ECG analysis for monocrotaline (MCT)-treated rats on day 0 (n = 23), day 14 (n = 23), and day 25 (n = 13) showing evolutionary changes during the development of pulmonary arterial hypertension (PAH). P values are for comparisons indicated. VG magnitude, ventricular gradient magnitude.
changed in MCT rats on day 14 but was significantly increased on day 25 (Fig. 4B).

**DISCUSSION**

The key finding of this study is that the development of PAH in rats is associated with a distinct evolution of ECG abnormalities. These ECG abnormalities were already present early in the development of PAH and preceded the onset of both RV hypertrophy and echocardiographic abnormalities. To our knowledge, this is the first report of serial three-dimensional electrocardiography detecting changes early in the development of PAH in animals with the use of a three-lead body surface ECG.

We used right heart catheterization as the gold standard for diagnosis of PAH in rats, similar to the guidelines for patient evaluation (3). Measuring mean CSA of RV cardiomyocytes served to determine RV hypertrophy and echocardiographic abnormalities. To our knowledge, this is the first report of serial three-dimensional electrocardiography detecting changes early in the development of PAH in animals with the use of a three-lead body surface ECG.

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Orthogonal lead system in its most simple form. Our longitudinal three-dimensional ECG analysis rendered variables unique to vectorcardiography, enhancing understanding of RV evolutionary changes during the development of PAH.

On day 14, initial changes in both depolarization and repolarization characteristics were already apparent. The decrease in QRS vector magnitude and the change in three-dimensional QRS-axis orientation imply a change in depolarization characteristics. The change in VG magnitude signifies a change in action potential duration heterogeneity in the ventricles, and the increased QRS-T spatial angle signifies a change in repolarization sequence. In the absence of ventricular conduction delays, these changes are most likely the result of increased cancellation of LV electromotive forces by an augmented RV contribution (Fig. 5) (15, 16).

On day 25, there were marked ECG changes in MCT rats, indicated by depolarization abnormalities, discordance of depolarization and repolarization, and decreased action potential duration heterogeneity. Heart rate was lowered further, and QRS-axis orientation changed dramatically. At the same time, mean QRS vector magnitude “normalized.” Changes in both QRS-axis orientation and “normalization” of mean QRS vector magnitude can be explained by an increased RV contribution to the resultant ventricular depolarization activity (Fig. 5). The further decrease in VG magnitude in MCT rats, despite a normalized mean QRS vector magnitude, can be understood by
taking a closer look at the significant increase in QRS-T spatial angle. The mean QRS-T spatial angle of 146° (SD 45°) in MCT rats on day 25 implies that direction of the T-axis is partially opposite to the QRS-axis, thereby decreasing ventricular gradient magnitude (10). These more pronounced late ECG changes are consistent with the observed abnormalities in LV/RV fractional shortening and the elevated RVSP values that were also present in the late stage of the experiment. In advanced PAH, moderate to severe RV hypertrophy is observed, often with RV dilatation and paradoxical septal movement (29, 31). Together, these anatomical and functional changes may have induced the impressive change in QRS-axis orientation on day 25 in MCT rats (Fig. 5). MCT administration does not affect LV remodeling or LV afterload (23). Hence, ECG changes reflect RV adaptation to the increased pulmonary vascular resistance.

The idea that sequential electrocardiography could detect cardiac changes in developing pulmonary hypertension was put forward by Bruner et al. (5), who observed a rightward shift in the frontal plane of the mean QRS-axis in rats 14 days after direct administration of MCT pyrrole (the active metabolite of MCT). Although the QRS-axis shift was in correspondence with the level of RV hypertrophy, elevated pulmonary artery pressures had been present for 7 days (5). However, two important differences should be noted between the study of Bruner et al. (5) and the current study. First, instead of using MCT pyrrole, we used MCT, causing a significant delay in development of pulmonary hypertension (5). Electrocardiographic changes observed in this study on day 14 are therefore not comparable to the changes observed by Bruner et al. (5) on day 14. Second, the three-dimensional QRS-axis orientation is the basis for QRS-axis orientation in any plane. Therefore, any change in QRS-axis orientation in a plane of choice (e.g., the frontal plane as used by Bruner et al.) can be a meaningful approximation of the true change in three-dimensional QRS-axis orientation when the QRS-axis is oriented in or close to this frontal plane at the time of each measurement. However, when the three-dimensional QRS-axis is oriented more perpendicularly to the plane of choice, a change in three-dimensional orientation may be both largely underestimated and overestimated by the change in QRS-axis orientation in this particular plane. A change in three-dimensional QRS-axis orientation is
although more accurate and reliable than a change in two-dimensional QRS-axis orientation. With the advent of state-of-the-art techniques such as continuous invasive telemetry, future research will likely unravel the true relationship between ECG changes and the onset of elevated pulmonary pressures. Our observation that elevated pulmonary artery pressures precede RV hypertrophy confirms prior reports that RV hypertrophy is a relatively insensitive marker of pulmonary hypertension (5). Lee et al. (21) established the presence of “compensated” RV hypertrophy after 14 days, using 5-wk-old male Wistar rats exposed to a 60 mg/kg dose of MCT. Others demonstrated that RV hypertrophy precedes neurohormonal activation and β-adrenoceptor downregulation (23). In our study, MCT rats showed a progressive decrease in heart rate under anesthesia during development of PAH. This may reflect the increased cardiodepressive effect of anesthesia in the presence of early neurohormonal changes.

MCT-induced PAH is broadly recognized as an experimental model for studying RV hypertrophy as well as treatment effects of PAH-attenuating medication. Although MCT has been shown to primarily affect the RV, with no known effects on LV remodeling or changes in potassium channel expression, a direct effect of MCT on myocardial electrical properties cannot be fully excluded (19, 24, 34). Although the presence of discordance between depolarization and repolarization is a global and rather aspecific marker of ventricular pathology, it is associated with an adverse long-term prognosis (33). Since this particular model only affects RV afterload, such discordance between depolarization and repolarization is most likely a direct consequence of resultant RV hypertrophy in the absence of RV ischemia. Ventricular repolarization sequence becomes abnormal in rats with PAH, given the high spatial angle (Table 1). In fact, changes in RV action potential duration and/or action potential duration heterogeneity are necessary to elicit such changes. However, these changes are by no means indicative of spatial differences in action potential duration distribution or repolarization within the RV. The exact mechanism underlying this phenomenon is beyond the scope of the current study. A RV load-dependent downregulation of voltage-gated potassium channels is likely involved (19, 21). Further research is necessary to appreciate changes in RV myocardium elicited by PAH.

A limitation in our study, which was essentially designed as a reversal protocol, is that the limited time of therapy as well as the relatively low dosages may have precluded a beneficial effect of bosentan, sildenafil, and fasudil on RV pressure overload (1, 17, 30).

In conclusion, developing pulmonary arterial hypertension is characterized by early ECG changes preceding RV hypertrophy, whereas severe pulmonary arterial hypertension is marked by profound ECG changes, associated with anatomical and functional changes in the RV. Three-dimensional ECG analysis appears to be very sensitive to early changes in RV afterload. Having established that developing pulmonary arterial hypertension in the rat is associated with distinct evolutionary ECG changes, this finding must now meet its clinical use by serial ECG analysis in a select group of patients at risk for developing pulmonary arterial hypertension.

**REFERENCES**


