Angiotensin II-induced sudden arrhythmic death and electrical remodeling

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Angiotensin II-induced sudden arrhythmic death and electrical remodeling. Am J Physiol Heart Circ Physiol 293: H1242–H1253, 2007. First published April 6, 2007; doi:10.1152/ajpheart.01400.2006.—Rats harboring the human renin and angiotensinogen genes (dTGR) feature angiotensin (ANG) II/ hypertension-induced cardiac damage and die suddenly between wk 7 and 8. We observed by electrocardiogram (ECG) telemetry that ventricular tachycardia (VT) is a common terminal event in these animals. Our aim was to investigate electrical remodeling. We used ECG telemetry, noninvasive cardiac magnetic field mapping (CMFM) at wk 5 and 7, and performed in vivo programmed electrical stimulation at wk 7. We also investigated whether or not losartan (Los; 30 mg·kg−1·day−1) would prevent electrical remodeling. Cardiac hypertrophy and systolic blood pressure progressively increased in dTGR compared with Sprague-Dawley (SD) controls. Already by wk 5, untreated dTGR showed increased perivascular and interstitial fibrosis, connective tissue growth factor expression, and monocyte infiltration compared with SD rats, differences that progressed through time. Left-ventricular mRNA expression of potassium channel subunit Kv4.3 and gap-junction protein connexin 43 were significantly reduced in dTGR compared with Los-treated dTGR and SD. CMFM showed that depolarization and repolarization were prolonged and inhomogeneous. Los ameliorated all disturbances. VT could be induced in 88% of dTGR but only in 33% of Los-treated dTGR and could not be induced in SD. Untreated dTGR show electrical remodeling and probably die from VT. Los treatment reduces myocardial remodeling and predisposition to arrhythmias. ANG II target organ damage induces VT.

Electrical remodeling involves acquired changes in cardiac structure or function that promote the occurrence of atrial or ventricular cardiac arrhythmias (22). On the molecular level, electrical remodeling involves changes in function and expression of membrane ion channels, gap-junction proteins, Ca2+-cycling proteins, and extracellular matrix composition. All these factors predispose to arrhythmogenic mechanisms such as early and delayed afterdepolarizations and reentry (13). The multifactorial origin of electrical remodeling has been extensively studied in cardiac ischemia and heart failure. However, electrical remodeling in hypertension is less well defined. Patients with hypertension-induced left-ventricular hypertrophy are at increased risk for arrhythmias, which contribute to a twofold increase in cardiovascular mortality (7). Monitoring electrical remodeling is challenging. The standard 12-lead electrocardiogram (ECG), ECG-based body surface potential mapping, and signal-averaged ECG are correlated with an increased risk to develop malignant arrhythmias. However, the positive predictive accuracy is unacceptably low or not sufficiently tested in randomized trials (14).

Multichannel cardiac magnetic field mapping (CMFM) reflects the magnetic fields generated by the myocardial electrical currents occurring during the cardiac cycle. CMFM signals have several advantages: 1) they are little influenced by the tissues between skin and heart, 2) they are sensitive to tangential currents that arise in the border zones of cardiac tissue with different electrophysiological properties such as ischemia, and 3) they consider the track of electrical vortex currents (10). The technical feasibility of recording CMFM signals in small animals has already been shown (31). Brisinda et al. (3) used CMFM to show repolarization changes in aging Wistar rats.

We investigated a double-transgenic, angiotensin (ANG) II-induced rat model (rats harboring the human renin and angiotensinogen genes; dTGR) of target organ damage. Untreated dTGR have four- to fivefold elevated circulating and local ANG II plasma levels and die suddenly between wk 7 and 8 (20). We performed ECG telemetry and found that the animals die of ventricular tachycardia (VT). Therefore, we performed a comprehensive study of CMFM and programmed electrical stimulation in these animals to identify the cause.

METHODS

Animal studies. We studied male transgenic dTGR that were generated on a Sprague-Dawley (SD) background (RCC, Itingen, Switzerland) and age-matched nontransgenic SD rats (Tierzucht Schönwalde, Schönwalde, Germany) (11, 36). The study adhered to American Physiological Society guidelines after local authorities had approved the experiments. In preliminary experiments, we observed that five dTGR outfitted with ECG telemetry died of VT. Prospective experiments were then done. Untreated dTGR (n = 10 at wk 5; n = 18 at wk 7) and SD (n = 8 each at wk 5 and 7) rats were killed at age 5 and 7 wk. Four-week-old dTGR were treated with losartan (Los, 30 mg/kg in the diet; n = 15) and were killed at wk 7. ECG, CMFM, and systolic blood pressure (by tail cuff) were determined at wk 5 and 7. In a second protocol, 7-wk-old rats (dTGR, n = 8; Los, n = 9; SD, n = 7) were subjected to in vivo programmed electrical stimulations.

Immunohistochemistry. Ice-cold acetone-fixed cryosections (6 μm) of cardiac tissue were stained by alkaline phosphatase antialkaline technique and immunofluorescence as described earlier (20). The sections were incubated with the following antibodies: anti-ED-1...
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RESULTS

RNA expression with TaqMan. For RT-PCR, RNA was isolated from left-ventricular tissue following the Trizol protocol (GIBCO Life Technology), and cDNA was transcribed with Superscript II according to the protocol. TaqMan analyses were conducted according to the manufacturer’s instructions by using an Applied Biosystems 7700 Sequence detector and qPCR Sybr Mastermix (Eurogentec). The PCR parameters were as follows: 2 min at 50°C, 10 min at 95°C, and 45 cycles of 15 s at 95°C and 1 min at 60°C. Each sample was measured in triplicate, and expression levels were normalized to 36B4 housekeeper expression. [The TaqMan primer sets for potassium channel subunits Kv4.3, human ether-a-go-go gene (HERG) channel, the sodium-calcium exchanger, and the gap-junction protein Cx43 are available on request.]

ECG and CMFM. Magnetic fields of the rat heart were recorded over the anterior chest wall by using a magnetic measurement system (Cryoton, Moscow, Russia) with seven sensors based on low-temperature superconducting quantum interference devices coupled with axial second-order gradiometers (baseline 55 mm, pickup coil diameter 20 mm). We also performed a standard six-lead ECG simultaneously with all CMFM measurements. Calculation of standard ECG time-interval parameters were performed by two independent operators based on averaged ECG lead II waveforms registered for CMFM time processing (30 s, 1,000 Hz sampling rate). All experimental details, including the correction for heart rate and definition of time points, are described in the supplement. (The online version of this article contains supplemental data.)

ECG telemetry and in vivo programmed electrical stimulation. For continuous ECG monitoring, ECG radiotransmitters (TA10ETA-F20; DSI) were placed intraperitoneally. The two leads were fixed subcutaneously in a standard lead II position. The data were stored for retrospective analysis by two independent cardiologists. Programmed electrical stimulation was performed to test for the inducibility of ventricular arrhythmias. We used a digital electrophysiology lab (EP Tracer; CardioTek). During inhalation anesthesia with isoflurane, the animals’ body temperature was kept constant by using a warming light. Two small octapolar electrophysiology catheters (CIBer mouse cath; NuMed) were placed in the right-atrial and left-ventricular cavities via the right jugular vein and the right carotid artery. Programmed ventricular stimulation was performed by using a standardized protocol that included trains of 10 basal stimuli (S1) followed by up to 3 extra stimuli (S2–S4), delivered with a coupling interval decreasing in steps of 5 ms until ventricular refractory was reached. The stimulation procedures were repeated at three different basal cycle lengths (150 ms, 120 ms, 100 ms). Occurrence and duration of inducible arrhythmias were documented. Only stimulation protocols with reproducible arrhythmias longer than five consecutive beats were considered positive. The numbers of animals with positive protocols as well as the relative portion of positive protocols from all performed stimulation protocols were compared between the groups.

Statistical analysis. Data are presented as means ± SE. Statistically significant differences in means values were tested by Mann-Whitney test using SPSS statistical software. A value of P < 0.05 was considered statistically significant.

RESULTS

Systolic blood pressure in untreated dTGR increased progressively from wk 5 to 7 (152 ± 3 at wk 5 to 198 ± 4 mmHg at wk 7; P < 0.05; Fig. 1A). Los reduced blood pressure to control level (117 ± 4 for Los vs. 119 ± 6 mmHg for SD; P < 0.05; Fig. 1A). At wk 5, untreated dTGR already showed increased heart weight (689 ± 11 mg) and cardiac hypertrophy index (Fig. 1B), expressed as heart weight-to-tibia ratio (26.5 ± 0.4 mg/mm), compared with SD rats (550 ± 24 mg and 21.2 ± 0.9 mg/mm, respectively; P < 0.05). The heart-weight differences were much more prominent at 7 wk (1,151 ± 16 mg for dTGR vs. 933 ± 17 mg for SD; P < 0.05). Los treatment normalized heart weight (905 ± 21 mg; P < 0.05).

Mortality of dTGR around wk 7 is ~40%. We witnessed nine deaths under continuous ECG telemetry, of which five were due to sustained VT degenerating to asystole (5/9 = 56%; Fig. 2A and supplemental Fig. 2, available in the online version of this document). The ECG in the remaining four animals showed ECG alterations like ST shifts hours before death and bradyarrhythmias, which finally resulted in asystole. This finding led to the present study in which dTGR were left untreated or received Los compared with SD controls (Fig. 2B). Standard ECG waveform analysis demonstrated prolonged QRS du-
ration and QT interval in untreated dTGR compared with controls; Los prevented these changes (Fig. 2B). However, the exact determination of the terminal T wave was difficult because of frequent superposition of the following P wave and the reduced information from one or few available leads.

We next analyzed whether the observed increased rate of spontaneous arrhythmias of untreated dTGR correlates with an increased inducibility of ventricular arrhythmias and whether Los treatment affects arrhythmia induction. Figure 3C shows representative recordings of programmed electrical stimulations. In untreated dTGR, reproducible ventricular arrhythmias were inducible in 7 of 8 (88%) animals and in 18 of 24 (75%) performed stimulation protocols (Fig. 3A). In SD controls, the same protocol never initiated significant arrhythmias. In contrast, Los treatment resulted in a significant reduction of arrhythmia induction [3 of 9 (33%) animals, 5 of 27 (19%) performed stimulation protocols; P < 0.05]. Ventricular effective refractory period (Fig. 3B) was significantly prolonged in untreated dTGR compared with SD controls and Los-treated rats (53.5 ± 2 vs. 45.4 ± 2 vs. 41.1 ± 1.7 ms; P < 0.05).

CMFM and ECG parameters are summarized in Table 1. We partitioned the CMFM recordings according to conventional ECG measurements. Heart rate was not significantly different within the age-matched groups. CMFM and ECG both showed that untreated dTGR (wk 7) had a significantly prolonged QRS duration and QT interval compared with controls. Los treatment normalized all time intervals. QT parameters, either corrected for heart rate (QTc) or not, showed similar results.

We also analyzed parameters reflecting the inhomogeneity of depolarization (inhomogeneity index for cardiac magnetic field distribution during late phase of QRS interval) and repolarization (Tpeak-Tend interval) by CMFM. The inhomogeneity index for late QRS was significantly increased in 5- and 7-wk-old dTGR (20.7 ± 3.7 and 24.5 ± 3.7 ms) compared with SD (5.3 ± 1.0 and 10.0 ± 1.5 ms; P < 0.005, respectively). At wk 7, when prolongation of the whole repolarization (QT interval) was not yet apparent, the peak of the dTGR T wave was significantly delayed compared with SD (39.3 ± 0.7 vs. 31.3 ± 0.6 ms; P < 0.001, respectively). At wk 7, QT and QTc intervals increased due to prolongation of the Tpeak-Tend interval in untreated dTGR (68.6 ± 2.3 vs. 54.7 ± 2.0 ms; P < 0.005), indicating an increase in global dispersion of repolarization. Los treatment completely normalized increased Tpeak-Tend values (51.8 ± 2.0 ms). We also found a significant rotation of magnetic field orientation in the beginning of the T wave (first 10 ms after QRS) in untreated dTGR from wk 5 to wk 7 (175 ± 11° to 132 ± 7°; P < 0.005), indicating change in the spatial course of repolarization.

CMFM for QRS and ST-T wave is shown for the groups (Fig. 4, A and B). The maps are a composite of all the animals in the groups and are analogous to relief-contour maps. Red lines are magnetic field vectors directed toward the chest, and blue lines are vectors leaving the chest. The corresponding
The time (milliseconds) of the QRS or ST-T interval is given on the abscissa. The ring number reflects the magnetic field strength. The maps visualize the increase in strength and duration of the QRS in dTGR compared with controls. The course of isolines and the positions of positive (red) and negative (blue) extrema were different in untreated dTGR compared with controls. The difference was particularly marked in the second part of depolarization (maps 13 to 18 ms), directly reflecting change in cardiac magnetic field distribution. Los treatment ameliorated these changes.

**Fig. 3.** A: inducibility of significant ventricular arrhythmias by in vivo programmed electrical stimulation. Given is the relative portion of stimulation protocols with inducible arrhythmias from all performed stimulation protocols. B: increased mean left-ventricular effective refractory period in untreated dTGR compared with controls and reversal by Los treatment. *P < 0.05 vs. SD; #P < 0.05 vs. dTGR. C: representative results of programmed electrical stimulation from all groups. One ECG is shown, simultaneously with endocardial right-atrial (RA) and left-ventricular (LV) electrograms and indicators of delivered electrical stimuli (Stim). Untreated dTGR showed reproducible inducibility of ventricular tachyarrhythmias, whereas in most cases the same stimulations showed no inducibility in Los-treated dTGR. In SD controls, no arrhythmias were inducible.

**Table 1.** Surface electrocardiogram and cardiac magnetic field mapping variables in double-transgenic and Sprague-Dawley rats at 5 and 7 wk and losartan-treated double-transgenic rats at 7 wk

<table>
<thead>
<tr>
<th>Variable</th>
<th>SD, 5 wk</th>
<th>dTGR, 5 wk</th>
<th>SD, 7 wk</th>
<th>dTGR, 7 wk</th>
<th>dTGR + Los, 7 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>468±8</td>
<td>439±16</td>
<td>355±13</td>
<td>365±22*</td>
<td>380±27</td>
</tr>
<tr>
<td>ECG</td>
<td>52.2±1.1</td>
<td>52.9±0.8</td>
<td>51.9±1.3</td>
<td>53.9±1.3</td>
<td>50.9±0.6</td>
</tr>
<tr>
<td>PQ duration, ms</td>
<td>17.0±0.6</td>
<td>21.4±0.4*</td>
<td>18.4±0.5</td>
<td>23.1±0.6#,+</td>
<td>19.3±0.3</td>
</tr>
<tr>
<td>QT duration, ms</td>
<td>105.0±1.2</td>
<td>115.5±4.12*</td>
<td>87.7±2.9</td>
<td>123.4±2.3#</td>
<td>83.5±2.3</td>
</tr>
<tr>
<td>QTc duration, ms</td>
<td>121.0±0.9</td>
<td>127.9±2.8*</td>
<td>88.2±4.4</td>
<td>125.0±5.1#</td>
<td>86.5±4.1</td>
</tr>
<tr>
<td>CMFM</td>
<td>5.3±1.0</td>
<td>20.7±3.7*</td>
<td>10.0±1.5</td>
<td>24.5±3.7#</td>
<td>15.4±1.8#</td>
</tr>
<tr>
<td>Mean inhomogeneity index (late QRS), arbitrary units</td>
<td>18.5±0.8</td>
<td>21.4±0.5*</td>
<td>19.0±0.9</td>
<td>23.0±0.5#,+</td>
<td>20.0±0.2</td>
</tr>
<tr>
<td>QT duration, ms</td>
<td>110.8±1.6</td>
<td>109.0±2.6</td>
<td>83.1±2.0</td>
<td>109.8±3.6#</td>
<td>83.6±5.0</td>
</tr>
<tr>
<td>QTc interval, ms</td>
<td>31.3±0.6</td>
<td>39.3±0.7*</td>
<td>27.9±0.7</td>
<td>39.6±1.6#</td>
<td>28.4±0.8</td>
</tr>
<tr>
<td>Tpeak-Tend interval, ms</td>
<td>64.5±2.4</td>
<td>58.7±3.2</td>
<td>54.7±2.0</td>
<td>68.6±2.3#,+</td>
<td>51.8±2.0</td>
</tr>
<tr>
<td>Mean magnetic field angle (T begin), degrees</td>
<td>344±5</td>
<td>355±11</td>
<td>340±6</td>
<td>312±7#,+</td>
<td>344±8</td>
</tr>
</tbody>
</table>

Values are means ± SE. dTGR, double-transgenic rats; SD, Sprague-Dawley rats; Los, losartan; ECG, electrocardiogram; CMFM, cardiac magnetic field mapping; *P < 0.05 dTGR vs. SD 5 wk; #P < 0.05 dTGR, dTGR + Los vs. SD 7 wk; +P < 0.05 dTGR 7 wk vs. 5 wk.
repolarization, particularly in untreated dTGR at 7 wk, by longer-lasting signal strength (number of isolines). The initial increase in magnetic field strength during the first 10 ms after J point was markedly reduced. The maps show the rotation of the magnetic field distribution in the first 10 ms of ST-T wave segment.

The range at ECG R_{Peak} was already markedly increased in dTGR compared with SD by wk 5 (Fig. 5A). At wk 7, the dTGR R_{Peak} was delayed. Interestingly, the range at ECG R_{Peak} was no longer markedly different in dTGR compared with the other groups, although cardiac hypertrophy had further increased at this time (Fig. 5B). The J point, indicating the end of the QRS interval, is given for SD. At wk 5, the ECG T_{Peak} (given for SD) was markedly delayed in dTGR without a difference in overall repolarization. In contrast, the whole repolarization of 7-wk-old dTGR was markedly prolonged. The ECG T_{Peak} was delayed by almost 12 ms in dTGR. Los treatment corrected these changes in repolarization.

“Butterfly” plots, as composites from CMFM waveforms, reflect differences in magnetic field range and time intervals corresponding to ECG QRS complex and T wave between the groups (Fig. 6). The main focus is the difference in the ST-T wave segment with regard to the dispersion of the T wave peak in different sensor positions. As shown in inserted magnifications of ST-T wave segment, the dispersion of T_{Peak} was increased in dTGR already at wk 5. The difference was more pronounced at wk 7 and was ameliorated by Los treatment.

We next analyzed molecular mechanisms of electrical remodeling in this model. We focused on left-ventricular gene expression of two voltage-gated potassium channels, namely Kv4.3 and the HERG channel, the sodium-calcium exchanger, and the gap-junction protein Cx43. Transient outward Kv4.3 expression was significantly reduced in dTGR at wk 5 and 7 (1.4 ± 0.1 and 3.7 ± 0.7 arbitrary units) compared with controls (2.1 ± 0.1 and 7.2 ± 1.2 arbitrary units; \( P < 0.05 \), respectively). In contrast, Los-treated dTGR at wk 7 had normal Kv4.3 expression (8.9 ± 1.3 arbitrary units). Delayed-rectifier HERG expression was also reduced in dTGR compared with age-matched controls (wk 5, 5.2 ± 0.6 vs. 6.6 ± 0.7; wk 7, 4.9 ± 0.5 vs. 5.7 ± 0.3). However, the reduction did not reach statistical significance. In contrast, Cx43 expression was significantly reduced in 7-wk-old untreated dTGR compared with SD (25.2 ± 1.8 vs. 40.5 ± 7.8 arbitrary units; \( P < 0.05 \)) and was normalized by Los treatment (48.1 ± 6.3 arbitrary units). Sodium-calcium exchanger expression was reduced in 5- and 7-wk-old untreated dTGR (2.1 ± 0.3 and 1.9 ± 0.2 arbitrary units) compared with controls (2.9 ± 0.2 and 3.1 ± 0.4 arbitrary units; \( P < 0.05 \), respectively), whereas Los-treated hearts showed a normal expression level (2.9 ± 0.4 arbitrary units).

We also analyzed matrix formation in the heart. Fibronectin (Fig. 7A) and collagen I (Fig. 7B) were increased in untreated dTGR at wk 5 and 7 (wk 5, 2.9 ± 0.4 and wk 7, 4.2 ± 0.04 arbitrary units; \( P < 0.05 \), respectively) compared with SD controls (both 1.0 ± 0.0 arbitrary units). Los-treated dTGR significantly reduced fibronectin and collagen I expression (1.7 ± 0.2 and 1.5 ± 0.2 arbitrary units; \( P < 0.05 \)). CTGF is a key regulator for matrix formation. Immunohistochemical analysis showed a slightly increased vascular CTGF expression.
at wk 5, which was much more pronounced at wk 7. TaqMan RT-PCR experiments confirmed these findings (wk 5, 3.0 ± 0.3 vs. 1.3 ± 0.2 arbitrary units; wk 7, 8.9 ± 1.0 vs. 2.3 ± 0.3 arbitrary units; P < 0.01, respectively; Fig. 8A). At wk 7, untreated dTGR often develop myocardial microinfarctions with patchy areas of necrosis. In contrast, this result was not observed in either the Los-treated dTGR or SD controls. Serial sections demonstrated that CTGF, collagen I, and macrophages were present at the site of the infarcted area (Fig. 8B). At wk 5, untreated dTGR already showed a significantly higher macrophage infiltration, which further increased at wk 7 and was completely abolished by Los treatment (wk 5, 9.2 ± 0.5 vs. 3.5 ± 0.2 cells per view field; wk 7, 13.2 ± 1.5 vs. 4.0 ± 0.4 cells per view field; Los, 5.3 ± 0.8 cells per view field; P < 0.05; Fig. 8B, inset).

We performed Cx43 immunostainings for an analysis of Cx43 distribution (Fig. 8C). Untreated dTGR showed pronounced changes in Cx43 immunoreactivity compared with SD controls. Los treatment resulted in a nearly normal Cx43 distribution pattern. In untreated dTGR, the structure of intercalated discs appeared disorganized, with broad and diffuse gap-junction plaques. There was an increased diffuse localization of Cx43 in the transitional junction zone and within the cytosol.
Fig. 7. Cardiac fibronectin (A) and collagen I (B) expression. Both matrix proteins were increased in 5- and 7-wk-old dTGR compared with SD. Los reduced matrix formation. Quantification by scoring the intensity of the immunoreactivity confirmed the results. Results are means ± SE. *P < 0.05 vs. 5-wk SD, +P < 0.05 vs. 5-wk dTGR, and #P < 0.05 vs. dTGR + Los and 7-wk SD.
Fig. 8. A: Connective tissue growth factor (CTGF) immunoreactivity and left-ventricular mRNA expression, which was increased in untreated dTGR, particularly at wk 7. Los normalized mRNA and protein expression. B: Patchy areas of necrosis in a 7-wk-old untreated dTGR. Serial sections demonstrated colocalization of CTGF, collagen I, and macrophages (ED-1+ cells). Semiquantification of infiltrated macrophages is given in the inset of B. Results are means ± SE. *P < 0.05 vs. 5-wk SD, +P < 0.05 vs. 5-wk dTGR, #P < 0.05 vs. dTGR + Los and 7-wk SD, and $P < 0.05 vs. 7-wk SD. C: Representative immunofluorescence staining of heart sections in 7-wk-old animals. The sections were stained with polyclonal connexin 43 (Cx43) antibodies. Untreated dTGR showed pronounced changes in Cx43 immunoreactivity compared with SD controls. Los treatment resulted in a nearly normal Cx43 distribution pattern. In untreated dTGR, the structure of intercalated discs seems to be disorganized, with broad and diffuse gap-junction plaques and diffuse Cx43 localization in the transitional junction zone (open arrowheads) and within the cytosol (closed arrowheads).
DISCUSSION

Our findings suggest that hypertensive dTGR may commonly die of VT. We also found that nontreated dTGR show VT, inducible by using in vivo programmed electrical stimulation. Los treatment sharply reduces induction of VT. In normal SD rats, no VT could be initiated by programmed electrical stimulation. Our findings suggest that ANG II leads to VT, probably through electrical remodeling. Furthermore, our findings underscore that CMFM is of use to follow the course of electrical remodeling.

By wk 8, untreated dTGR did not survive. Changes in conventional time-interval variables, interpreted along lines familiar to clinicians, indicated the presence of alterations in the depolarization and repolarization process. The ECG, as a comparative gold standard, confirmed these findings. However, substantially more information on the early processes of electrical remodeling was obtained from the CMFM studies. Contour mapping and butterfly plots reflected marked inhomogeneity among the animals that progressed over time. We also detected alterations in the potassium channel Kv4.3 and exposed changes in the gap-junction protein Cx43. We found a marked increase in interstitial and perivascular fibrosis, CTGF expression, and increased cardiac inflammation. Los treatment normalized the gene-expression alterations, the cardiac damage, the CMFM signs of electrical remodeling, and the arrhythmia vulnerability in programmed electrical stimulation studies.

Wijffels et al. (37) introduced the term electrical remodeling in 1995. They described the occurrence of cardiac arrhythmias due to acquired changes in cardiac structure or function. Inflammation, fibrosis, and changes in tissue architecture are known to play an important role in this pathophysiological process (33). We showed previously (25) that dTGR feature cardiac hypertrophy with increased wall thickness and diastolic dysfunction. There is growing evidence that diastolic dysfunction on the basis of altered Ca\(^{2+}\) cycling is directly linked to increased arrhythmogeneity (1). Increased fibrosis is another important factor that induces slowing or even blocks electrical conduction, thereby increasing the susceptibility to arrhythmias (35). Elevated inflammation markers were associated with states of electrical remodeling in an earlier study (30). In our model, untreated dTGR with terminal cardiac damage commonly develop microinfarctions, with variable areas of damaged cells, fibrosis, and inflammation. Such areas also contribute to an arrhythmogenic substrate (15).

We also found a significant dysregulation of ion channels and proteins implicated in increased arrhythmogeneity. We focused our analysis on important ion channels determining repolarization and the main ventricular gap-junction protein Cx43. Various studies showed that gene expression of potassium channel subunits Kv4.3 and HERG correlate with the activities of \(I_{\text{to}}\) and \(I_{\text{Kr}}\) currents (16, 29). A pronounced down-regulation of Kv4.3 represents a characteristic finding in electrical remodeling in the hypertrophied, especially failing heart (23). With CMFM, we found a delay in the T-wave peak, indicating prolongation of the early phase of repolarization. This observation correlates well with our finding of a down-regulated Kv4.3 (\(I_{\text{to}}\)) potassium channel, which is the main repolarizing ion current in rat ventricular myocardium in this phase. Interestingly, this delay in early repolarization did not lead to significant QT prolongation at 5 wk, indicating that overall repolarization was not prolonged. In contrast, QT and QTc were significantly prolonged in 7-wk-old dTGR based on a significantly increased \(T_{\text{peak}}-T_{\text{end}}\) interval. This interval was shown to correlate with increased dispersion of repolarization and increased incidence of arrhythmias (9, 19). However, CMFM from multiple sensor positions over the thorax provided additional information about spatial distribution of myocardial electrical signals. We found an increased regional dispersion of the T-wave peak in butterfly plots mainly in preterminal 7-wk-old dTGR. Nakai et al. (21) reported similar magnetocardiography findings in patients after myocardial infarction.

The observed prolongation of the QRS complex has been associated with an increased risk of arrhythmias and sudden cardiac death (17). The finding reflects slowed myocardial conduction, which is induced by fibrosis, inflammation, and alteration in Cx43 abundance and localization (2, 27, 28) Correspondingly, the inhomogeneous CMFM of the late QRS complex was already increased by wk 5. The increase in field strength at ECG \(R_{\text{peak}}\) in 5-wk-old untreated dTGR also attests to the sensitivity of the CMFM technique. The increased signal strength reflects the physiological response of a still-organized and functioning myocardium to increased afterload (5). Although hypertrophy was further elevated at 7 wk, the CMFM signal-strength range decreased with progressive cardiac damage. This finding may result from the progressive myocardial deterioration, including enhanced fibrosis, scarifications, and inflammation. Rotation of the cardiac magnetic field, especially in the ST-T interval, was also found in patients with left-ventricular hypertrophy and cardiac ischemia (4, 12). The reasons for these changes in magnetic field distribution could be related to changes in underlying myocardial electrical currents. Interestingly, these changes were already visible in QRS and ST-T maps in untreated dTGR at wk 5. Thus our findings are in agreement with the concept of altered ion-channel and gap-junction gene expression and the occurrence of electrical remodeling.

The observed noninvasive CMFM findings are substantiated by the direct evidence for an increased inducibility of ventricular arrhythmias by programmed electrical stimulation in 7-wk-old dTGR. Although this method does not reflect the complex mechanisms that led to the documented spontaneous sustained VT, it is an established method to show the capability of the myocardium to initiate and maintain tachyarrhythmias. The prolongation of ventricular refractoriness is probably the result of ANG II-mediated action potential prolongation and meets the observed prolongation in repolarization. Interestingly, Regan et al. (26) described the same effect of prolonged ventricular refractoriness in SD rats under infusion of 4-aminopyridine, a nonselective potassium channel blocker.

ANG II induces fibrosis, inflammation, an increase in sinus rate and pacemaker activity in the His-Purkinje-System, changes in Ca\(^{2+}\) cycling, a decrease in conduction velocity as a result of cell-to-cell-uncoupling, inhibition of inward Na\(^{+}\) current, increased activity of the sympathetic nervous system, the release of aldosterone, the inhibition of repolarizing potassium channels, and increased dispersion of repolarization (6). In humans, angiotensin-converting enzyme inhibitors, AT1 receptor blockers, and mineralocorticoid receptor blockers have all exhibited antiarrhythmic potential (32). We cannot exclude a blood pressure-dependent influence on electrical
remodeling in our model. Nevertheless, we believe that blood pressure-independent ANG II actions account for the electrical alterations. Studies from mice with cardiac-restricted high ANG II levels and heart-specific mineralocorticoid receptor overexpression showed sudden cardiac death, atrial and ventricular arrhythmias, impaired arteriovenous conduction, and an acquired long QT syndrome despite normal blood pressures (8, 24, 38). We observed similar events in our dTGR model.

Conclusions. Our data underscores the propensity for ANG II to induce VT. We believe that the study of our model allowed a closer view of the mechanisms involved. We showed that the CMFM technique is sensitive to image electrical remodeling in rats with cardiac damage and a predisposition to potentially lethal arrhythmias. ANG II-induced electrical remodeling is part of the pathophysiological process in hypertensive heart disease and accounts for an increased arrhythmia burden in these patients. Several clinical CMFM studies suggested that multichannel CMFM systems can detect an increased risk in life-threatening arrhythmias (18, 19, 34). We believe the perspectives of the CMFM technique lie in determining nonhomogeneous aspects of depolarization and repolarization before the conventional ECG can detect these changes. CMFM could facilitate identifying high-risk individuals within the enormous number of hypertensive patients to subject them to an appropriate pharmacological treatment. As shown in our study, it could also help evaluate the benefit of therapeutic approaches.

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