Arrhythmogenic substrate in hearts of rats with monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy

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Benoist D, Stones R, Drinkhill M, Bernus O, White E. Arrhythmogenic substrate in hearts of rats with monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy. Am J Physiol Heart Circ Physiol 300: H2230–H2237, 2011. First published March 11, 2011; doi:10.1152/ajpheart.01226.2010.—Mechanisms associated with right ventricular (RV) hypertension and arrhythmias are less understood than those in the left ventricle (LV). The aim of our study was to investigate whether and by what mechanisms a proarrhythmic substrate exists in a rat model of RV hypertension and hypertrophy. Rats were injected with monocrotaline (MCT; 60 mg/kg) to induce pulmonary artery hypertension or with saline (CON). Myocardial levels of mRNA for genes expressing ion channels were measured by real-time RT-PCR. Monophasic action potential duration (MAPD) was recorded in isolated Langendorff-perfused hearts. MAPD restitution was measured, and arrhythmias were induced by burst stimulation. Twenty-two to twenty-six days after treatment, MCT animals had RV hypertension, hypertrophy, and decreased ejection fractions compared with CON. A greater proportion of MCT hearts developed sustained ventricular tachycardias/fibrillation (0.83 MCT vs. 0.14 CON). MAPD was prolonged in RV and less so in the LV of MCT hearts. There were decreased levels of mRNA for K+ channels. Restitution curves of MCT RV were steeper than CON RV or either LV. Dispersion of MAPD was greater in MCT hearts and was dependent on stimulation frequency. Computer simulations based on ion channel gene expression closely predicted experimental changes in MAPD and restitution. We have identified a proarrhythmic substrate in the hearts of MCT-treated rats. We conclude that steeper RV electrical restitution and rate-dependent RV-LV action potential duration dispersion may be contributing mechanisms and be implicated in the generation of arrhythmias associated with in RV hypertension and hypertrophy.

The monocrotaline (MCT)-induced model of pulmonary arterial hypertension and RV hypertrophy is well established (e.g., Refs. 14, 23). MCT is a pyrrolizidine alkaloid from the plant Crotalaria spectabilis. A single injection of MCT results in injury to the vascular endothelium of the lung, pulmonary hypertension, and RV hypertrophy and failure occurs within 3–4 wk (15, 24). Heart failure can be identified by clinical symptoms that have been linked to hemodynamic indexes of right heart failure (15). Following MCT treatment, the RV action potential is prolonged (9, 26, 32). This is associated with decreased K+ channel activity, although studies disagree precisely which channels (26, 27, 32, 41). Interestingly, the changes in ventricular gradient and QT interval seen in human PAH (17, 19, 20) are also seen in the MCT model (16, 27, 32). These observations would predict that the MCT model is proarrhythmic, but this has not previously been directly investigated. The purpose of this study was to test the hypothesis that the MCT model of pulmonary hypertension has a proarrhythmic substrate and, if so, to investigate associated mechanisms to better understand arrhythmias associated with RV hypertension and hypertrophy.

MATERIALS AND METHODS

Animal model. All experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986, with the approval of the UK Home Office. Male Wistar rats (200 g) received a single intraperitoneal injection of MCT (60 mg/kg in saline) or an equivalent volume of saline. Animals were weighed weekly for 3 wk postinjection and then daily. Ethical permissions required intervention on the observation of clinical signs of heart failure (e.g., dyspnea, cold extremities, lethargy, and weight loss) to prevent unplanned mortality (see Refs. 9, 16). Control (CON) animals were killed on equivalent days, postinjection.

In vivo measurement of RV function. Rats were anesthetized with isoflurane (5%) and oxygen. Following induction of anesthesia, they were placed on a heated pad to control body temperature. Anesthesia was then maintained using 1.5–2.5% isoflurane. While the animals were under an OPMI pico surgical stereomicroscope (Carl Zeiss), a midline incision was made in the neck and the left and right jugular veins were isolated. A 1.4-F combined pressure-conductance catheter (model SPR-839; Millar Instruments) was advanced into the RV via the right jugular vein and secured in place. The catheter was connected to a pressure-conductance unit (MPVS-300; Millar Instruments), and the data were acquired on a Power Lab 8/30 (AD Instruments) attached to a computer running LabChart Pro (AD Instruments). The pressure-volume loops were analyzed using PVAN 3.6 software (Millar Instruments) to determine the cardiac hemodynamics. A cannula was inserted into the left jugular vein to inject a bolus (20 µl) of hypertonic saline (15%) to determine the parallel conductance of surrounding tissue (30). Before cardiac catheterization, the Millar device was calibrated for pressure using a mercury manometer. At the end of the experiment, fresh heparinized blood was...
collected from the animal and used to fill a cuvette (Millar P/N 910–1048) to calibrate for volume.

Measurement of monophase action potentials and electrical restitution curves. Isolated hearts were mounted on a Langendorff perfusion system and perfused at 0.11 ml s⁻¹ g⁻¹ with a modified Krebs-Henseleit solution containing the following (in mmol/l): 118.5 NaCl, 25 NaHCO₃, 11.1 glucose, 4.2 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄·H₂O, and 1 CaCl₂ continuously gassed with 95% O₂-5% CO₂ (pH 7.4) at 37°C. Hearts were paced at the right atrial-ventricular junction, and monophasic action potentials (MAPs) were recorded on the epicardial surface of the RV and LV using Franz-type (12) spring-loaded contact electrodes. MAP duration (MAPD) was measured at 25, 50, and 90% repolarization. In one set of hearts, electrical restitution curves were generated by stimulating hearts with a computer-driven S1–S2 protocol whereby 20 S1 pulses with a cycle length of 200 ms were followed by the application of a S2 stimulus at an interval that was reduced from 200 to 100 ms in 20-ms decrements and from 100 to 40 ms by 10-ms decrements. The MAP generated by the S2 stimulation was measured, and the shortest S1–S2 interval generating a MAP was assumed to be the effective refractory period. Computer-driven S1–S2 stimulation protocol whereby 20 S1 pulses with a cycle length of 200 ms down to 40 ms in decrements of 10 ms was applied, and external stimulation was then stopped and the heart’s intrinsic pacing allowed to resume. Stimulus intensity during burst pacing was increased until tachycardia or fibrillation was induced (e.g., Ref. 28). ARRHYTHMIAS were defined by the presence of tachycardia (VT, a rapid intrinsic heart rate >10 Hz) or ventricular fibrillation (VF). The duration of the VT/VF stimulation interval of 20 ms was assumed as nonsustained arrhythmia if it lasted 90 s or as sustained if it lasted 90 s. The dominant frequency of arrhythmias was assessed by fast-Fourier analysis.

Measurement of ion channel mRNA by real-time RT-PCR. Hearts from 12 CON and 14 MCT animals were removed, and the RV and LV free wall were excised and snap frozen in liquid nitrogen between 90 s or as sustained if it lasted 90 s. The dominant frequency of arrhythmias was assessed by fast-Fourier analysis.

Computer simulations. We utilized a mathematical model of cardiac electrophysiology developed by Pandit et al. (31) to investigate the effects of changes in ion channel expression on the action potential in RV heart failure. The Pandit model is based on experimental data obtained from isolated rat LV myocytes at room temperature (22°C) and contains Hodgkin-Huxley formulations for the major transmembrane ion channels (I_Na, I_CaL, I_K1, I_Na,b, I_CaL,b, and I_K1) as well as a description for sarcolemmal pumps (I_Na,K and I_CaL,TP), the sodium-chloride exchanger (I_NaCl), and a multicompart-ment model of intracellular calcium handling (31). Here, a description of I_Ks was incorporated in the model and the ion channel gating kinetics of I_CaL, I_Na,b, I_K1, I_Ks, and I_Ks were scaled by Q10 factors of 2.1, 2.66, 2.66, 2.18, and 2.18, respectively, to allow simulation of LV action potentials at 37°C, as recently done by Noujaim et al. (29). A formulation of the T-type calcium current I_CaT was incorporated as in Faber and Rudy (10). RV (and MCT-treated LV) action potentials in the CON and MCT group were obtained by scaling the relevant whole cell ion channel conductances according to their respective mRNA expression obtained from our PCR studies and normalized to the control expression in the LV (6). We only retained those changes in ion channel expression between CON and MCT right hearts that were statistically significant and for which the corresponding current was present in the mathematical model. Supplemental Table S2 summarizes the whole cell ion channel conductances of interest in the four ventricular types and the computed APD at 90% repolarization (Supplemental Material for this article is available online at the Am J Physiol Heart Circ Physiol website). The extracellular Na⁺, K⁺, and Ca²⁺ concentrations were assumed to be 143.5, 5.4, and 1.0 mM, respectively, to match the Tyrode solution used in our MAP experiments. All other parameter values are as described in the original model (31). The model equations were solved using a forward Euler method with an adaptive time step varying between 0.001 and 0.01 ms in real-time PCR.

RESULTS

RV hypertension and hypertrophy. MCT treated animals were killed upon showing clinical signs of heart failure, 22–26 days postinjection. They displayed significantly greater heart and lung weights, RV (but not LV) weights, and RV-to-LV weight ratios (Table 1). Millar catheter recordings from the RV

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Table 1. Whole animal and organ weights in CON and MCT animals

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<tr>
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<th>CON (n = 18)</th>
<th>MCT (n = 14)</th>
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<tr>
<td>Body weight, g</td>
<td>346 ± 8</td>
<td>293 ± 71*</td>
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<tr>
<td>Heart weight, g</td>
<td>1.70 ± 0.06</td>
<td>1.99 ± 0.06*</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>1.75 ± 0.09</td>
<td>2.69 ± 0.06*</td>
</tr>
<tr>
<td>Heart/body weight, mg/g</td>
<td>4.91 ± 0.10</td>
<td>6.83 ± 0.20†</td>
</tr>
<tr>
<td>RV weight/body weight, mg/g</td>
<td>0.76 ± 0.02</td>
<td>1.04 ± 0.07†</td>
</tr>
<tr>
<td>LV weight/body weight, mg/g</td>
<td>1.61 ± 0.03</td>
<td>1.06 ± 0.06 (P = 0.835)</td>
</tr>
<tr>
<td>RV weight/LV weight, g/g</td>
<td>0.04 ± 0.02</td>
<td>0.95 ± 0.05†</td>
</tr>
<tr>
<td>Lung weight/body weight, mg/g</td>
<td>5.04 ± 0.24</td>
<td>7.17 ± 0.34†</td>
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Values are means ± SE. RV, right ventricle; LV, left ventricle. *P < 0.01, †P < 0.001, control (CON) vs. monocrotaline (MCT).
of anaesthetized animals, on the day of death, showed significantly increased RV diastolic and systolic pressures and volumes in MCT animals, together with a significant reduction in ejection fraction (Table 2).

**MAPs.** At a stimulation frequency of 5 Hz, the MAPD was significantly longer in the RV of MCT hearts compared with CON hearts at early, mid, and late repolarization (Fig. 1, A and B; *P < 0.001). Novel observations were that MAPD90 was correlated with heart weight-to-body weight ratio (HW:BW; $R^2 = 0.90; P < 0.0001$) for CON (○) and MCT (●) hearts. B: RV MAPD duration of MCT hearts was significantly prolonged at each level of repolarization (**P < 0.0001, CON vs. MCT). C: MAP duration at 90% repolarization was significantly correlated with the heart weight-to-body weight ratio (HW:BW; $R^2 = 0.90; P < 0.0001$) for CON (○) and MCT (●) hearts. D: LV MAPD duration in failing hearts was significantly prolonged at each level of repolarization (*P < 0.05, **P < 0.01, CON vs. failing; n = 17 CON and 14 MCT hearts).

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<tr>
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<th>CON (n = 8)</th>
<th>MCT (n = 10)</th>
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<tbody>
<tr>
<td>HR, beats/min</td>
<td>429 ± 9</td>
<td>393 ± 15</td>
</tr>
<tr>
<td>SV, µl</td>
<td>33.7 ± 8.6</td>
<td>36.6 ± 4.3</td>
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<tr>
<td>CO, µl/min</td>
<td>14,683 ± 3,903</td>
<td>14,458 ± 1,906 (P = 0.957)</td>
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<tr>
<td>EDP, mmHg</td>
<td>4.73 ± 0.81</td>
<td>8.0 ± 1.2*</td>
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<tr>
<td>ESP, mmHg</td>
<td>24.1 ± 3.06</td>
<td>79.2 ± 6.2‡</td>
</tr>
<tr>
<td>Ved, µl</td>
<td>47.3 ± 14</td>
<td>97.7 ± 14.7*</td>
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<tr>
<td>Ves, µl</td>
<td>24.5 ± 10.1</td>
<td>70.6 ± 12.5*</td>
</tr>
<tr>
<td>EF, %</td>
<td>66.2 ± 8</td>
<td>38.4 ± 3‡</td>
</tr>
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Values are means ± SE. HR, heart rate; SV, RV stroke volume; CO, cardiac output; EDP, RV end diastolic pressure; ESP, RV end systolic pressure; Ved, RV end diastolic volume; Ves, RV end systolic volume; EF, ejection fraction. *P < 0.05, †P < 0.01, ‡P < 0.001, CON vs. MCT.

**Altered ion channel expression in MCT hearts.** Gene expression of ion channels that contribute to the rat action potential was measured using real-time RT-PCR (Fig. 2 and Supplemental Table S1). In the RV, the expression of the major Na+ channels (Nav1.5) was unchanged. Gene expression of the major Ca2+ channels subtype (L-type) was decreased but was increased for the minor (T-type) Ca2+ channel. mRNA expression for the genes encoding for many K+ channels was reduced. In contrast, markers for heart failure (atrial and brain natriuretic peptide, collagen type 1, ratio of β-to α-myosin heavy chain) were all increased. Changes in MCT LV gene expression were broadly similar to RV but less numerous, such that of 18 mRNAs measured, 16 were significantly changed in the RV but only 10 in the LV, relative to the corresponding CON chamber (see Supplemental Table S1).

Predictive computer simulations based on the significant changes in Ca2+ and K+ ion channel gene expression (see Supplemental Table S2) were able to closely model action potential prolongation in the RV (Fig. 3A) and the lesser prolongation in LV (Fig. 3B). Analysis of the contribution of individual ion channels predicted that the differential lengthening of the RV and LV action potential in MCT myocytes was primarily due to the differential depression of $I_{to}$ and $I_{K1}$ currents. By altering currents in isolation we observed that...
those giving the closest responses to the ensemble changes, and thus the most influential in the simulated APD changes were \( I_{\text{to}} \) and \( I_{\text{K1}} \). When an S1–S2 type APD restitution experiment was simulated, it was observed that the RV, MCT restitution curve had a steeper slope than the three other simulations (Fig. 3C) and that the RV-LV dispersion of APD was greater in MCT hearts (Fig. 3D). Similar observations in vivo might contribute to a proarrhythmic substrate.

**Sustained arrhythmias in MCT hearts.** When isolated hearts underwent burst pacing, CON hearts usually restored intrinsic rhythm after a brief period of tachycardia (e.g., Fig. 4A), the mean duration of these events was 1.90 ± 0.53 s. In contrast, MCT hearts suffered sustained arrhythmias that did not terminate spontaneously (e.g., Fig. 4B). Arrhythmias appeared more easily provoked in MCT hearts as there was a tendency (\( P = 0.11 \), data not shown) for MCT hearts to require a smaller increase in stimulation intensity to provoke arrhythmias. Once initiated, the proportion of MCT hearts showing sustained arrhythmias (90 s before intervention) was eightfold greater than CON hearts. Thus sustained tachycardia or fibrillation was

Fig. 2. Relative expression of mRNA for genes encoding for the major Na\(^+\), Ca\(^{2+}\), and K\(^+\) channels associated with the rat action potential in samples from the RV of normal (open bars) and MCT (closed bars) hearts. Transcript expression is normalized to the housekeeper gene 18S and relative to the normalized transcript expression level in a calibrator sample (***\( P < 0.001 \), *\( P < 0.05 \), CON vs. failing, statistical significance for two-way ANOVA; \( n = 12 \) CON and 14 MCT hearts ). Expression of K\(^+\) channel genes in the RV of MCT hearts is generally depressed. See Supplemental Table S1 for additional RV and all LV data.

Fig. 3. Computer modeling of the intracellular action potential of rat ventricular myocytes using the model of Pandit et al. (31). When the relevant ion channels in the model are scaled from control levels (○) with respect to significant changes in gene expression in MCT hearts (●) (see Ref. 6 and Supplemental Table S2), there is a prolongation of the RV (A) and LV (B) action potential consistent with changes in MAPD measured in MCT hearts. C: simulated S1–S2 APD restitution curves for CON RV (○) and LV (△) and MCT RV (●) and LV(▲). D: simulated differences in RV and LV APD in MCT (●) and CON (○) hearts. C and D are based on the parameters that generated APDs shown in A and B. Simulation predicts steep APD restitution in MCT RV compared with CON and a greater APD dispersion between ventricles in MCT hearts.
significantly more common in MCT hearts ($P < 0.05$; Fig. 4C). The dominant frequency of the arrhythmias in RV MCT was significantly lower than the other ventricles (Fig. 4D).

MAP restitution and dispersion. When S1–S2 protocols were applied to generate electrical restitution curves (Fig. 5; A and B), it was observed that the slope of the restitution curve was significantly steeper and the effective refractory period significantly longer in the RV of MCT hearts (Fig. 5, C and D, $P < 0.001$). In contrast, the slope of the restitution curve of the LV of MCT hearts was similar to CON (Fig. 5B). This heterogenic response in the RV and LV of MCT hearts caused a large MAPD dispersion between the ventricles which altered dramatically with S1–S2 interval (Fig. 6). Using dynamic restitution protocols, we found similar behavior (data not shown).

DISCUSSION

The principal novel findings of our study are as follows: 1) the MCT model of pulmonary hypertension and RV hypertrophy has a proarrhythmic substrate; 2) electrical remodeling occurs in the LV of MCT hearts as well as RV, but to a lesser degree, resulting in increased electrical heterogeneity; and 3) MCT hearts display steeper RV action potential restitution and enhanced RV-LV dispersion of APD. We predict that these mechanisms contribute to the proarrhythmic state.

Our in vivo and whole heart measurements revealed substantial RV hypertension, hypertrophy, dilation, and an almost halved ejection fraction. These findings together with observed clinical signs and increased expression of heart failure marker genes indicate heart failure, as previously reported (5, 15, 24). Our findings show that the predominant effect of MCT was in the RV but that there were also effects in the LV. This is consistent with recent studies (e.g., Refs. 8, 25) that have found mechanical properties of the LV were altered in this model, possibly via neurohormonal and biomechanical mechanisms.

MAP changes. Our observation that the RV MAP duration was prolonged is consistent with action potential and QT interval measurements in previous studies (9, 26, 32). The correlation between the degree of cardiac hypertrophy and APD is a novel observation in this model and is similar to that between impaired mechanical function and hypertrophy recently reported in MCT hearts (40). We also observed prolongation of LV action potentials by MCT, contrary to the findings of Lee et al. (26). This was perhaps because the previous study (26) did not account for regional variations in LV APD (7) (our own LV data was only taken from the subepicardial myocardium) and because their recordings were made with 10 mM EGTA in the recording pipette, which would be expected to greatly influence Ca$^{2+}$-activated currents and APD.

Gene expression of ion channels and computer simulations. Previous studies have investigated gene expression of selected K$^+$ (27, 32, 41) and Ca$^{2+}$ channels (36), principally in the MCT-treated RV. Ours is the first to combine measurement of Na$^+$, Ca$^{2+}$, and K$^+$ channels in both the RV and (subepicardial) LV within a single study. When measured, studies agree that mRNA levels for Kv1.2, 1.5, 4.2, and 4.3 are decreased (27, 32, 41, and present study) but Kv1.4 (27, 32) and Kv 2.1 (32) are not always depressed. There is evidence that MAPD and K$^+$ channel remodeling is linked to a metabolic shift associated with pyruvate dehydrogenase kinase activity (32).
Our finding that the RV of MCT hearts has increased expression of T-type Ca\(^{2+}\) channels is consistent with the observations of Takebayashi et al. (36) and suggests its role in the proarrhythmic state of MCT hearts warrants further investigation given the upregulation of this ion channel and its role in arrhythmogenesis in other types of heart failure (22).

Computer simulations of electrical activity were based on changes in gene expression, which may not always reflect ion channel activity and may thus be a limitation of our study (see Ref. 35 for discussion). However, there is good agreement in the differences between CON and MCT hearts in Kv4.2/4.3 mRNA (27, 32, and present study) and Kv4.2/4.3 protein (32) and changes in \(I_{\text{to}}\) current (26). In the present study, the simple scaling of changes in ionic conductance to changes in mRNA expression, initially described by Chandler et al. (6) was remarkably effective at predicting the scale of changes in RV and LV APD and the APD restitution characteristics we measured experimentally and suggest that changes in both \(I_{\text{to}}\) and \(I_{\text{K1}}\) are important for action potential prolongation.

Proarrhythmic consequences of altered electrical properties. We have demonstrated that the MCT model is proarrhythmic; there was a tendency for arrhythmias to be more easily triggered and, once triggered, they were much more likely to be sustained. In MCT-treated hearts, the RV has a dramatically prolonged action potential with steep restitution characteristics while the LV APD is only slightly prolonged with more normal restitution characteristics; thus the RV-LV APD dispersion is highly variable at physiological heart rates. This observation is consistent with increased APD dispersion predicted from changes in ECG-derived ventricular gradients in MCT hearts (16). Action potential prolongation (39), a steeper restitution curve (21, 34), and increased dispersion of APD (1) are known to be mechanisms that predispose to arrhythmias by generating a substrate for reentry and were all displayed in MCT hearts. The lower dominant frequency of arrhythmias (Fig. 4D) is consistent with the increased refractory period (Fig. 5D) we measured in the MCT RV. However, the cycle length of the dominant frequencies is shorter than the measured refractory periods; this may reflect the rapid and chaotic pacing history of the arrhythmic events. Comparisons of the dominant frequencies between MCT and CON should be made cautiously due to the different timescales over which they were measured.

Fig. 5. Electrical restitution in CON and MCT hearts. A: example of a S1–S2 electrical restitution experiment. MAPs were recorded from the epicardial surface of the RV, and the heart was paced at 5 Hz (indicated by regularly spaced lines above each MAP). Following the final (S1) stimulus at 5 Hz, an S2 stimulus was given with varying delay (indicated by arrow above the final MAP); this is the S1–S2 interval. When the S2 stimulus falls within the absolute refractory period of the tissue, the S2 stimulus fails to elicit a MAP. B: restitution curves showing mean MAP duration at 90% repolarization for various S1–S2 intervals in CON (RV, ○; LV, △) and MCT (RV, ●; LV, ▲) hearts. MCT LV MAPs demonstrated a shallow restitution curve compared with MCT RV. C: maximum slope of the restitution curves for RV CON and MCT hearts. D: effective refractory period (shortest S1–S2 interval to elicit a MAP) in RV of CON and MCT hearts. RV of MCT hearts had significantly steeper restitution curves and longer effective refractory periods than CON hearts. (***P < 0.001, CON vs. MCT; n = 8 CON and 7 MCT hearts).

Fig. 6. Effect of stimulation frequency on MAP dispersion in CON and MCT hearts. Duration of the epicardial RV and LV MAP was simultaneously measured in CON (○) and MCT (●) hearts at different stimulation frequencies. Difference in RV and LV MAP duration at 90% repolarization measures the MAP dispersion. In CON hearts, there was no significant difference between RV and LV MAP at any basic cycle length. In contrast, there was a large RV and LV difference in MCT hearts that changed steeply with stimulation frequency. (***P < 0.01, CON vs. MCT for S1–S2 intervals 200–100 ms inclusive; n = 8 CON and 7 MCT hearts).
A steeper restitution curve on its own could promote wave breaks and sustain VF, and while APD dispersion and altered refractoriness could be important in creating the initial conduction block leading to reentry, it should be noted that the RV-LV APD dispersion in MCT hearts is very unstable with regard to stimulation frequency. Therefore, in general, it seems reasonable to conclude that the APD dispersion and restitution characteristics we observe in MCT hearts are at least in part associated with the proarrhythmic substrate. However, the initiation and stability of VT/VF would seem also to depend on mechanisms operating at short cycle lengths where our data suggest APD dispersion is less pronounced. It should also be noted that changes in the anisotropic properties of the intact heart due to the amount and location of the gap junction protein connexin 43 have been reported in MCT-treated hearts (37). These together with alterations in Ca\(^{2+}\) handling (e.g., Refs 9, 23) are likely to also contribute to electrical remodeling. Therefore, although we have identified a proarrhythmic substrate, further studies are required to fully clarify mechanisms.

**Study implications.** Heart failure is characterized by compromised pumping function, particularly when demand is increased (33), and this phenomena is displayed in the MCT model (24). It was elegantly shown that the rate-dependent decline in force is associated with a frequency-dependent fall in the myofilament Ca\(^{2+}\) sensitivity related to increased levels of phosphorylation of troponin-I (24). However, a frequency-dependent fall in Ca\(^{2+}\) transient amplitude was also reported (9) that may be directly related to the marked frequency-dependent fall in APD we now report in the RV of MCT hearts by abbreviating Ca\(^{2+}\) entry through L-type Ca\(^{2+}\) channels and promoting the extrusion of Ca\(^{2+}\) via NCX (33).

Our RT-PCR investigation has highlighted potential targets for further detailed electrophysiological study. Future studies of ion channel kinetics in single myocytes are likely to be required to fully explain the alterations in restitution characteristics we have observed. Cardiac arrhythmias are associated with several RV conditions such as arrhythmogenic RV cardiomyopathy (3) and the Brugada syndrome (2) in addition to with several RV conditions such as arrhythmogenic RV cardiomyopathy. However, the initiation and stability of VT/VF would seem also to depend on mechanisms operating at short cycle lengths where our data suggest APD dispersion is less pronounced. It should also be noted that changes in the anisotropic properties of the intact heart due to the amount and location of the gap junction protein connexin 43 have been reported in MCT-treated hearts (37). These together with alterations in Ca\(^{2+}\) handling (e.g., Refs 9, 23) are likely to also contribute to electrical remodeling. Therefore, although we have identified a proarrhythmic substrate, further studies are required to fully clarify mechanisms.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


